Scharlau

The wise choice

COMPLETELY REVISED EDITION!

Handbook of Microbiological Culture Media

Dehydrated Culture Media ■ Additives ■ Supplements ■ Culture Media Ingredients ■ Reagents ■ Stains

■ Edition No. 11 ■

First Edition, 1994
Second Edition, 1995
Third Edition, 1996
Fourth Edition, 1999
Fifth Edition, 1999
Sixth Edition, 2001
Seventh Edition, 2002
Eighth Edition, 2003
(second print 2004)
Ninth Edition, 2006
Tenth Edition, 2007
Eleventh Edition, 2011

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Depósito Legal:

Table of contents

Preface

We have the pleasure of introducing our new Microbiological Culture Media Handbook Edition no. 11.
With practical usability in mind, we have decided to continue with the spiral binding, as this format occupies minimum shelf and work space. Our book cover is water resistant to withstand the harsh conditions on the working bench.

We gratefully acknowledge the contributions made by the microbiologists, users of our products and our distributors.
Special thanks to Dr. Sancho, Rosa Baldrís, Xavier Torralba, Paquita Rogers, Michael Summerford, Albert Vidal, Ana Martín and all others who have contributed to the creation of this new Handbook edition.

This edition coincides with the move of our production to a larger plant that allows Scharlau to increase its production capacity and to reach a new level of productivity.

Werner Scharlau
Chairman
Scharlab S.L.

What's new?

Since we published our first Microbiology Handbook in Spanish in the year 1994 under the ADSA Micro brand many changes have taken place.

Microbiology Handbook Edition 11

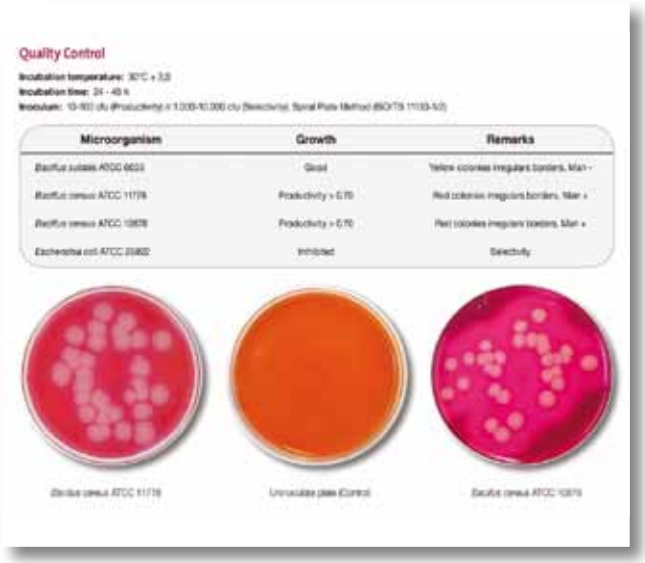
Our handbook is now available in several forms: in printed form as a bound book, on CD and on our website in the form of an eBook.

- The hard copy is spiral bound which means it occupies **minimal space** on the laboratory bench. (A4).
- The cover is made from water resistant plastic material, **impervious to wet** surfaces and splashes or spillages.
- The paper used has a mat finish to **avoid** annoying light **reflections** and is also water repellant.

The **eBook** on our website www.scharlab.com is simple to read and thanks to the **index** and the **search function,** information is easily and quickly accessed.

eBook of our new handbook

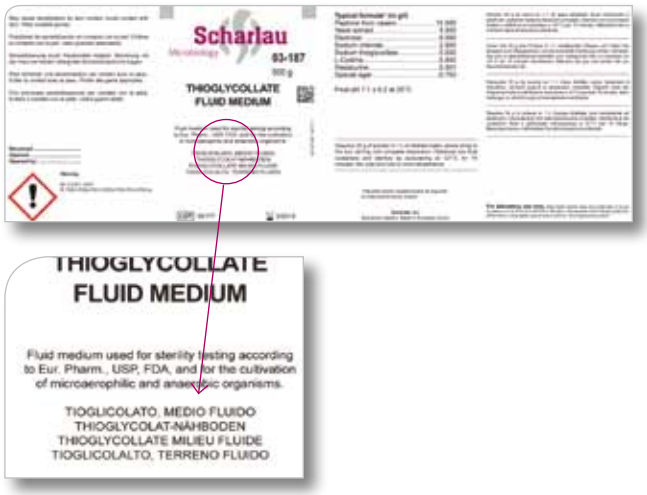
New to this edition is also the incorporation of a **table defining the strains** used for quality control in each medium.



We have also included practical **tables, sorted by industry,** which will help users select the right media for each microorganism to be analysed.

What's new?

On each label we now indicate if the medium is in compliance with a certain **reference method** (see page 11 for abbreviations table).



Change of media names

In order to make it easier to find certain media and following the latest international recommendations, we have decided to change a number of media names. See our index alphabetical index to find easily every media.

PAC-O-VAC

To guarantee that our dehydrated media maintain the same properties as fresh material (free flowing, no loss of growth promotional properties) we are supplying each bottle **vacuum** packed in an outer bag.

Supplements in unique vials

Supplements are available in our unique press-shake-use vials. This is another example of our effort to maximise ease-of-use of our products for our customers.

New freeze-dried supplements

New in this catalogue are the supplements in freeze dried form. Less practical than our unique press-shake-use vials but more economical.

Noteworthy is the fact that we offer the necessary and sterilised solvents along with the supplements. This enables the user to start the dissolution of the supplement immediately, saving the tedious work of measuring and sterilising the solvents.

Method 1

Method 2

What's new?

Diluents in FlexiBags

Food industries and large laboratories with high throughput of samples will welcome the availability of ready-made diluents in larger containers. We offer our diluents in 2, 3 and 5 L sterilised bags, ready for use.

Pre-weighed dehydrated media in sachets

Small aluminium sachets containing the exact quantity of powder to make up 500 mL of reconstituted medium. Ideal for customers that always want fresh material. Comes in boxes of 5 units.

Harmonised media

Recently the American, European and Japanese Pharmacopoeias have come to an agreement to create harmonised specifications for a number of media used in the Pharmaceutical Industry. Scharlau has revised and adapted all its media accordingly, so that customers using our media will comply with the latest regulations (see below).

Table of harmonised media

Art. No	Pack Size	Description Scharlau	Description as per Ph. Eur. 6.0 (2008)	Description as per Ph. Eur. 7 (2011) 7 th ed.
01-030-500	500g	Baird Parker Agar	Medium O	not mentioned
01-309-500	500g	BGA (ISO) (Brilliant Green Agar)	Medium L	not mentioned
02-494-500	500g	Buffered Peptone Water	Buffered sodium chloride-peptone solution pH 7.0	Buffered sodium chloride-peptone solution pH 7.0
01-160-500	500g	Cetrimide Agar	Medium N	Cetrimide Agar
01-680-500	500g	Columbia Agar		Columbia Agar
01-056-500	500g	Deoxycholate Citrate Agar	Medium J	not mentioned
02-064-500	500g	E. E. Broth	Medium E	Enterobacteria Enrichment Broth-Mossel
02-105-500	500g	Lactose Broth	Medium D	not mentioned
02-519-500	500g	Lactose Sulphite Broth	Medium R	not mentioned
01-118-500	500g	MacConkey Agar	Medium H	MacConkey Agar
02-611-500	500g	MacConkey Broth	Medium G	MacConkey Broth
01-116-500	500g	Mannitol Salt Agar		Mannitol Salt Agar
01-483-500	500g	Potato-Dextrose Agar		Potato-Dextrose Agar
01-540-500	500g	R2A	Medium S	not mentioned
02-668-500	500g	Rappaport Vassidialis Broth		Rappaport Vassidialis *Salmonella* Enrichment Broth
03-289-500	500g	Reinforced Clostridial Medium (RCM)	Medium P	Reinforced Medium for Clostridia
01-166-500	500g	Sabouraud Chloramphenicol Agar	Medium C	not mentioned
01-165-500	500g	Sabouraud Dextrosed Agar	Medium C	Sabouraud-Dextrosed Agar
02-165-500	500g	Sabouraud Dextrosed Broth		Sabouraud Dextrosed Broth
03-187-500	500g	Thioglycollate Fluid Medium		Thioglycollate Fluid Medium
02-629-500	500g	Tetrathionate Bile Brilliant Green Broth	Medium I	not mentioned
01-200-500	500g	TSA (Tryptic Soy Agar)		Casein Soya Bean Digest Agar
02-200-500	500g	TSB (Tryptic Soy Broth)	Medium A	Casein Soya Bean Digest Broth
01-192-500	500g	SI (Triple Sugar Iron Agar)	Medium M	not mentioned
01-295-500	500g	VRBD Agar (Violet Red Bile Dextrose Agar)		Violet Red Bile Glucose Agar
01-220-500	500g	VRBLD (Violet Red Bile with Lactose Dextrose Agar)	Medium F	not mentioned
01-211-500	500g	XLD Agar	Medium K	Xylose, Lysine, Deoxycholate Agar

Other sources of support

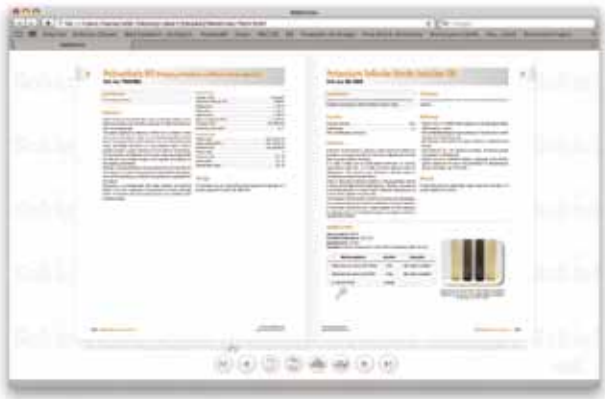
Scharlau's website www.scharlab.com, today, provides a single location where all our up-to-date technical information can be found.
From here you can download

- **Certificates of analysis**
- **Certificates of suitability**
- **Safety data sheets**
- **Leaflets**

Furthermore you will be able to read our catalogue and handbook and all other literature in **eBook** form.
Don't forget to have a look at the FAQ section and also watch any of our **video-tutorials.**

New Scharlab web - Leaflets

New Scharlab web - Certificates of analysis



New Scharlab web - eBook

New Scharlab web - Safety Data Sheets

New Scharlab web - Video tutorials

Documentation

Transmissible Spongiform Encephalopathy (TSE)

Many culture media formulations incorporate raw materials of animal origin meaning there is a theoretical risk of animal spongiform encephalopathy transmission.
Scharlau culture media comply with the guidelines recommended by the European Department for Quality of Medicines (EDQM) on the manufacturing of drugs: Resolution of the Public Health Committee Partial Agreement, Resolution AP-CSP (99-4) and the European Pharmacopoeia section 5. 2. 8 minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products.
This is achieved by making sure that all raw materials of animal origin are sourced only from manufacturers that can prove absence of TSE risk by providing us with a **Certificate of Suitability.** This certificate guarantees that the peptone raw materials come from TSE-free countries like New Zealand, Australia or the United States and that during the production processes contamination does not occur.
Scharlau can provide, on request, a **TSE-Certificate.**

BSE/TSE free certificate

Certificate of suitability

Certificates of Suitability are granted by the Certification Secretariat of the European Directorate for the Quality of Medicines (EDQM).
These certificates can only be granted to the manufacturers of raw materials of animal origin, not to the manufacturers of the culture media. If we, as a manufacturer of culture media, want to guarantee to our users the absence of TSE, the safest way is to buy our raw materials from only those manufacturers that are able to provide us with this certificate of suitability. A pemium price is paid for these high quality, certified, raw materials.
To obtain the Certificate of Suitability, the manufacturer of any animal based raw materials must prove to EDQM that the products are being tested in accordance with the monographs of the European Pharmacopoeia. The manufacturer has to provide the EDQM with a complete dossier and detailed description of the source of the animal tissues used, manufacturing methods, traceability, Quality System in place and a declaration of its willingness to be inspected upon request by the EDQM.
The Certificate of Suitability certifies that a raw material is suitable for use in medical products.
All animal based raw materials with risk of transmitting TSE employed in the manufacturing of culture media at Scharlau have this certification.

Certificate of analysis

Our Certificates of Analysis (CoA) are lot specific and one of the most comprehensive certificates in the market. Raw material is tested before manufacturing. During and after manufacturing we perform basic tests, such as pH testing. Once the lot has been manufactured it is kept in the quarantine zone until the separate quality control laboratory has performed the final QC. Our testing procedures comply with ISO 11133 part 2 and the requirements of the USP (United States Pharmacopoeia) and EP (European Pharmacopoeia) and whatever other directives we indicate on a given product.
Batch specific **recovery data,** obtained for every control strain with the help of a spiral plater, are included on each certificate. Our certificates also carry **colour pictures,** showing typical growth of some of the control strains.
Certificates can be easily **downloaded** from our website www.scharlab.com

CoA for agars *CoA for broths*

Compliance with reference methods

In the product description in this handbook, on the technical sheets and on the labels you will find which directives and pharmacopoeiae our media comply with. Depending on the application of each medium, different directives are relevant and are to be followed (ISO, FDA-BAM, AOAC…).
Whenever any of our formulations are in compliance with or recommended in any reference method, we use the following abbreviations (see next page).

It is understood that in order to guarantee consistent lot to lot performance we need to adjust our formulations in every batch. This is due to the fact that most ingredients are of natural origin and can vary substantially from lot to lot. The formulations indicated in our literature and labels have to be considered typical compositions as allowed in the ISO 11133 part 2.

Documentation

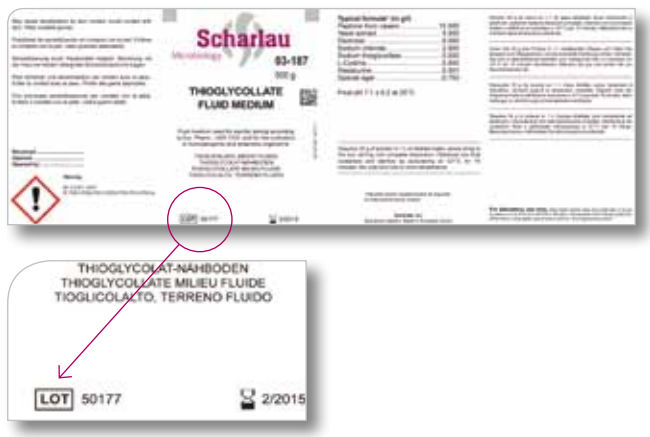
Technical data sheets

These are available from our website www.scharlab.com in pdf format. To make sure the latest information is always available, all the data in this format come from our central data base and is updated automatically, as soon as any changes are made.

Video tutorial in our web

Lot number

The Lot number is printed on each label as well as on the new Datamatrix bar code labels. The new bar code contains the article number, lot number and expiry date, which will allow automation of inventory or the purchasing process. Thanks to the comprehensive information on our bar codes we can trace the person, the day and the raw materials that were used in the production of each lot.



Compliance with reference methods: Abbreviations

Abbreviation	Document	Institution / Organization
APHA		American Public Health Association (Washington DC, USA)
AOAC	Official Methods of Analysis of AOAC International	Association of Official Analytical Chemists
ASM		American Society for Microbiology (WashingtonDC, USA)
ASTM		American Society for Testing Materials
AWWA		American Water Works Association (Washington DC, USA)
BAM	Bacteriological Analytical Manual	Food and Drug Administration (Washington DC, USA)
BP	British Pharmacopoeia	British Pharmacopoeia Commission, The Stationery Office (London)
CEN		Centre Europeen pour la Normalisation (Brussels)
CLSI		Clinical Laboratory Standards Institute (Vilanova, PA, USA)
CMPH	Clinical Microbiology Procedures Handbook	American Society for Microbiology (ASM)
COMPF	Compendium of Methods for the microbiological examination of foods	APHA
DIN		Deutsche Institut für Normung (Berlin)
EDQM		European Directorate for the Quality of Medicines
EN	European Norme	Centre Europeen pour la Normalisation (Brussels)
EP	European Pharmacopoeia 5th Ed.	EDQM (Strasbourg)
FDA		Food and Drug Administration (Washington DC, USA)
FIL		Federation International Laitière (Brussels)
IDF		International Dairy Federation (Brussels)
IFU	IFU Microbiological Methods	International Federation of Fruit Juice Producers (Switzerland)
HP	Harmonized Pharmacopoeias (European, American and Japanese)	EDQM (Strasbourg)
	European Pharmacopoeia 6th Ed., Supplement 6. 3	
ISO		International Standardization Organization (Geneva, Switzerland)
NCLSS		National Committee for Clinical Laboratory Standards (Vilanova, PA, USA)
NMKL		Nordisk Methodenkommittee fur Lebensmittel
SL	Schweizerisches Lebensmittelbuch (Swiss Food Manual)	Bundesamt für Gesundheit, Direktionsbereich Verbraucherschutz (Bern)
SMD	Standard Methods for the Examination of Dairy Products	APHA, (Washington DC, USA)
SMWW	Standard Methods for the examination of Water and Wastewater	APHA, AWWA, WPC
USP	United States Pharmacopeia - National Formulary USP 28	USP Convention Ltd. (Rockville MD, USA)
WEF		Water Envinonment Federation (Washington DC, USA)

Documentation

Transmissible Spongiform Encephalopathy (TSE)

Some of our culture media contain raw material from animal origin, which poses a potential risk of TSE. Scharlau acted quickly in response to this threat and it was decided following the first outbreak, to minimise the risks by:

a) Where possible substitute raw materials of animal origin for raw materials of **plant origin.**

b) Switching to **raw material** suppliers from countries that have not been affected by TSE like the **USA, Australia or New Zealand.**

c) Buying raw materials with risk of TSE infection only from manufacturers that can provide us with a **Certificate of Suitability.**
This Certificate of Suitability is granted by the EDQM (European Directorate for the Quality of Medicines) to the manufacturers of the raw materials which can document their sourcing and manufacturing processes as well as their quality control in order to guarantee the absence of TSE.

Based on the documentation from our raw material suppliers with Certificates of Suitability, Scharlau issues a **BSE/TSE free certificate** for all its media.

BSE/TSE free certificate

www.scharlab.com

From our website users will be able to access and download

- Our handbook in eBook format
- Technical data sheets in English or Spanish
- Topic specific leaflets (Food, Water…)
- Product leaflets (Supplements, Media in bags…)
- Safety data sheets (MSDS)
- Certificates of Analysis (CoA)
- TSE free certificates
- FAQ
- Video tutorials

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GHS

GHS: Globally Harmonised System of Classification and Labelling of Chemicals

Until recently, different classification and labelling systems coexisted in the world. One and the same product could be classified differently in the United States and in Europe.
This is why the United Nations published the GHS in 2002 in the so called "Purple Book" in order to create one system valid for all the countries. As a result of this, new pictograms, "safety phrases" and "risk phrases" appear on our labels. Safety Data Sheets have also been provided for each product.
Most culture media are not classified as dangerous goods and will not require any pictograms. Only those media containing hazardous additives will be classified as dangerous mixtures. The same holds true for a number of additives and supplements.

In the following we will try to sum up the most important issues regarding GHS.

The GHS was born in the UN with the purpose of facilitating world-wide commerce whilst at the same time protecting human health and the environment, unifying the different criteria used for classification of dangerous substances that coexist in the world. Each country or economic community has its own legislation and therefore the same substance can be classified, harmful, not dangerous or toxic depending on the place of classification.

CLP: Classification Labelling and Packaging of Substances and Mixtures

The European Union has adopted the GHS by means of a new regulation (EC) no. 1272/2008, also called CLP, that came into force the 20 of January 2009 in all the Member States, and the hope is that it will also be adopted in Norway, Iceland and Liechtenstein in the near future. The CLP will replace the present system of classification and labelling, defined by the directives 67/548/EEC (DSD) and 1999/45/EC (DPD) for substances and mixtures respectively These directives will become invalid the 1 of June 2015.

The implementation of the CLP is marked in the calendar by two important dates:

1 December 2010

Obligation to classify and to label substances according to the CLP.

1 June 2015

Obligation to classify and to label mixtures according to the CLP.

New Scharlau labels

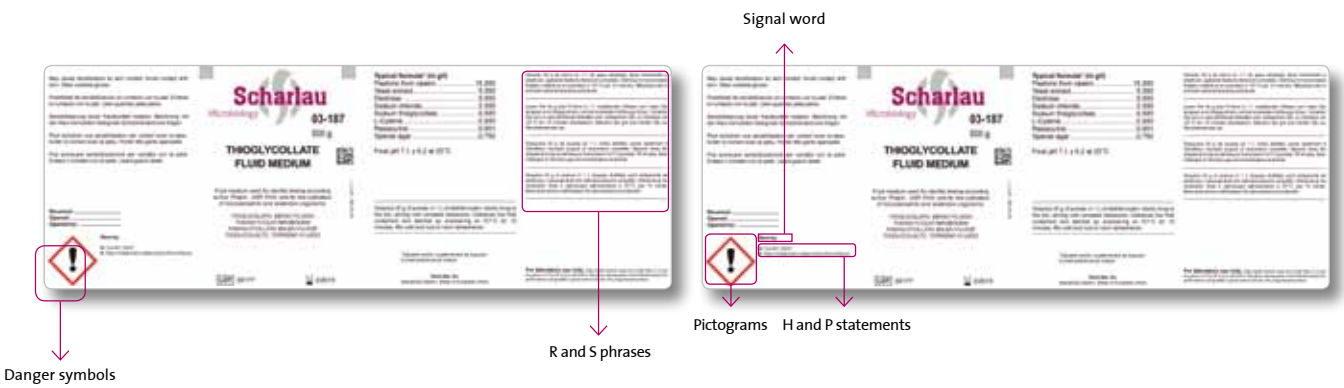
For the past few months Scharlau has been working on its classification system so that it conforms to CLP. We have also designed new labels that incorporate the new pictograms and the hazardous or precautionary statements. As of today, all our products, leave our production facilities labelled in accordance with CLP.

As a result of the application of CLP we have introduced several changes in elements of our labels:

- Pictograms. The traditional danger symbols with an orange background are replaced by new pictograms on a white background surrounded by a red frame.
- The hazard statements (H) replace the traditional risk phrases (R).
- The precautionary statements (P) replace the present safety phrases (S).
- Signal word. The text that accompanied the pictograms, e. g. "Easily Flammable" disappears. Nevertheless, a signal word prefaces new statements H and P indicating the severity of potential hazards. This word can be "Danger" or "Warning".

Old label

New label



The history of modern microbiology

The existence of microorganisms was hypothesized for many centuries and well before their actual discovery in the 17th century. Scholars from many ancient civilisations believed that diseases could be spread by unseen entities. Quarantining and shunning of diseased individuals and burning of infected bodies that had died from disease were practiced by ancient peoples.

However the real science of microbiology began in tandem with the development of the microscope. The first person to report seeing microbes was an Englishman **Robert Hooke.** This was in the early 1660's and in 1665 he published "Micrographia", which described the microscopic world for the first time. However he was unable to "see" bacteria with his crude microscope.

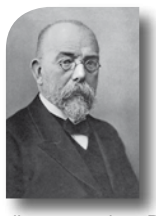
Meanwhile in the Netherlands **Anton van Leeuwenhoek** was the first to see and describe bacteria, which he called "animalcules", or little eels. Up until his death in 1723 he worked tirelessly revealing the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi and bacteria using a single lens of 300-500 fold magnification making these descriptions even more remarkable. Microscopes, however were rare and advancements in microbiology slow.

The theory of the day that held sway and was debated vigorously was that of "spontaneous generation" i. e. that life could spontaneously occur. It was refuted by **Francesco Redi,** who showed that fly maggots do not arise spontaneously from decaying meat, if the meat is covered. **Lazzaro Spallanzani** also disputed the theory by showing that boiled broth could not give rise to microscopic forms of life.

It was not until the latter half of the 19th century, when high-magnification microscopes of good optical quality became more widely available that the science of microbiology began in earnest. One of the most notable scientists to emerge was **Ferdinand J. Cohn** who in 1875 effectively founded the science of bacteriology (a branch of microbiology that studies bacteria). His main contribution was the classification system for bacteria and he coined the name "Bacillus".

Louis Pasteur would probably be considered today as the greatest biologist of the nineteenth century. It was he who finally ended the debate regarding "spontaneous generation". A genius at devising definitive experiments, he used swan necked sterile flasks, filled with broth and successfully showed that life could only be generated from existing life. Pasteur also showed that fermentation, a process used in baking and brewing, was caused by microorganisms. He performed numerous experiments to discover why wine and dairy products became sour, and proved that bacteria were to blame. As a result he went on to develop the process for sterilizing milk and this was named after him "pasteurization". He is also credited with the development of vaccines for (rabies and anthrax).

Pasteur called attention to the importance of bacteria in everyday life and if they could turn wine and milk bad, maybe they could cause human illnesses the "Germ theory" of disease. Pasteur's attempts to prove this theory were unsuccessful and it was left to **Robert Koch,** a German scientist, to provide this proof.



Koch did this by successfully isolating and cultivating anthrax bacteria from a diseased animal. He then injected this pure culture into mice, reproducing the disease and finally, the re-isolated the bacteria from the infected animal. These four steps became known as Koch's Postulate and we still in general follow these dictates today. Because anthrax was such a commercially important disease and his techniques were easily verifiable, he became famous. Like Pasteur he had his own institute and together they developed the basic techniques used in microbiology today.
These include sterile culture techniques, pure culture techniques, the use of Petri dishes, inoculation needles and solid medium, where agar and gelatine were used to produce a solid surface, (the use of Agar-agar as a means to solidify media is attributed to **Dr. Hesse** and his wife Angelina, who joined Koch's lab in1881).
Koch also discovered the etiological agents of cholera and tuberculosis.

Gram stains and other staining procedures were developed in Koch's lab and it was here, while studying differential staining, that **Paul Ehrlich** came up with the idea of a "magic bullet". He reasoned that if different chemical dyes could bind to different components of different eukaryotic cells and differentiate bacteria, perhaps there existed chemicals that could selectively kill certain pathogens without harming the host cells i. e. a targeted drug or "magic bullet". He embarked on a search to find the cure a "magic bullet" for Syphilis and after testing hundreds of chemicals finally found one he named Salvarsan. This discovery laid the ground for antibiotics and other chemotherapeutic agents.

Despite the advances in microbiology during this golden age, it was rarely possible to administer life saving therapy to an infected patient. Eventually in 1928 **Alexander Fleming** accidentally discovered the antibiotic substance penicillin produced by the fungus Penicillin notatum. When it was finally recognised for what it was, the most efficacious life-saving drug in the world, it spawned the development of a

The history of modern microbiology

huge pharmaceutical industry, (post World War II) producing large volumes of synthetic penicillins that would reduce the incidence of many diseases and infections that had been the scourge of mankind. These included pneumonia, tuberculosis, meningitis, syphilis, gangrene and many others.

Because Koch and Pasteur mainly focused on microorganisms having direct medical relevance, their work did not reflect the true diversity of the microbial world. It was only by the late 19th century and the work of **Martinus Beijerinck** and **Sergei Winogradsky,** the founders of general microbiology (an older term encompassing microbial physiology, diversity and ecology) that the true breadth of microbiology was revealed. Beijerinck made two major contributions to microbiology, the discovery of viruses and the development of enrichment culture techniques **(Dmitri Ivanovski** discovered viruses in 1892 but failed to report his findings). While his work on the Tobacco Mosaic Virus established the basic principles of virology, it was his development of enrichment culture methods that had the most immediate impact on microbiology allowing cultivation of a wide variety of different microbes with very different physiologies.

Meanwhile it was Winogradsky who was the first to develop the concept of chemolithotrophy and to thereby reveal the essential role played by microorganisms in geochemical processes He was also responsible for the isolation and description of nitrifying and nitrogen-fixing bacteria.

Work with viruses however was limited by the magnification of available microscopes. In the 1930's two German scientists **Max Knoll and Ernst Ruska** invented the Electron Microscope. This microscope used electrons, not light, to see the object being studied.
During the1940's the electron microscope was further developed and could magnify objects by a million fold. In that decade cultivation methods for viruses were also introduced. The knowledge of viruses grew rapidly and by the 1950's and 60's many viral diseases such as polio, measles, mumps and rubella were under control due to the introduction of vaccines.

Whilst in the past the study of microbiology has in the main focused on diseases and their cure leading to huge progress in the eradication of these, many microbes are responsible for beneficial processes. These include such processes as industrial fermentation e. g. (the production of alcohol, vinegar and dairy products). This type of production has long been practised albeit not on an industrial scale however our advancement in knowledge of microbes particularly with regard to molecular microbiology and genetic engineering has allowed us to harness microbial production on a scale and for a variety of substances like never before.

One of the main areas of applied microbiology is biotechnology. This generally implies "engineered" production of a substance that has involved some sort of genetic engineering (movement of a gene sequence to a microbe). The microorganisms are then used as living factories to produce pharmaceuticals and other substances that could not otherwise be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood clotting factors, clot dissolving enzymes and a number of vaccines.

Ironically, microbes are used to produce biotechnological important enzymes such as Taq polymerase, reporter genes for use in other genetic systems and novel molecular biology techniques such as the yeast two-hybrid system.

Microbes are used for the industrial production of amino acids, biopolymers, etc. and as vectors for cloning in higher organisms such as plants.

Microorganisms are also used in the microbial biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The ability of each microorganism to degrade toxic waste depends on the nature of each contaminant. Therefore, in general, a mixture of bacterial species and strains are used.

Recent research has suggested that microorganisms could be useful in the treatment of cancer. Various strains of non-pathogenic clostridia can infiltrate and replicate within tumours. Clostridial vectors can be safely administered and their potential to deliver therapeutic proteins has been demonstrated in a variety of preclinical models.
The field of microbiology can be said to be still in it's infancy relative to older biological disciplines such as zoology and botany and offers great potential to solve key problems and answer fundamental scientific questions for mankind today.

Microbiology at Scharlau

Manufacture of dehydrated culture media was started in 1979 in Barcelona by Dr. José Sancho, lecturer at the University of Barcelona, together with two other partners. The company was named MCM S.A. (Medios de Cultivo para Microbiología S.A.) and was set up in a small rented space in Avenida del Hospital Militar. Initially the company encountered financial difficulties as investment in research and development of culture media formulations used up all available funds and the partners had to increase the capital several times. In the early eighties the other partners sold their shares to Alfonso Deselaers S.A., a laboratory consumables company with a good sales network in Spain. MCM was renamed ADSA=MICRO and started successfully competing with foreign brands of dehydrated culture media in Spain. At that time Spain still had very high import duties and ADSA=MICRO was the only Spanish manufacturer of these products. Unfortunately Alfonso Deselaers S.A. went into insolvency in 1991, and Dr. Sancho once again had to look for a new partner.

In 1992 Scharlau Chemie S.A., a manufacturer of laboratory chemicals, formed a new partnership with Dr. Sancho to re-establish the production of dehydrated culture media.

Scharlau Chemie S.A. itself was founded in 1949 and was owned by a German national, Mr. Paul Scharlau. After importing and distributing laboratory chemicals from a German manufacturer, the company now began its own production in Spain with the aim of offering products of quality comparable to that of imported reagents.

In the following years the company went through an important transformation. Production was transferred from Barcelona to Sentmenat, where the laboratory chemicals plant is located. The portfolio of products was expanded, the brand name changed from ADSA=MICRO to Scharlau, the handbook was translated into English and exports increased to 80% of total production.

In 2002 Scharlau Chemie S.A. set up a new production facility within the same premises.

In 2008 Scharlau Chemie S.A. and its distribution company Scharlab S.L. merged.

The main activity of Scharlab S.L. is the distribution of laboratory consumables and small laboratory equipment in Spain. This is done by means of a network of over 30 salespeople, who take care of 6000 regular customers. At the same time Scharlab S.L. exports its laboratory chemicals and microbiology products to over 100 countries world wide. This is done through a network of local distributors and partners in the different countries.

In 2009 production of dehydrated media increased again and was moved to a new site. This new facility provides more space, and also using improved manufacturing processes and increased automation will allow Scharlau to triple its current production levels.

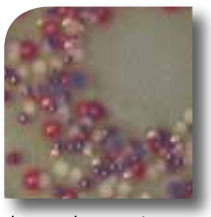
Recognising the increasing demand for prepared media in countries with higher labour costs, Scharlau was quick to respond and bring to the market media in prepared form.
Scharlau was one of the first to adopt the innovative blister-packaging for contact and filtrations plates. With a shelf life of over 6 months this meant it could be sold both in Spain and exported abroad.

Then we added prepared media in bottle and tubes. The protective cap that covers the septum plus the shrink wrap film that holds this cover in place guarantees that the septum of the bottles is sterile. The user does not need to disinfect the septum with alcohol.

Before coming on the market with a range of supplements, our R&D staff focused on how we could improve on what was currently available in the market in terms of safety and easy of use. The result has been our revolutionary CASE supplement vials.
These press-shake-use vials offer the users convenience next to none. The supplement in powder form is stored safely and isolated in the cap. By pressing the cap the powder falls on to the sterilised solvent and is ready to use.

Microbiology at Scharlau

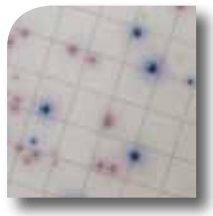
Entry into the chromogenic media market began with a Presence/Absence Test Kit for Coliforms and *E. coli* in water samples, sold under the BACOLI brand name. The bottle is supplied, containing the broth, so that the user only needs to add the water sample, incubate and read under UV light. The broth contains fluorogenic and chromogenic substances that allow detection of total coliforms and *E. coli* at the same time. The chromogenic media line has been extended with the addition of two new media for Coliform and *E. coli* detection.



Colinstant Chromogenic Agar is recommended for all types of samples, where testing for the presence of coliforms is required, specially in foodstuffs and the other CCA (Chromogenic Coliform Agar) is recommended for detection of coliforms in water, e. g. drinking water, wastewater, river and sea water.

Both media can be supplemented with our CV supplement to make it even more selective for coliforms and *E. coli*, inhibiting accompanying pseudomonas and enterococci.

CCA is the chromogenic medium of choice according to the Spanish Ministry of Consumption and Health for the control of water for human consumption.



With regard to **Media Test Fill,** for the pharmaceutical industry, we offer our TSB also in gamma-irradiated bottles, triple sealed. First our outer Pac-O-Vac vacuum pack and in addition a flow-pack bag. Users will be able to save on advance preparation of the medium and will be able to use it in clean rooms.

For large food testing laboratories we offer prepared broth media and diluents in **FlexiBags** of 2 l, 3 l or 5 l. capacity. These FlexiBags are connected to automatic dispensers for easy filling of the Stomacher bags.

Pre-weighed dehydrated media in sachets. Small aluminium sachets containing the exact quantity of powder to make up 500 ml of re-constituted medium. Ideal for customers that always want fresh material. Comes in boxes of 5 units.

Guidelines for correct procedures and practices in the microbiology laboratory

The following instructions provide guidelines for the best procedures and practices in microbiology. Every microbiological laboratory is responsible for their proper implementation.

A. Personal

A. 1. Personal hygiene is the basic element needed for safe handling of microorganisms.

A. 2. All microorganisms must be presumed potentially pathogenic, regardless of their nature.

A. 3. Handling of microorganisms should be conducted in such a way that will not incur any environmental contamination or hazard. For example, aerosols should always be avoided and pipettes must be submerged immediately in disinfectant after use.

A. 4. Technicians, users must be aware of the risks and should know how to prevent them.

A. 5. Care should be taken to ensure that both, the user and the equipment itself are perfectly safe from any potential risk.

B. Equipment in general

B. 1. Standard equipment for the sterilization of any potentially hazardous material must be available (autoclaves, jars with disinfectant for pipettes, dry heat ovens etc.).

B. 2. Protective clothes must be used exclusively in the working area, and should never be worn while in public areas.

B. 3. Toilets and other facilities must be available close to the working areas, and separate from those used by the general public.

B. 4. Any device which may potentially produce aerosols must be always handled in microbiologic safety cabinets like Laminar Air Flow.

B. 5. In case of any accident, all necessary substances and equipment, either to minimize or neutralize the risks must be readily available.

C. Basic characteristics of the laboratory

C. 1. The laboratory or plant should not be located in a passage or corridor so that any non-laboratory personnel cannot easily enter.

C. 2. It must be equipped with an appropriate ventilation system, mechanically regulated if possible, so as to create a flow of air from the areas of low risk through the contaminated areas, and then finally after filtration using HEPA filters, discharged outside the building.

C. 3. The laboratory must be built of easily washable, waterproof and disinfectant-resistant surfaces.

D. Administrative procedures

D. 1. Systems to confirm and ensure the effectiveness of the biological safety protocols carried out by qualified personnel.

D. 2. Training programmes regarding safety during or at work, adapted to the level required by every operator / technician.

D. 3. Adequate medical surveillance and facilities.

D. 4. Procedures for the verification and maintenance of the ventilation system and the equipment in general.

D. 5. Emergency procedures and systems for use in case of any accident.

D. 6. Strategies to restrain entry and exit of non- laboratory personnel, to maintain the safety standards of the laboratory and the plant.

D. Administrative procedures (continuation)

D. 7. Procedures for the transportation, postage and reception of biological material (postage regulations and related matters).

D. 8. Training of personnel to act as security officers.

D. 9. Systems to eliminate any potentially hazardous residues.

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Guidelines for correct procedures and practices in the microbiology laboratory

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Reception of goods

Our CoA (Certificate of Analysis) should be downloaded from our website www.scharlab.com.
Most media are not dangerous but those which are labelled with a pictogram carry a potential risk and the MSDS (Material Safety Data Sheet) should be downloaded and read carefully
Our new Datamatrix barcode labels contain the article number, lot number and expiry date. With a proper bar code reader this data can be automatically input into an inventory database.
Our labels allow appropriate notes to be made on the container e. g. the opening date, the name of the person handling etc.
Where possible our media should be used in order of expiry date.
We supply our dehydrated media in 500 g PE bottles with a tamperproof cap and in a vacuum Pac-O-Vac bag. Bottles with no Pac-O-Vac bag must be rejected.
Alternatively we supply our dehydrated media in sachets that make up 500 ml of solution. These sachets come in boxes of 5. We recommend this product when the requirement is for media used infrequently.

Storage of dehydrated media

Dehydrated culture media should be stored safely, protected from humidity or moisture, light and heat, which are the most frequent causes of deterioration. SCHARLAU media are supplied in opaque, waterproof, screw-cap plastic bottles, with a special stopper in the cap, which eliminates the need for an intermediate cap. Ensure a good seal by keeping the bottle top clean and tightening the cap firmly. The packaging material used (PE), unfortunately, offers no complete barrier against moisture and depending on the storage conditions and the time scale, media may become more or less humid.
To avoid this Scharlau has packaged its media bottles in an additional Pac-O-Vac vacuum bag, ensuring that our media arrive in your lab with the same low moisture content as that measured at the time of bottling.

It is important to open the bottles in a dry atmosphere and close them immediately after their use. Refrigeration is not necessary even if low temperatures prolong the medium's effectiveness. The nature of Scharlau's containers make our culture media suitable for prolonged storage in cool, dry places. The optimum temperature is 15-25°C (59 - 77°F). If the media are moist they become stiff and hard, lose their properties, and can even allow direct growth of microorganisms. Under such circumstances they should not be used. Although they usually have a dry and powdery appearance, the media may occasionally appear moist and oily, but never hard, without there being any alteration in their composition (e. g. as in MRS, Tributyrin Agar, etc.).
Dehydrated media have a shelf life of 3-6 years, depending on the product. Media should be consumed within 12 months of opening the bottle for the first time, as long as the medium has still a valid shelf life.
Loss of quality is easily noted by observing homogeneity, free flow characteristics and colour changes.

Storage of supplements

SCHARLAU offers its supplements in 2 presentations. One offers maximum convenience as the supplement in powder form is mixed with the solvent just before use by pressing on the cap.
The other presentation is in freeze dried form. Supplements come in one vial and the solvent in a separate vial.

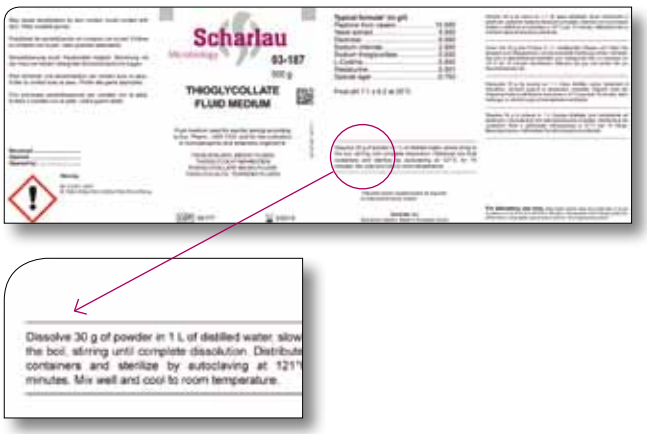
Guidelines for correct procedures and practices in the microbiology laboratory

Both presentations need to be stored in the refrigerator at 2-8°C (35 - 47°F). The shelf life of each supplement is written on the packaging and is generally between 3-5 years.

The appearance and quality of the prepared medium usually depends on the method used for rehydration and storage. It is therefore very important to follow the recommendations stated below.

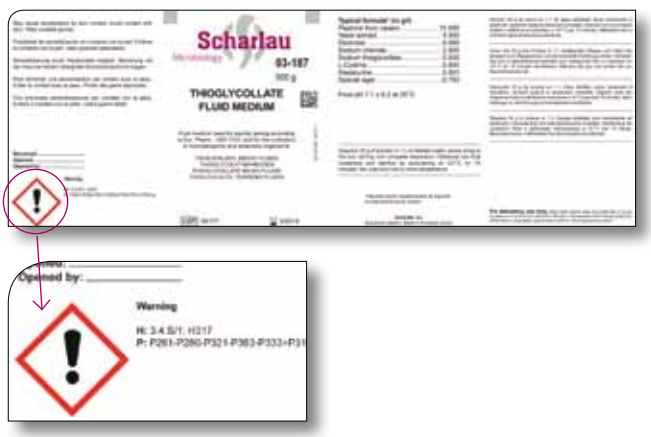
Preparation of culture media

Directions for the individual reconstitution are detailed on the container of every medium and must be strictly followed.



Safety

The reconstitution process must be thoroughly documented: medium description, brand and article number, lot number, opening date, physical condition of medium, operator and date.
A safety mask must be worn for protection from potentially hazardous dust. Safety symbols on the medium bottle should be checked and if necessary work carried out under a hood.



Weighing

Scales with an accuracy ± 0, 1 g should be used for weighing the medium. Clean the surface of the scales after weighing. Dehydrated media are very corrosive and should be removed with water, 70% ethanol or detergent.

Rehydration

The water used for reconstitution purposes should comply with pharmaceutical grade water specifications. Whether water purification has been carried out by distillation or de-ionization, it is important that it is free from nutrients and inhibitors. If the water used is tap water, the chlorine must be neutralized with sodium thiosulphate prior to distillation. Ion exchange resins may be very contaminated by microorganisms so that de-ionized water needs to undergo further treatment before being appropriate for use in microbiology.
The purified water must be stored in containers made from inert materials (neutral glass, PE). Care must be taken that no carbon dioxide dissolves in it, turning the water acidic nor that there is any growth of algae whose metabolites may inhibit growth of microorganisms.

The solution or suspension must be completely homogeneous and the exposure to heat, if necessary, should be minimal.
In any case, it is always helpful to heat the water previously to 50-60°C (122-140°F), to help dissolve the media.

For the preparation of the culture media the most recommended technique is the following:

Dissolution

Add the necessary amount of powder to half the volume of water needed. The water should have already been warmed to 50-60°C (122-140°F). Stir the mixture thoroughly until the powder dissolves. Use the remaining water to dissolve the powder sticking to the walls or sides of the container and continue stirring and heating if necessary. Heat must be applied directly and gently. Constant agitation will prevent the components from sticking to the walls of the container. Use a container with a capacity of approximately 2 to 3 times the volume of the medium, to allow plenty of space for handling. Sometimes, it is necessary to boil the medium for more than 1 minute. In such cases, the volume of evaporated water must be restored.

Media not containing agar or any solidifying agents (Broths and Nutrient-Solutions) tend to dissolve easily and rapidly in preheated water, however sometimes boiling is necessary to obtain complete dissolution. These media have a tendency to form a concentrated syrup or suspension which settles at the bottom of the container. This thick suspension usually remains at the bottom, risking the destruction of the medium contents by hydrolysis, caramelization and pH drift when the heat is applied. Therefore, thorough mixing should be carried out before heating.

Media containing solidifying agents (Agars and Fluid Media) must be preheated before sterilization. Media containing agar must be brought to boiling point, since agar is insoluble in water below 98-100°C (208-212°F).

Guidelines for correct procedures and practices in the microbiology laboratory

It is useful to let the agar soak in preheated water for 5 to 10 minutes before heating, in order to allow it to swell and to ensure its solubility and uniform distribution and dissolution.

The following highly recommended procedure can be carried out.
Remove the rehydrated medium from the source of heat when the mixture begins to boil, letting it stand for a while and then quickly bring it to the boil again. This may be repeated 2 or 3 times with constant agitation. The complete dissolution of the agar will be indicated by the absence of granules in the container.
The solution or suspension must be completely homogeneous and the exposure to heat, if necessary, should be minimal.
In any case, it is always helpful to heat the water in advance to 50-60°C (122-140°F), to help dissolve the media.
Dissolution of culture media directly during sterilization in the autoclave is a frequent and totally incorrect practice. It alters the medium's qualities and often causes the uneven dissolution of the agar into layers with different concentration gradients, pH drifts and browning.

Nowadays, heating the rehydrated medium in a microwave oven in order to melt the agar or dissolve the medium components is a very common procedure. In this case also, it is recommended that the agar-medium soak for some time before using the microwave and to use containers suitable for the volume of medium to be prepared. Using a microwave with 800 Watt for 4 minutes is enough to achieve the total dissolution or melting of the agar. However, it must be noted that since this is a static procedure, a concentration gradient will appear which will create stratification. Thus, it may be necessary to mix the rehydrated medium vigorously to homogenize the solidifying agent before using or sterilizing it in the autoclave.
Microwave heating can never be adopted as a substitute for autoclave sterilization.

Dehydrated culture media usually maintain their characteristics if reconstituted properly. Nevertheless, verification is encouraged since some of them (colour, pH, etc.) may slightly change subject to the conditions of reconstitution and the type of water used.

These recommendations are particularly important when referring to fluid media, since such media contain small amounts of solidifying agent and are therefore very delicate. Cooling becomes as important as heating, as any violent process may induce the formation of flakes and clouds. A slow and gentle cooling process is recommended.

Media containing phosphate buffers together with glucose or other carbohydrates may darken if overheated when in concentrated solution. Precipitates may also appear when using poor quality water.

Special attention must be paid to all media containing agar at a pH below 5, since it hydrolyses and looses the ability to form a gel(solidify). In these cases, unnecessary heating must be avoided.

Glassware and plastic material

Glassware should be made from borosilicate to withstand the usual heating temperatures (100-121°C, 212-250°F) for the reconstitution of media. Only volumetric glassware with a calibration certificate should be used.
The glassware must be free from detergents as these may inhibit the growth of microorganisms. Glassware should be washed with hot water and detergent, thoroughly rinsed and then dried before each use. Stubborn residues may have to be removed from the glassware with more aggressive solutions like potassium dichromate. One way to test for detergent residues is by rinsing the containers with a bromothymol blue indicator solution which will change colour to yellow in the presence of acidic detergent residues or to blue-green/blue in the presence of alkaline detergent residues.

Depending on use, glassware may require additional sterilisation.
Also the volume of the container must be double the volume of medium to be reconstituted, in order to facilitate stirring and addition of supplements or other additives.

Disposable plastic material should be packaged in dust proof bags and if possible in individual packaging. Should sterilised plastic material be required, the correspondent proof of sterilisation (colour label inside the packaging) or a certificate must be supplied.
Plastic ware for repeated use should be treated in the same way as glassware.

Sterilization

For each medium the instructions given on every container must be followed, bearing in mind that they refer to quantities of up to 1 litre. As for bigger volumes, autoclaving and heat penetration conditions of the medium must be taken into consideration.

In all cases autoclaving must be monitored regularly by checking manometers and thermometers, and also noting even distribution of heat. Nowadays this can be done mechanically (thermocouples), chemically (thermal indicators) or biologically (thermoresistant spores). Considering that overheating is one of the main causes of culture media alteration, the treatment of large quantities of media and prolonged exposure to heat must be avoided if possible. For large autoclaves preheating is recommended before the introduction of the medium in the autoclave and also letting the temperature drop to 70-80°C (158-176°F) before removing the medium, so as to avoid severe temperature fluctuations. Although the use of cold water to cool media is widespread, it is not recommended for media containing agar since it causes flake and cloud formation.

Guidelines for correct procedures and practices in the microbiology laboratory

Containers used for sterilization purposes must have a large head space for air to allow foaming. If screw-cap containers are used, the caps must be loosely closed to allow inner and outer pressure compensation.
All containers should be chemically inert to prevent pH alteration of the contents. Borosilicate glass containers are highly recommended.
Media containing carbohydrates darken a little after heat sterilization; therefore, sterilization should be carried out at a temperature below 120°C (248°F) if possible.

Should an autoclave not be available, sterilization can be done in a pressure vessel (at 100°C, 212°F for 30 minutes). In this case it is useful to re-sterilize the medium after three days, if the medium allows for such treatment.
Some media containing selective substances do not need sterilization. The preparation process is quicker since they are ready for use after dissolution. Since these media are usually not very stable and preparation is brief and easy, only the exact quantities required should be prepared.

Additions to the medium (if any) after sterilization must be done aseptically, that is, additives should have been previously sterilized. Additives are generally thermolabile if not they would be part of the dehydrated medium's composition. It is therefore necessary to let the medium cool to 50-60°C (122-140°F) before their addition, so as not to alter the additive while still allowing for thorough mixing and dissolution. Once the supplement has been added, reheating must be avoided.
Ideally distribution into final containers should be completed before sterilizing, to avoid any unnecessary handling of the sterile medium. However since it is rarely possible to do so, handling should be done in safety cabinets instead. The sterile cabinets or rooms should not be exposed to any radiation, nor any kind of highly active reagent that could affect the medium's components. Excessive steam condensation in the final containers can be avoided by distributing the medium into these at temperatures close to solidification, i. e. between 45 to 50°C (113 to 122°F). See tables on the right.

Conversion factors between pressure units

	atm	bar	psi
atm	1,000000	1,013250	14,695900
bar	0,986900	1,000000	14,503700
psi	0,068040	0,068947	1,000000

Autoclave steam pressures and corresponding temperatures

Steam Pressure			Temperature
atm	psi	bar	ºC
0,0000	0	0,0000	100,0
0,0680	1	0,0689	101,9
0,1361	2	0,1379	103,6
0,2041	3	0,2068	105,3
0,2722	4	0,2758	106,9
0,3402	5	0,3447	108,4
0,4082	6	0,4137	109,8
0,4763	7	0,4826	111,3
0,5443	8	0,5516	112,6
0,6124	9	0,6205	113,9
0,6804	10	0,6895	115,2
0,7484	11	0,7584	116,4
0,8165	12	0,8274	117,6
0,8845	13	0,8963	118,8
0,9526	14	0,9653	119,9
1,0206	15	1,0342	121,0
1,0886	16	1,1032	122,0
1,1567	17	1,1721	123,0
1,2247	18	1,2410	124,1
1,2928	19	1,3100	125,0
1,3608	20	1,3789	126,0
1,4288	21	1,4479	126,9
1,4969	22	1,5168	127,8
1,5649	23	1,5858	128,7
1,6330	24	1,6547	129,6
1,7010	25	1,7237	130,4
1,7690	26	1,7926	131,3
1,8371	27	1,8616	132,1
1,9051	28	1,9305	132,9
1,9732	29	1,9995	133,7
2,0412	30	2,0684	134,5

Values are for steam pressure only and any air in the autoclave invalidates temperature data.

Guidelines for correct procedures and practices in the microbiology laboratory

Addition of supplements to medium (see our leaflet for text)

Those components of media which are heat sensitive and therefore cannot be sterilised are not incorporated in the formula and are sold separately. These sterile supplements (freeze-dried antibiotics) or additives (egg yolk emulsions…) are generally kept in a fridge and should be allowed to reach room temperature before addition to the reconstituted and sterilised medium. If too cold, these supplements can cause flaking or gelling of the medium and thus prevent formation of a proper mixture.
Supplements should be dissolved thoroughly prior to addition to the medium. Sterile additives should also be checked visually for absence of microbial growth (blood, egg yolk emulsions).

The medium should be cooled to 45-50° C (113-122°F) before adding any supplements.

Preparation of media with pH values below 6.0

Special care is necessary when preparing culture media of pH values below 6.0 as there is the possibility that the agar may hydrolyze during the heating process (MRS Agar, Orange Serum Agar, Rogosa Agar, Lysine Medium) . This may reduce the gel strength and also trigger a number of other chemical reactions that may alter the performance of the medium.
Hydrolysis can be avoided by adjusting the pH to 7.0 prior to heating and to re-adjust the pH to the original value after sterilisation or heating has been carried out.
Another option would be to add 5 g/l of bacteriological agar (07-004) to the medium before reconstitution.

Pouring agar plates

The medium should be allowed to cool to 45-48° C (113-118°F) prior to filling the Petri dishes. Higher temperatures of the medium can lead to an excess of water condensation ocurring on the plates.
The medium in its container is mixed homogeneously using a magnetic stirrer. Pouring 18-20 ml of medium into each petri dish, ensures a medium thickness of 3-4 mm.
Petri dishes are best placed on a cool, even and horizontal surface and allowed to solidify
after having replaced the lids on each plate.
Plates should never be stacked until the medium has solidified. Solidification takes place from the edges towards the centre.
To remove air bubbles, briefly fan the surface of the plate with the flame of a Bunsen burner.
Before storing, the preparation date and medium description should be noted on the bottom of the plate.

Drying of prepared plates

Condensed water may have formed on the plate. This water should be removed by drying the plates, before any surface inoculation can be performed.
Do not remove condensation water that may have adhered to the lid of the plate. Make sure this water flows back on to the plate.
Remove the lid, turn the plates upside down in an incubator, place the lid on the edge of the inverted plate and start the drying process with warm circulating air at 37°C (99°F) for 20-30 minutes.
The exact time depends on a number of variables and should be validated by each user. Over-drying will result in wrinkled surfaces and strongly impaired media efficiencies (inhibition of Gram-negative microorganisms) and still wet media may enable some microorganisms to move during the growth phase and form more than one colony.

Agar Slant tubes

Certain tests are performed best by culturing on a surface inside a tube. Such a surface can be created by filling the tube with sterilized agar medium and letting it solidify in an inclined position to create a slanting surface of approximately 3 cm in length. Convenient slant racks are sold commercially to obtain reproducible slanting surfaces.

Media for anaerobes

To ensure an anaerobic atmosphere all air must be eliminated from the medium. In order to achieve this, heat the medium in a water bath for 15 minutes with the cap or lid loosely fitted. Thereafter allow the medium to cool to 45-48° C (113-118°F) then quickly tighten the cap or lid. .

Storage of ready prepared culture media

You may decide to buy prepared media or use dehydrated media to produce your own prepared media.
Commercially purchased prepared media will carry an expiry date. These media should be stored ideally between 4-12°C (39-54°F), in the dark and inside a tight polyethylene bag to reduce the risk of dehydration and contamination.
Prepared media with added blood, egg yolk and antibiotics should be stored at even lower temperatures of between 2 and 8°C (36-46°F).

Although it is best to use up all freshly prepared media, it is common practice to produce in excess and to store for later use.
Media prepared in the lab should be stored under the same temperature conditions as indicated above for commercially purchased prepared media.
Media should be allowed to cool down before storage, to reduce water condensation. To achieve the longest possible shelf life (generally 4-6 weeks) placing plates and tubes in hermetically sealed screw-capped containers is recommended.
In some cases, as in thioglycollate media and almost all media for anaerobes, storage at room temperature is recommended to reduce aeration.

- Solid media are best stored in bottles rather than in poured plates.
- However, if plates are poured, the following should be observed:
- Refrigerate the medium immediately after solidification, parallel to

conducting sterility controls with plates from the same batch.

- If the media are to be stored for a longer period, seal each plate individually with adhesive tape and, if possible, store in single containers.
- If storage is to last more than a few days, the plates should be wrapped in plastic bags to avoid dehydration of the medium.

One way to reduce water condensation is by storing prepared plates upside down. The colder the medium is when plates are poured, the less condensation water will be formed and hence the risk of contamination is also reduced. Media which have been stored in the refrigerator should be allowed to warm up gradually. It is recommended keeping them at room temperature for a few hours before inoculation, to allow condensation to clear from the container and to make sure growth can be initiated swiftly.

A loss of water in excess of 15% may affect growth and such plates should not be used. Large volumes of condensed water, or the retraction, cracking and precipitation or crystallization of the medium are all indications of loss of water. If you observe any colour changes you should also discard the plates. Each laboratory should establish its own expiration dates for each culture medium and standards forits particular preparation, packaging and storage conditions.

Re-melting solid media

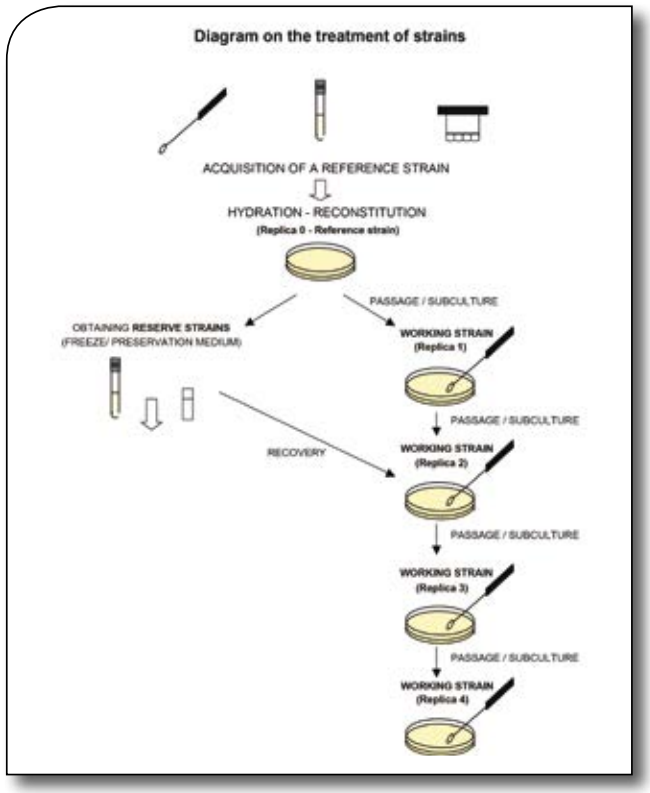
Although most solid culture media can be stored ready prepared and sterilized and can be remelted and poured into plates whenever necessary, this procedure should not be applied to media which do not need sterilization or to those with a pH value of 5.0 or less, since it alters their properties.
Remelting is done in a boiling water bath or by fluent steam autoclaving for 30 minutes. Do not apply direct heat. However, it must be emphasized that remelted media are prone to precipitation and darkening if held in a melted state for more than 1 hour and at temperatures ranging from 45 to 65°C (113 to 149°F). Such media may also undergo nutritional impairment for growth of the desired microorganisms. It is therefore recommended to avoid remelting more than once whenever possible.

One of the quickest remelting methods for culture media is the use of microwave ovens. We recommend placing the bottle or tube with loosened cap in a water bath inside the microwave oven. In this way heat transfer is done more evenly. Validate the necessary time, which should be in the range of 2-3 minutes for a 100 ml bottle.

A common and erroneous practice consists of exposing the bottle to strong radiation for short periods of time, which results in partial remelting, sudden boiling and overflowing of the medium and an overall alteration of media properties.

Melted media should be used as promptly as possible. Under no circumstances should they be remelted a second time.

Test strains



Many quality control procedures are performed using test strains of microorganisms. Protocols outlined by diverse organisations in, e.g. the European Pharmacopoeia (EP), the United States Pharmacopoeia (USP) or the Standard Methods for the Examination of Water and Wastewater (SMWW) recommend certain test strains to be used for the quality control of media. Organisations such as (ATCC, NCTC, DSM…) in each country maintain collections of strains which they either sell directly or through other specialized companies.
Any strain purchased from such an internationally recognized collection is called a **reference or master strain.**
A strain obtained directly from a master strain is called a **reserve strain or primary culture.**
Subcultures obtained from these reserve strains or primary cultures are called **working strains or secondary cultures,** and these are the ones used in quality control. No more than two serial secondary cultures should ever be prepared.

Reference or master strains can be acquired in a number of presentations:

- Vials with caps that contain between one and ten freeze-dried pellets or impregnated discs.
- An injection vial with one freeze-dried pellet
- A loop impregnated with the test strain
- A swab impregnated with the test strain

Every laboratory should maintain a database of its reference strain stocks.

Guidelines for correct procedures and practices in the microbiology laboratory

Irrespective of the presentation, reference strains are stored in a fridge at 2-10°C (35-50°F).

Reconstitution of reference or master strains should be carried out following the supplier's instructions.

The most common methods are:

Reconstitution of freeze-dried strains (pellets) in screw-cap vials:

- Place a disc in 1 to 2ml of broth (TSB or as indicated by the supplier) and leave at room temperature for 5 minutes.
- Inoculate on to a solid medium (as indicated by the supplier and depending on the strain, TSA, Columbia Sheep Blood, Chocolate Agar…) by means of a Pasteur pipette and then streak plate the inoculum using the standard method. .
- Incubate, as indicated by the supplier and depending on each strain, at 37°C or 28°C (99-82°F) in an incubator in an aerobic, anaerobic or microaerophilic atmosphere.
- Check purity and non-contamination by the macroscopic appearance of the colonies.

Reconstitution of freeze-dried strains in crimp-top vials:

- Rehydrate with 1-2ml of broth (TSB or as indicated by the supplier) by means of a syringe and leave at room temperature for 5 minutes.
- Inoculate on to a solid medium (as indicated by the supplier and depending on the strain, TSA, Columbia Sheep Blood, Chocolate Agar…) by means of a Pasteur pipette and then streak plate the inoculum using the standard method.
- Incubate, as indicated by the supplier and depending on each strain, at 37°C or 28°C (99 or 82°F) in an incubator in an aerobic, anaerobic or microaerophilic atmosphere.
- Check purity and non-contamination by the macroscopic appearance of the colonies.

Reconstitution of freeze-dried strains on a loop:

- Remove the seal that protects the handle.
- Place the handle on the plate so as to guarantee contact with the surface of the agar for 10-15 seconds, applying gentle pressure (as indicated by the supplier and depending on each strain, TSA, Columbia Sheep Blood, Chocolate Agar…). Rehydration will be visible by the release of the film contained in the handle.
- Exhaust in classical flute.
- Incubate, as indicated by the supplier and depending on each strain, at 37°C or 28°C (99 or 82°F) in an incubator in an aerobic, anaerobic or microaerophilic atmosphere.
- Check purity and non-contamination by the macroscopic appearance of the colonies.

Reconstitution of freeze-dried strains on a swab:

- Allow the rehydration liquid to be released by pressing the upper part of the cap.
- Press with your fingers the bottom of the device in order to homogenize the pellet with the rehydration fluid.
- Saturate the swab with the resulting suspension.
- Inoculate a plate (as indicated by the supplier and depending on each strain, TSA, Columbia Sheep Blood, Chocolate Agar…) and then streak plate the inoculum with a loop.
- Incubate, as indicated by the supplier and depending on each strain, at 37°C or 28°C (99 or 82°F) in an incubator in an aerobic, anaerobic or microaerophilic atmosphere.
- Check purity and non-contamination by the macroscopic appearance of the colonies.

* Should the swab be sold without a reconstituting liquid, proceed as indicated for loops

Storing of Reserve Strains or Primary Cultures:

Cryopreservation of reserve strains

Prepare a milky suspension of the strain from a pure solid culture obtained from the reference strain and place it, using a sterile swab, in the cryotube containing beads.

- Shake vigorously for one minute and let it settle down.
- Aspirate the supernatant with a sterile pipette leaving only beads in the cryotube.
- Identify the strain as indicated in the chapter below.
- Freeze the properly labelled cryotubes and place them in an assigned position according to the data base record.

Preservation in solid medium

- Stab inoculate or streak a slant tube,containing the preservation medium with the help of a loop from a pure agar medium culture (solid medium is not suitable for Neisseria, Haemophilus and Streptococcus).
- Store in a dark place at room temperature.
- Identify the strain as indicated in the chapter below.

Identification of reserve strains or primary culture:

- Laboratory internal references (Number of location)
- Name of microorganism
- Reference number of the collection
- Expiration Date:
  - 1 year if kept in a solid storage medium
  - 3 years, if kept in cryotubes at temperatures below -20°C (-4°F)

Recovery of reserve strains or primary culture:

Reserve strains or primary culture stored using cryopreservation systems (cryovials of approx. 25 beads in a cryopreservative)

- Extract 1 to 3 beads under aseptic conditions from the frozen cryotube and place on an appropriate nutritive solid medium
  - Columbia Blood Agar: Anaerobic / Streptococcus / Neisseria…
  - Chocolate Agar: Haemophilus
  - TSA / PCA / TLH…
- Rub on the surface of the medium to obtain a thick inoculation line then extend by streaking using a loop to obtain isolated colonies t.
- Incubate, depending on each strain, at 37°C or 28°C (99 or 82°F) in an incubator in an aerobic, anaerobic or microaerophilic atmosphere.
- Check purity and non-contamination by the macroscopic appearance of the colonies.

Guidelines for correct procedures and practices in the microbiology laboratory

Reserve strains or primary culture stored in solid medium (preservation medium)

- Inoculate in solid medium. Depending on the strain in TSA, Columbia Sheep Blood, MacConkey Agar or equivalent) with the help of a loop or with a Pasteur pipette by, stab inoculation or streak inoculation.
- Incubate, depending on each strain, at 35°C or 25°C (95 or 77°F) in an incubator in an appropriate atmosphere.
- Check purity and non-contamination by the macroscopic appearance of the colonies.

Obtaining working strains or secondary cultures:

Working strains are those used for routine quality control and are kept in solid culture media in petri dishes or slant tubes and stored in the refrigerator for a period of time that ensures the viability of the microorganism. These cultures come from the reconstitution of the reference strain, of a reserve strain or from subculture of another working strain.

Working strains contained in plates/tubes are identified as follows:

Not more than 4 replications from the initial reference strain should be carried out, thus ensuring that phenotypic and physiological characteristics of the microorganism are maintained.

Therefore, a working strain from a reference strain will be "1", if it comes from a reserve strain it will be a "two" and if it comes from a working strain, the number will be the one that comes after the number written on the plate used for the sub-culture.

Replications can be carried out a maximum of 4 times. After that, a new reserve strain (primary culture) will have to be recovered and reconstituted and the new working strain will be noted on the plate by the number "2".

Replications of working strains should be carried out every 15-20 days by streak plating and incubated according to the type of microorganism.

Quality control of prepared media

Every new batch produced is QC tested by comparing it with another lot of the same medium which has passed QC and with a non-selective reference medium like TSA, TSB or Blood Agar.

Quality control includes physical parameters as well as sterility tests, growth performance and selectivity.

Physical parameters are comprised of pH measurement, appearance, volume or thickness of the layer, colour, transparency, gel strength and moisture.

Recovery of both wanted and unwanted strains is measured to check for productivity and selectivity.

Growth can be tested in quantitative, semi-quantitative and qualitative manners. Protocols can be found in Pharmacopoeias (EP, USP, JP and in the ISO 11133 part 2).

Trouble shooting

Typical alterations due to incorrect reconstitution

Effect	Possible Causes
Drift in pH	Overheating due to: a) Excessive sterilization. b) Heterogeneous mixing. c) Repeated remelting. d) Improper storage. Inadequate water. Chemically contaminated containers. pH determination carried out on hot medium (allow to cool and check again).
Incomplete solubility	Inadequate water. Insufficient soaking or incomplete mixing which causes overheating. Container too small to allow adequate mixing and/or convection.
Darkening	Overheating. Repeated remelting. Heterogeneous mixing - overheating.
Incomplete gelling Soft gelling	Incorrect proportions of product to volume of water Agar not properly dissolved. pH drift due to overheating. Failure to compensate due to inadequate mixing. Repeated remelting - overheating.
Loss of growth promotion or differential properties	Overheating. Repeated remelting - overheating. Inadequate mixing. Disturbance of the formula by the inoculum (addition of inhibitors or excessive electrolytes).

Guidelines for correct procedures and practices in the microbiology laboratory

Safe working procedures

One should assume that any sample may contain infectious microorganisms. This is why precautions should be taken to avoid microbiological infections. All containers, plates, tubes or bottles that have come into contact with microorganisms have to be sterilized before being discarded.

Laboratories that handle infectious samples or test strains should follow the biosafety guidelines established for the suspected microorganism. Four biosafety levels have been established:

Summary of Recommended Biosafety Levels for Infectious Agents 1

BSL	Agents	Practices	Safety Equipment (Primari Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human dissease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" preacutions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in the text

1 U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 4th Edition. Washington: GPO, May 1999.

- Suitable disinfectants should be used to decontaminate surfaces.
- Small sized corrosion resistant equipment (glassware or plastic ware) can be decontaminated by immersion in a freshly produced disinfectant solution. Single use ware should not be re-used.

Guidelines for correct procedures and practices in the microbiology laboratory

Disposal of biological waste

Waste is defined as any solid, liquid or gaseous material that is no longer used and will either be recycled, disposed of or stored in anticipation of treatment and/or disposal.

Storage

- Prior to disposal, all biohazardous waste should be maintained and stored separately from general waste and from other hazardous wastes. Containers used to store biohazardous waste should be leak-proof, clearly labelled with a red or orange universal biohazard symbol and sealed tightly when transported. In certain cases, it may be necessary to double-bag the waste to prevent leakage. Any biohazardous sharps, such as infectious needles and scalpels, must be placed in containers that are puncture-resistant, leak-proof on all sides and the bottom, and close-able. These containers can then be placed in a standard biohazard bag.

Disposal options

- There are three main disposal options:
  - Render the waste non-infectious by **autoclaving** and dispose it in the general waste stream. If autoclaving is not possible, decontaminate with **chemical disinfectants** or by boiling for 20 minutes before disposal.
  - On-site **incineration,** if possible.
  - **Transportation** of locally-generated waste to a distant appropriate facility.

Incineration is the preferred disposal option. Not only does this method render the waste non-infectious but it also changes the form and shape of the waste. Sterilization is an effective method for decontaminating waste, but it does not alter the appearance of the waste. Steam sterilization in an autoclave at a temperature of 121°C (250°F) for at least 15 minutes destroys all forms of microbial life, including high numbers of bacterial spores. This type of complete sterilization can also be accomplished using dry heat which requires a temperature of 160-170°C (320-338°F) for 2-4 hours. However, it must be ensured that heat comes in contact with the material to be rendered sterile. Therefore, bottles containing liquid material should have loosened caps or cotton plug caps to allow for steam and heat exchange within the bottle. Biohazard bags containing waste should be tied loosely. Once sterilized, biohazardous waste should be sealed in appropriate containers, labelled as disinfected waste and disposed of in an approved facility.
Biological waste should be clearly labelled prior to disposal and complete records should be maintained.

Sterilization

Sterilization is defined as the destruction or removal (by filtration) of all microorganisms and their spores, whereas disinfection is the destruction of many microorganisms but not usually bacterial spores. Sterilization is usually achieved with the help of heat whereas chemical agents are employed in disinfection.
Sterilization and disinfection are part of the daily routine of microbiological laboratories and constitute a vital activity which ensures that cultures, containers, media and equipment are treated in such a way that only inoculated organisms will grow while all others are eliminated.

Sterilization by heat

This can be achieved by autoclaving, by exposing articles to dry heat in hot air ovens or boiling.

Autoclaving

Autoclaves can sterilize anything that can withstand a temperature of 121oC for 30 minutes. A pressure cooker used in homes for cooking purposes can also be used as a makeshift autoclave.
Containers having clinical material are subjected to heat treatment in the autoclave after which these are emptied and washed and put back into service. Only autoclaves designed for laboratory work and capable of dealing with a mixed load should be used. Porous load and bottle fluid sterilizers are rarely satisfactory for laboratory work.
There are two varieties of laboratory autoclaves:

- Pressure cooker type.
- Gravity displacement models with automatic air and condensate discharge.
- **Pressure-cooker type laboratory autoclaves**

  The most common type is a device for boiling water under pressure. It has a vertical metal chamber with a strong metal lid which can be fastened down and sealed with a rubber gasket. An air and steam discharge tap, pressure gauge and a safety valve are fitted to the lid. Water in the bottom of the autoclave is heated by external gas burners, an electric immersion heater or a steam coil.
  - ***Operating instructions***
    - Ensure that there is sufficient water inside the chamber.
    - Load the autoclave and fasten the lid keeping the discharge tap open.
    - Adjust the safety valve to the required temperature and turn the heat on.
    - Allow the mixture of air and steam to pass out freely till all the air has been discharged.
    - Close the air discharge tap and let the steam pressure rise within the chamber till it attains a temperature of 121°C (1.5 kg/cm^2).
    - Hold the pressure for 15 minutes.
    - Turn off the heat and let the autoclave cool.
    - Slowly open the air and steam discharge taps after the pressure gauge has reached zero.
    - Allow the material to cool before these are handled (usually agar bottles take a few hours before these become safe to handle).
- **Autoclave with air discharge by gravity displacement**

  These are usually rectangular in shape and arranged horizontally. These autoclaves have a jacket around the chamber.

• ***Operating instructions***

- Bring the jacket of the autoclave to operating temperature.
- Load the chamber, close the door and open the steam valve so that steam can freely enter the top of the chamber. Air and condensation automatically flow out through the drain at the bottom.
- When the drain thermometer reaches the required temperature, allow a further time period for the load to reach the required temperature (this has to be determined initially and periodically for each autoclave).
- Continue the autoclave cycle for the holding period.
- Close the steam valve and let the autoclave cool till a temperature of 80oC is reached.
- Gradually and gently open the autoclave enabling the steam to escape and allow the load to cool further.

• Hot air oven

A hot air oven is electrically operated and should be equipped with a fan to ensure:

• Uniform temperature inside. The required temperature for sterilization is generally 160°C (320°F) for one hour.

• Operating instructions

- Arrange the material to be sterilized loosely and evenly on the racks of the oven allowing free circulation of air and thereby even heating of the load.
- Do not pack the load tightly since air is a poor conductor of heat.
- Switch on the power supply and control the temperature of the oven by adjusting the thermostat.
- Note the time when the desired temperature is reached (heating-up time).
- Hold the load in the oven at this temperature for a defined period of time (holding period). This is usually 60 minutes at 160oC.

- Do not overheat since it will char the cotton plugs and paper wrappings.

Autoclaves and hot air ovens can be used for disinfection of infectious waste before it is discarded. In addition, waste can be disposed of by boiling in detergent or by burial.

• Boiling in detergent

In the absence of an autoclave, most specimen containers can be boiled in water having detergents to decontaminate. This process kills the vegetative bacteria but fails to destroy the spores and certain viruses. The easiest way to get best results is to add washing powder or sodium carbonate crystals, 60 grams to one litre of water in a big container and boil specimen containers in it for a minimum of 30 minutes.

Table 2: Preferred methods of sterilization for common-use articles

Autoclaving Animal cages Sugar tubes Lab. Coats Filters Instruments Culture media	**Hot air oven** Glass ware Beakers Flasks Petridish Pipette Slides Glass syringes Test tubes Powders
Rubber Gloves, stopper, tubing	**Wood** Tongue depressor, applicator
Glass Slides, syringes, test tubes Enamel metal trays Wire baskets	

Guidelines for correct procedures and practices in the microbiology laboratory

Disinfection

Disinfection can be undertaken either chemically or by boiling. Boiling is an effective method to disinfect equipment e.g. needles and syringes, if autoclaving facilities are not available. Equipment which has already been cleaned should be boiled for 20 minutes. Chemical disinfection is used for heat-sensitive equipment that is damaged at high temperatures. Commonly-used chemical disinfectants include chlorine releasing compounds; ethyl and isopropyl alcohol, quaternary ammonium compounds and glutaraldehyde.

Table 1: Disinfectants and their mode of application*

Target	Disinfectant	Strength to use (disinfectant/material V/V)	Application	Time of exposure
Skin	Ethanol	70%	Direct	2 minutes
	Iodine	1%	Direct	2 minutes
	Povidone iodine	1%	Direct	2 minutes
	Quaternary ammonium comp		Direct	2 minutes
Blood	Cresol (pH 9)	5%	2:1	6 hours
	Ca hypochlorite	1%	2:1	6 hours
Urine	Cresol (pH 9)	5%	1:1	4 hours
Sputum	Cresol (pH 9)	5%	1:1	4 hours
Faeces	Cresol (pH 9)	5%	2:1	6 hours
	Hypochlorite	1%	3:1	6 hours
	(Na/Ca)			
	Ca hydroxide	20%	2:1	6 hours
Work benches	Lysol	5%	Direct	4 hours
	Cresol	1%	Direct	4 hours
	Hypochlorite	5%	Direct	4 hours
	Chloramine-T		Direct	4 hours
Glassware	Hypochlorite	1%	Direct	4 hours
Lab instruments	Hypochlorite	0,10%	Direct	4 hours
	Isopropanol	70%	Direct	4 hours

* Based upon: Basics of quality assurance: WHO/EMRO, 1992, page 162

Table 3: Disinfection of specific equipment

Container/material	Method of choice for decontamination	Alternative method of decontamination
Reusable stool container	Autoclaving 121°C for 30 minutes	Fill the jar having stool with 5% solution of phenol and keep for 24 hours
	Empty into lavatory*	Empty into lavatory*
Reusable containers of CSF, pus, sputum	Autoclaving	Boiling in detergent
Urine bottles (afeter emptying in lavatory*)	Autoclaving	Fill with 2% phenol or 1% bleach, leave for 4 hours, clean with detergent
Blood containers	Autoclaving	Soak overnight in strong disinfectant (5% cresol; 1% CA hypochlorite, 1;2 V/V)
Glass microscope slides**	Autoclaving	Soak overnight in 5% phenol

* If the lavatory is connected to a septic tank, phenol or other antiseptics should not be put into the lavatory.
** Glass microscope slides which have been used for the diagnosis of tuberculosis should be discarded after keeping them soaked in detergent overnight.

Differentiation of culture media

Differentiation of culture media

SCHARLAU names for culture media are based on their final consistency or appearance. Thus, a medium is named as:
Agar: Solid media with an agar content of 1% or more.
Fluid Medium (FM): Semisolid media with an agar content of less than 1%.
Broth: Liquid culture media with undefined organic components (peptones, organ and tissue extracts, etc…)
Nutrient Solution: Culture media with a defined chemical composition.
Selective Medium: Medium designed to enhance the growth of one type of microorganism and also inhibit the growth of other types with antibiotics or other substances .
Differential Medium: Medium that allows distinction or differentiation between similar culture types.
Enrichment Medium: Medium that aids the growth of a defined microorganism over the rest in the inocule.
General Medium: Medium that supports the growth of a very wide range of microorganisms without special nutritional needs.

Membrane filtration media

Utilization of Membrane filter techniques has offered some undoubted advantages, in overcoming particular problems arising in Microbiology. Membrane filtration allows testing of large volumes of liquid samples with very low microbial counts. It enables separation of microorganisms from the culture medium and even the exchange of microorganisms between two media without affecting their growth.
Moreover, the MPN technique is more precise in these media and finally, the membrane filter with colony growth may be stored and filed for future reference.
The membrane filtration system is accepted by most pharmacopoeias as an alternative or the single method to examine antimicrobial or strong inhibitor activity in samples, since a proper wash of the filter removes all substances that interfere with microbial growth and a microbial count can be carried out accurately.
Also the wide range of filters and filter quality available allows the examination of any product, since membrane filters are available that are resistant to almost all solvents.

Technique

Essentially, the technique consists of filtering the sample through a filter with a suitable porosity (0,22 microns for bacteria and 0,45 microns for fungi) using pressure or suction, in such a way that the microorganisms are retained on the membrane.
If the fluid that has been filtered contains inhibitors, the membrane is washed several times with a rinsing liquid to remove them. The membrane is removed aseptically and placed on the culture medium.
For sterility controls, the membrane is incubated directly on the standard medium. For these tests refer to: Thioglycollate Broth (Ref. 2-186), Thioglycollate USP Fluid Medium (Ref. 3-187), Tryptone Soy Broth (Ref. 2-200), Sabouraud USP Broth (Ref. 2-165).

Should an enumeration of colonies be desired, incubate the filter on a solid medium or on a pad soaked in liquid medium, but ensure that the lower surface touches the culture medium and that there are no air bubbles or air gap in between.
Usually, general media may be used for these techniques. However, there are several culture media specifically developed for MF, specially in microbiological water testing.

Melting of agar media and preparation of agar plates

These media are most often available in glass containers (vials,tubes) with screw caps and in a variety of volumes to facilitate melting and immediate use.

The melting of the medium must be carried out according to the manufacturer's instructions, either in a water bath or microwave oven. Never apply direct heat to melt a culture medium. The melting temperatures and times depend on the shape of the container, the volume of medium and the heat source.

Before melting of the culture medium, the screw cap must be loosened to avoid breaking the container.

The medium should be melted and used only once. Agar media should not repeatedly melted as their characteristics change with each re-melting. Avoid overheating as well as heating for a prolonged period above all with alkaline or acidic media.

Once the medium is melted, aseptically pour it into the petri plates. Any remaining medium should be discarded and in no event should any medium be remelted if it has begun to solidify.

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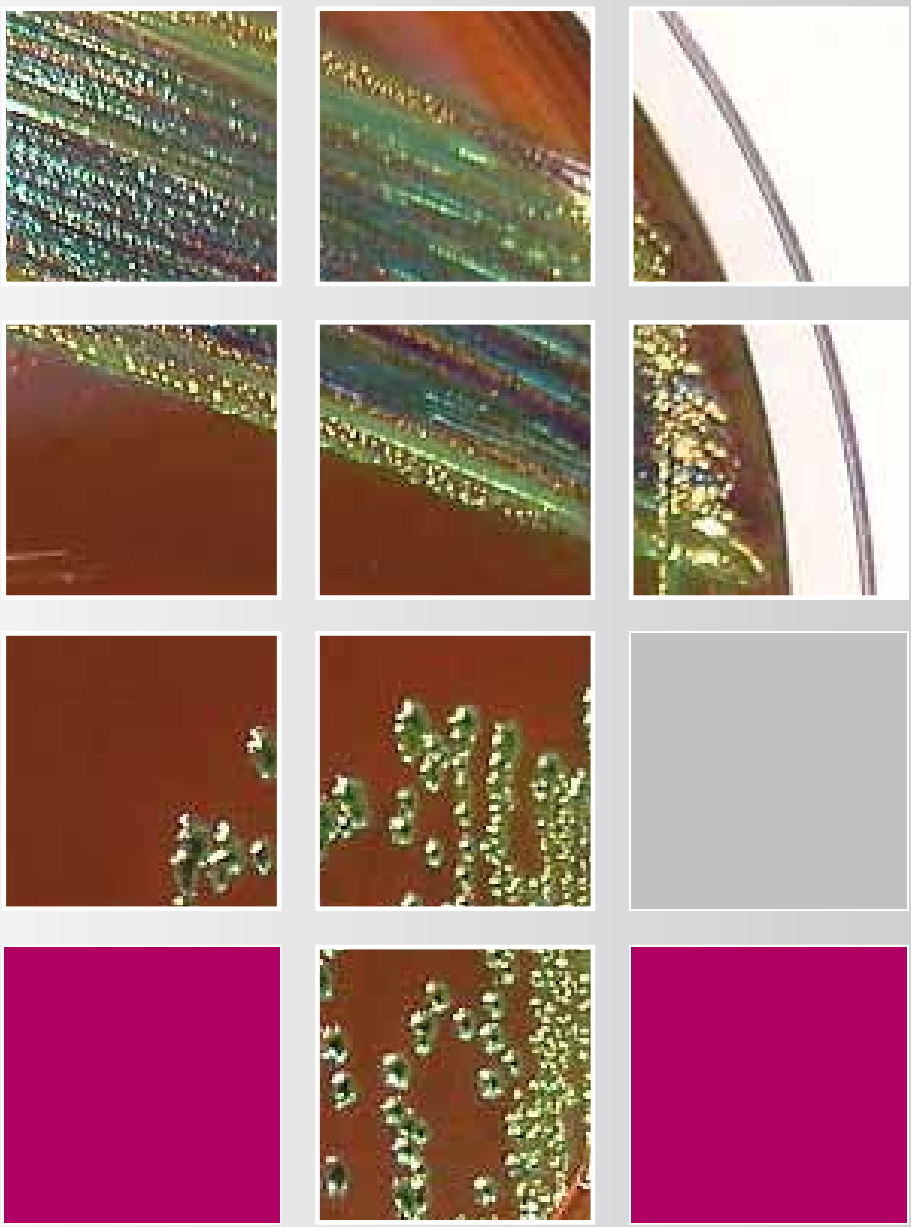
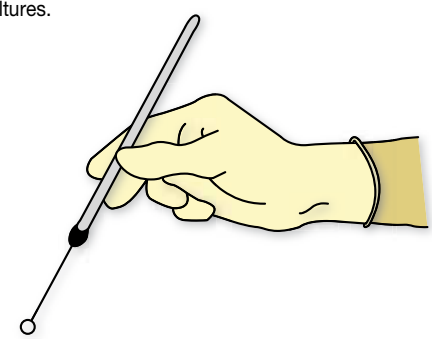


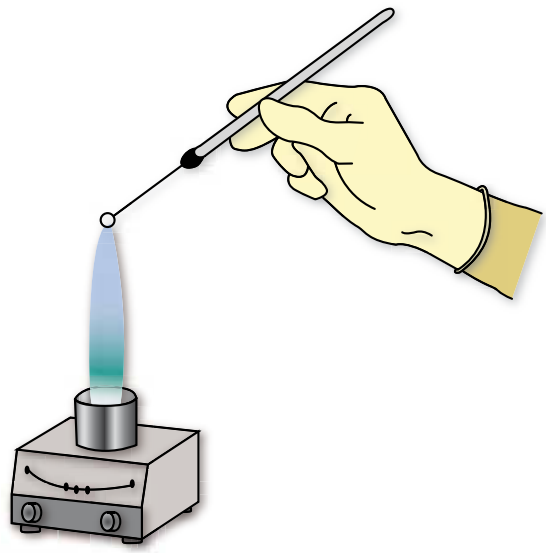
Basic Techniques

Staining Methods

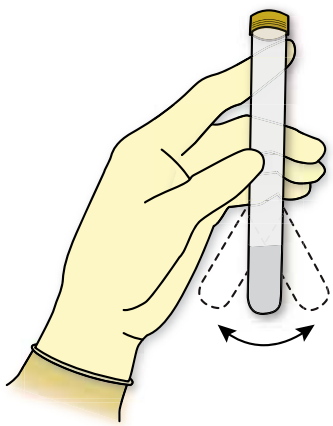
1. The handle is held and handled like a pencil.
   A loop is usually used for seeding liquid cultures and a straight wire for solid cultures.



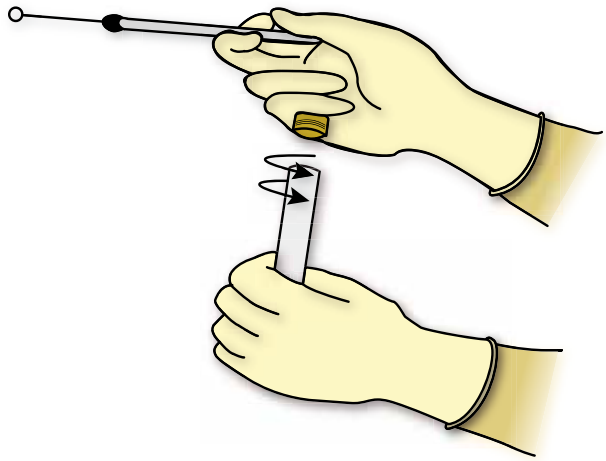
2. The wire is introduced in the upper portion (the hottest) of the Bunsen burner flame and maintained at an angle of about 60º. The wire is heated until red hot and then allowed to cool near the flame.



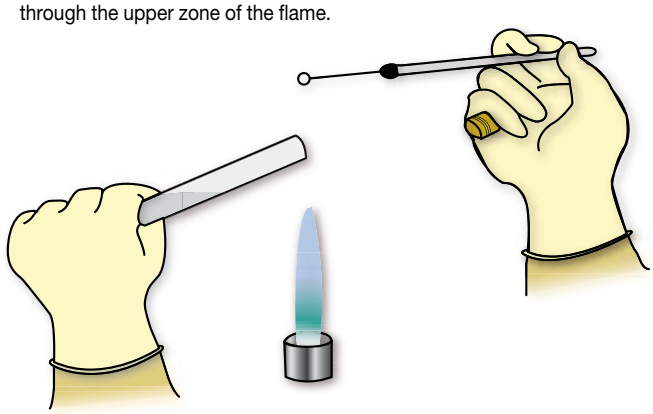
3. With your free hand take the tube with the liquid culture. Gently shake the broth from side to side ensuring that the microorganisms are in suspension.



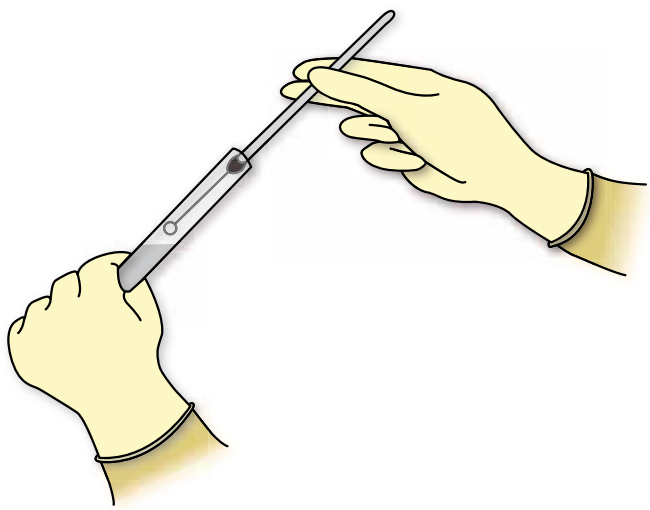
4. Remove the stopper of the tube with the free fingers on your hand holding the loop.



5. To sterilise the mouth of the tube, flame it passing it two or three times through the upper zone of the flame.

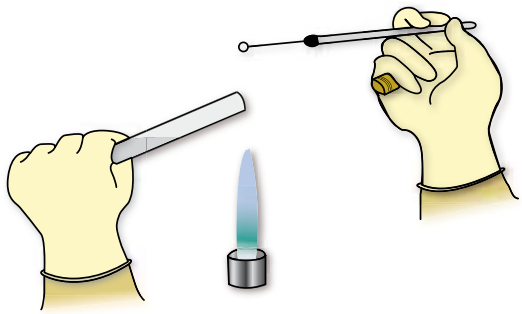


6. Insert the sterile inoculation loop inside the tube and remove a small amount of the culture. In liquid media it is possible that there is a hissing noise when the inserted wire loop is too hot.



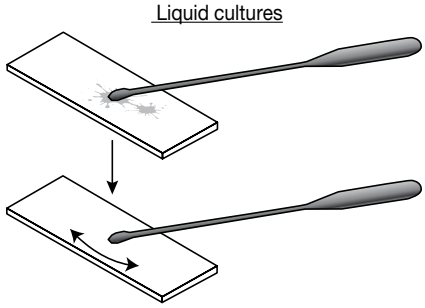
Staining Methods

7. Flame the spout again and cover with the cap still held in the same hand as the loop. The tube is returned to its rack.



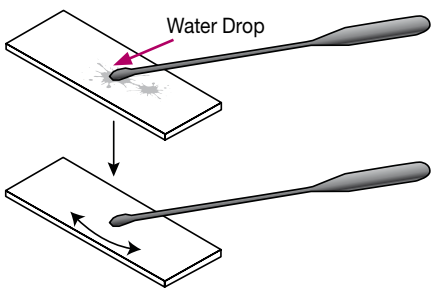
8a. In liquid cultures, place the volume contained on the loop on a slide. The drop is spread on a small area (smear) and dried.

Liquid cultures

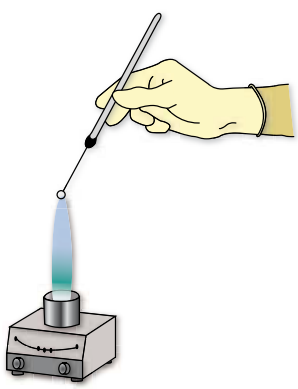


8b. With solid cultures, a small portion of the growth is mixed carefully with a water/saline droplet on the slide, previously prepared for that purpose. The mixture is spread on a small area and allowed to dry.

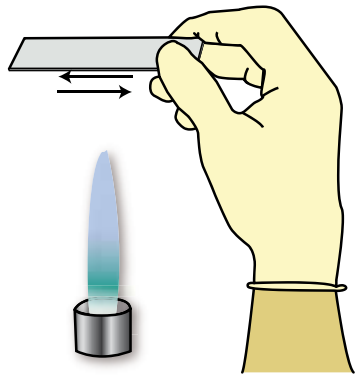
Agar cultures



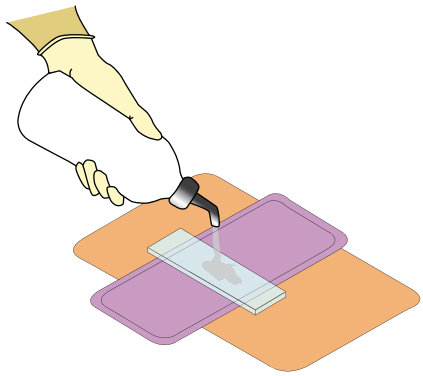
9. The inoculation loop is flamed again and returned to the stand or rack to cool.



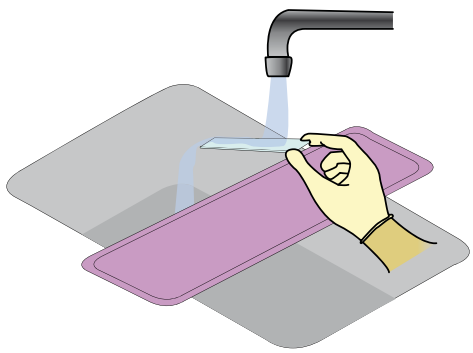
10. The dry smear is passed a few times through the hotter portion of the flame, being careful not to overheat.



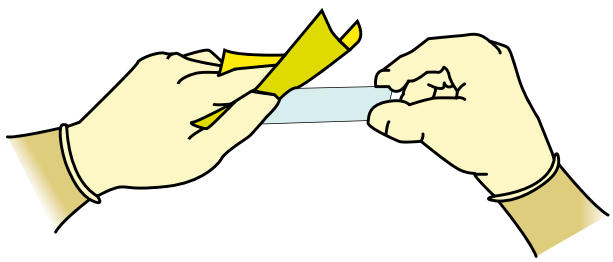
11. Apply only the amount of stain needed to cover the smear and leave it for 30 seconds.



12. Remove the stain and gently rinse off the excess, under running water.

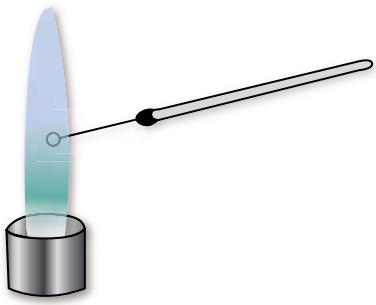


13. Dry the smear by placing the slide between filter paper or blotting paper, but do not rub.

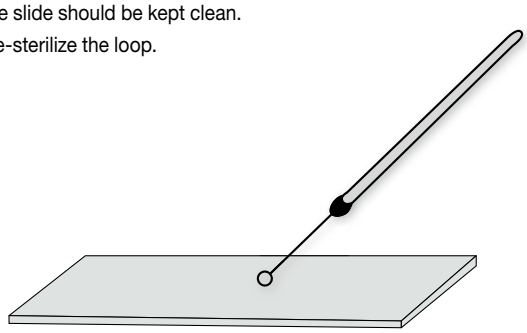


Preparation of a smear

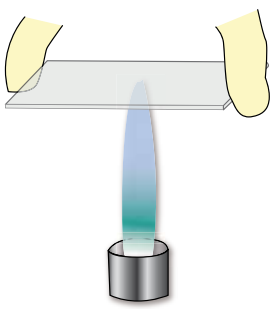
Sterilise the loop by flaming then allow it to cool.



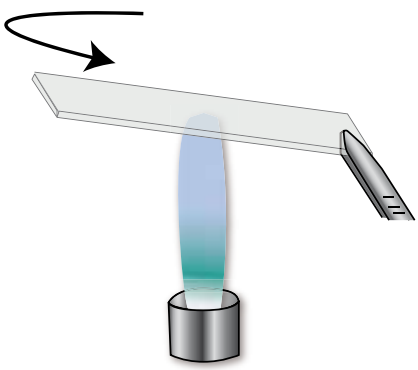
Spread a drop or content of the loop on the slide. The ends and edges of the slide should be kept clean.
Re-sterilize the loop.



The film is dried in air or, if necessary, keeping it above the Bunsen burner flame.

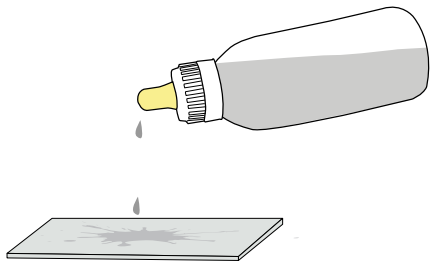


The dry film (smear) is fixed to the slide by passing it slowly three times through the Bunsen burner flame. The preparation should be cooled before proceeding to staining.

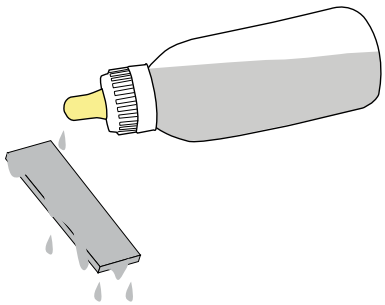


Gram staining

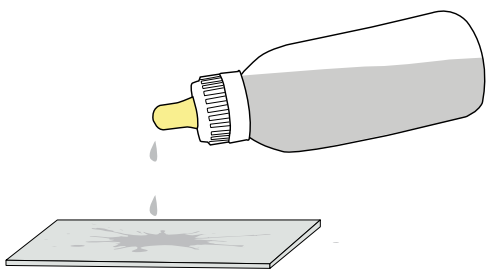
Cover the smear with Hucker's crystal violet solution and leave for 1 minute.



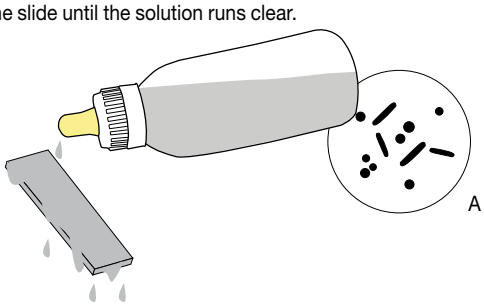
The dye is removed by washing with Burke's iodine solution eliminating any excess colour.



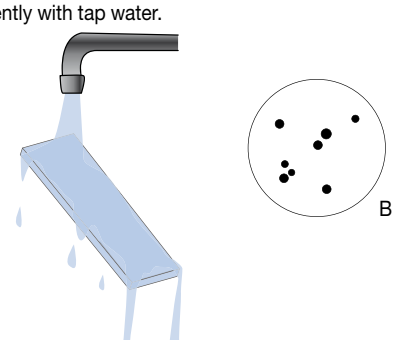
Cover the smear with Burke's iodine solution and leave for 1 minute.



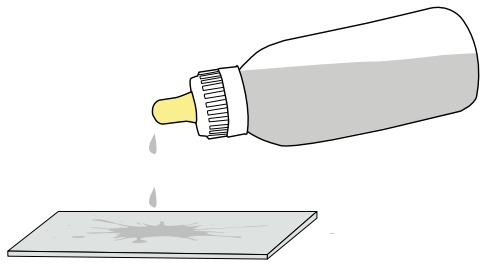
Wash gently with water and decolouriser poured drop by drop on one end of the slide until the solution runs clear.



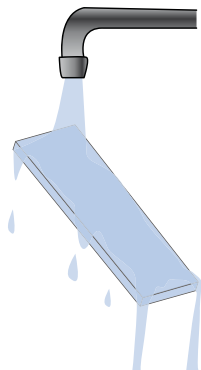
Wash gently with tap water.



Cover the preparation with safranin solution and leave for 1 minute.



Wash gently with tap water until the water runs clear.
Dry, without rubbing, using filter paper

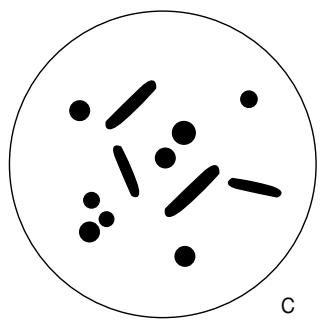


The circles illustrate the appearance of a smear of a mixed culture at different stages of staining:

A - Before applying the decolouriser all appear as gram-positive organisms.

B - After decolourising gram-negative organisms become invisible.

C - They appear again as gram-negative organisms after the counterstain is added.



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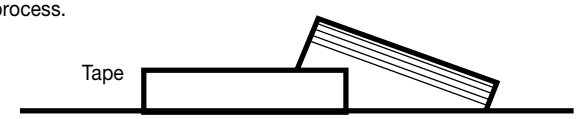
Inoculation of culture media

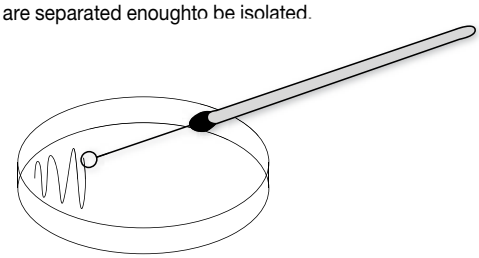
Screening with a loop

This is a qualitative isolation technique which, with practice, is faster than the serial dilution method and can give equivalent results for isolating strains. A key requirement in this technique is that the media surface is dry. Freshly prepared plates are inadequate and must undergo a drying process.

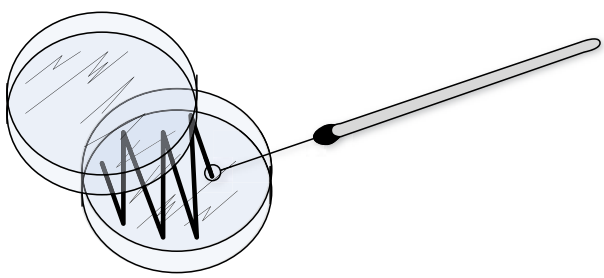


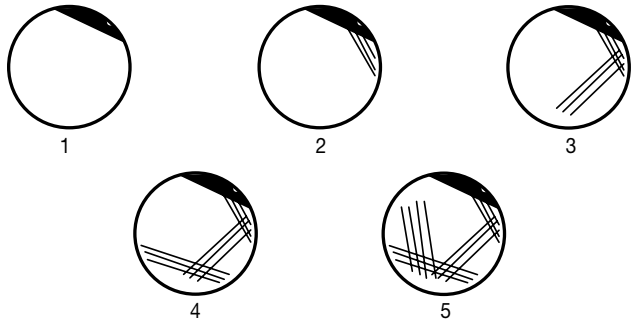
Drying of the surface of culture medium

1. With a loop take a drop of sample to be analyzed and place it at the edge of a regular agar plate. For a solid sample, first deposit on the plate a drop of ¼ Ringer's or sterile water and proceed in the same way as with the liquid sample.
2. Without lifting the loop draw a zig-zag line from end to end of the plate (see illustration) until the entire surface of the plate is used up and the sample on the loop is also used up. The strokes should be as close together as possible without touching, reaching (50 - 60 strokes / plate). You should not press the loop as the agar surface can be broken.
3. The number of colonies that appear will decrease along the line, until finally they are separated enoughto be isolated.

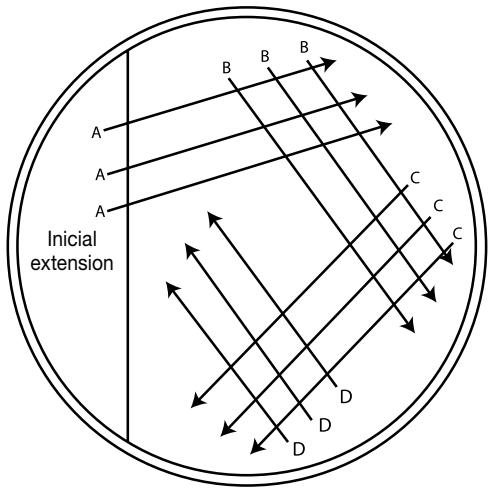


This method can be performed using different patterns at the time of streaking the solid medium. Below are some of the most common.



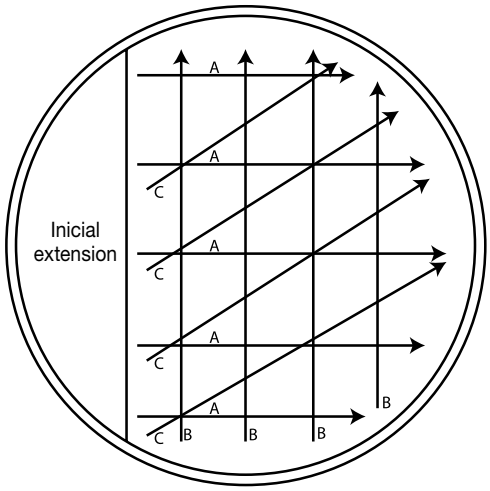


Method for four-phase streaking technique.



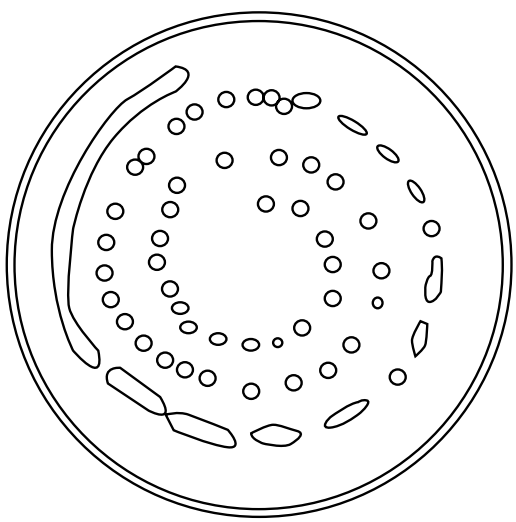
Normal procedure for the four-phase streak isolation technique. From the initial sample streak, the progressive reduction in cell numbers of the inoculum is ensured by the flaming of the loop every time the streak direction is changed.

Inoculation of culture media



Standard procedure for isolation using streak plating on selective media.
In this type of medium the inoculum must be large or more concentrated and there is no need to sterilize the loop between each set of streaks.

Quantification of the inoculum by streaking has been attempted using calibrated inoculating loops that collect a fixed volume of liquid. There have been many quantification methods published and some have been officially adopted by certain agencies or regulatory organisations, but the only one seems to have been widely accepted is called **"The Spiral Plate Count Method."**
In outline the method consists of a micropipette that releases a given volume (1-50 mL) along the radius of an agar plate rotating at a constant speed. The result is seeding in a constant line that follows the pattern of the Archimedes spiral. The automatic seeding is usually associated with an optical reader of colonies and a microcomputer containing data readings of the number of colonies after incubation along with those provided by the technician regarding the dilution of the sample. A statistical calculation is made of the number of CFU / mL of sample.

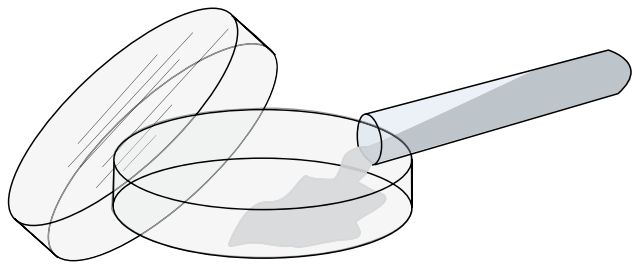


Spiral Plate Technique

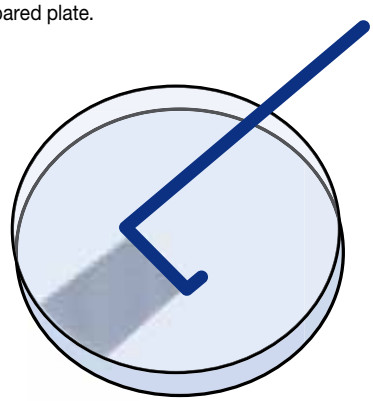
This system has the advantage of almost complete automation, which allows a significant reduction in labour costs, however it is limited by the optical reader which is very likely to introduce errors. In the market there are different models of these devices with different degrees of accuracy, mainly due to the statistical model employed.

Other types of inoculation

If the inoculum is a suspension of coarse particles difficult to pipette or simply an inoculum whose volume exceeds 0.1 mL, the **pour plate** technique is recommended.



In this technique, the inoculum is deposited at the bottom of an empty petri dish and melted agar cooled to 50-55°C is poured over it. To homogenize the mixture it is gently swirled, following a figure 8 on a flat surface immediately after the addition of the agar. Once solidified the plates are inverted and incubated as usual. The colonies appear within the agar adequate growth is obtained as the thickness of the layer does not prevent access to oxygen. As a method of counting, the system is fast and efficient provided that the plates have a colony density ranging between 3-300 colonies/plate. The main drawback is that all the colonies in these circumstances have a lenticular shape and are difficult to differentiate, but they are able to still be subcultured without toomuch trouble. Another drawback is that if growth is rapid the colony can break the agar and then radically change the conditions of cultivation. Another drawback is the possibility of no growth or much lower populations than expected due to exposure of the sample to excessive temperatures (<55°) from the melted agar.
The **"spread plate technique"** avoids this problem as an aliquot of the sample is spread at room temperature on the surface of the medium of a previously prepared plate.

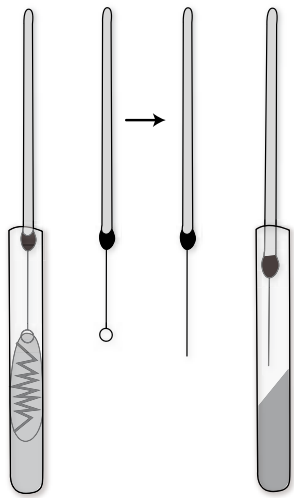


Seeding by surface spreading

Inoculation of culture media

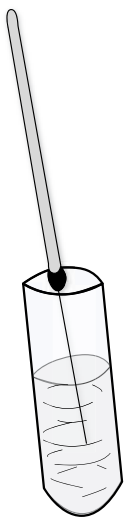
If the Drigalsky rod has not cooled sufficiently it can cause the same effects as the pour plate method, due to the volume for spreading being very small (~ 0.1 mL). There is also the risk, if the procedure is carried out too quickly or inexpertly, that part of the sample volume is withdrawn with the rod and therefore the entire sample is not inoculated.

In the laboratory, cultures are generally maintained in tubes, which provide the same features as the plate but occupy less space in refrigerators, laboratory benches or cabinets.
In tubes colonial morphology can not be easily distinguished, but they maintain moisture more efficintly. Culture medium may solidify as normal or at an **angle**, known as **"deeps"** and **"slants"** respectively and these two formats determine the type of inoculation.



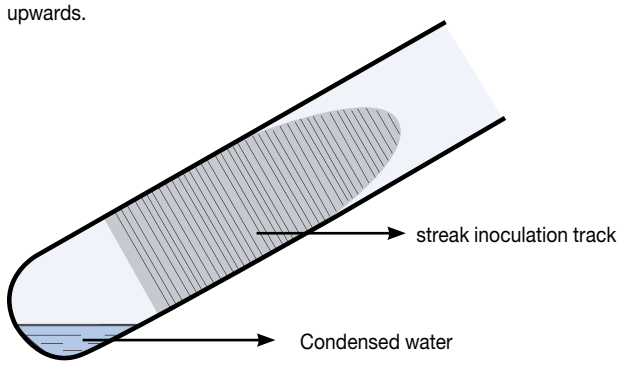
Streak inoculation Stab inoculation

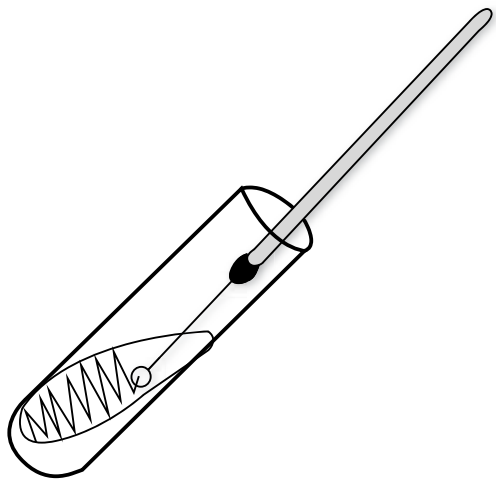
Types of tube inoculation



Stab inoculation

Slant tubes are inoculated by streaking without trying to use up the inoculum on the loop. It is important to start the streak in the inner zone of the inclined surface, avoiding contact with the condensate, and streak upwards.

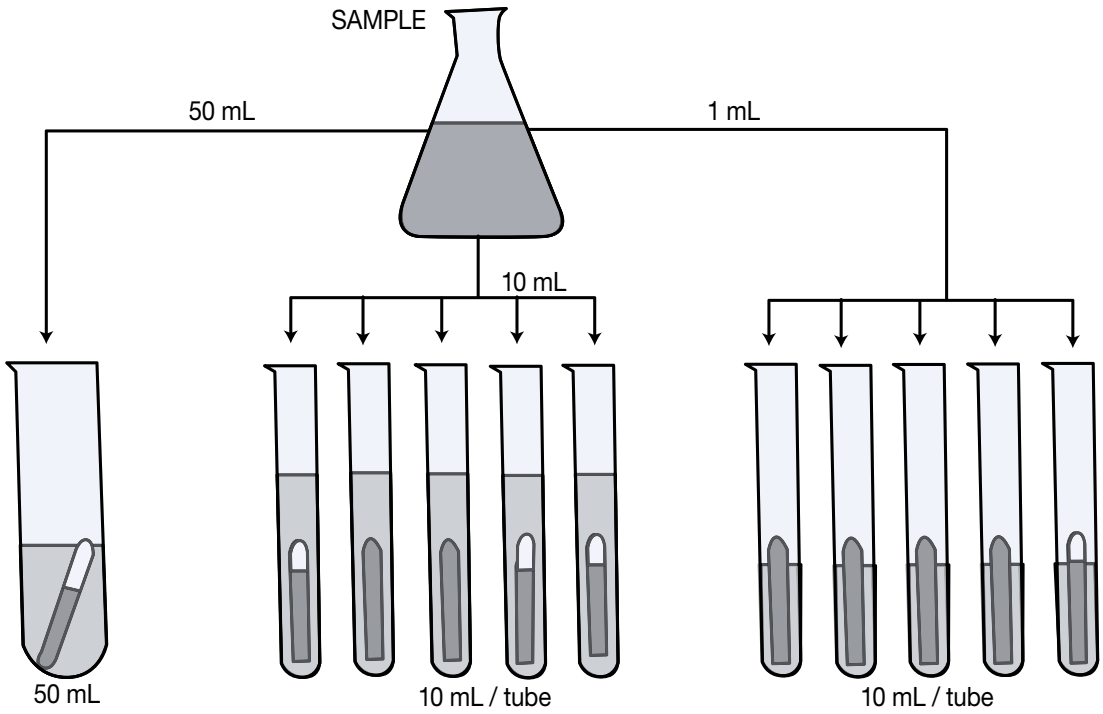




inoculating the slant tube.

If the tube agar has solidified in normal position, it should be **stab inoculated** deep into the butt using a loop or needle, the latter being made of the same material as the former but the wire is straight. With the tip of the loop, take the inoculum and stab deep and clean into the agar (~ ¾ of the depth of the medium), trying to enter and exit through the same point. This type of inoculation is used for certain physiological tests TSI, O / F, motility, etc.) Or to verify the type of growth in relation to atmospheric oxygen.
Sometimes the sample population is so low that it requires the testing of large volumes. In these cases **double-strength** liquid medium is used which is diluted with an equal volume of the sample. This will result in a normal concentration of medium inoculated with a large sample volume. If testing is for the enumeration of the original population, this technique is combined with the most probable number technique using selective media, if necessary.

Inoculation of culture media



Lactose broth + Bromocresol Purple

DOUBLE STRENGTH

Lactose Broth+ Bromocresol Purple

SINGLE STRENGTH

- Incubate for 48 h at 30 ° C

READINGS: POSITIVE = Acid and Gas (Yellow)

NEGATIVE = no gas and no change.

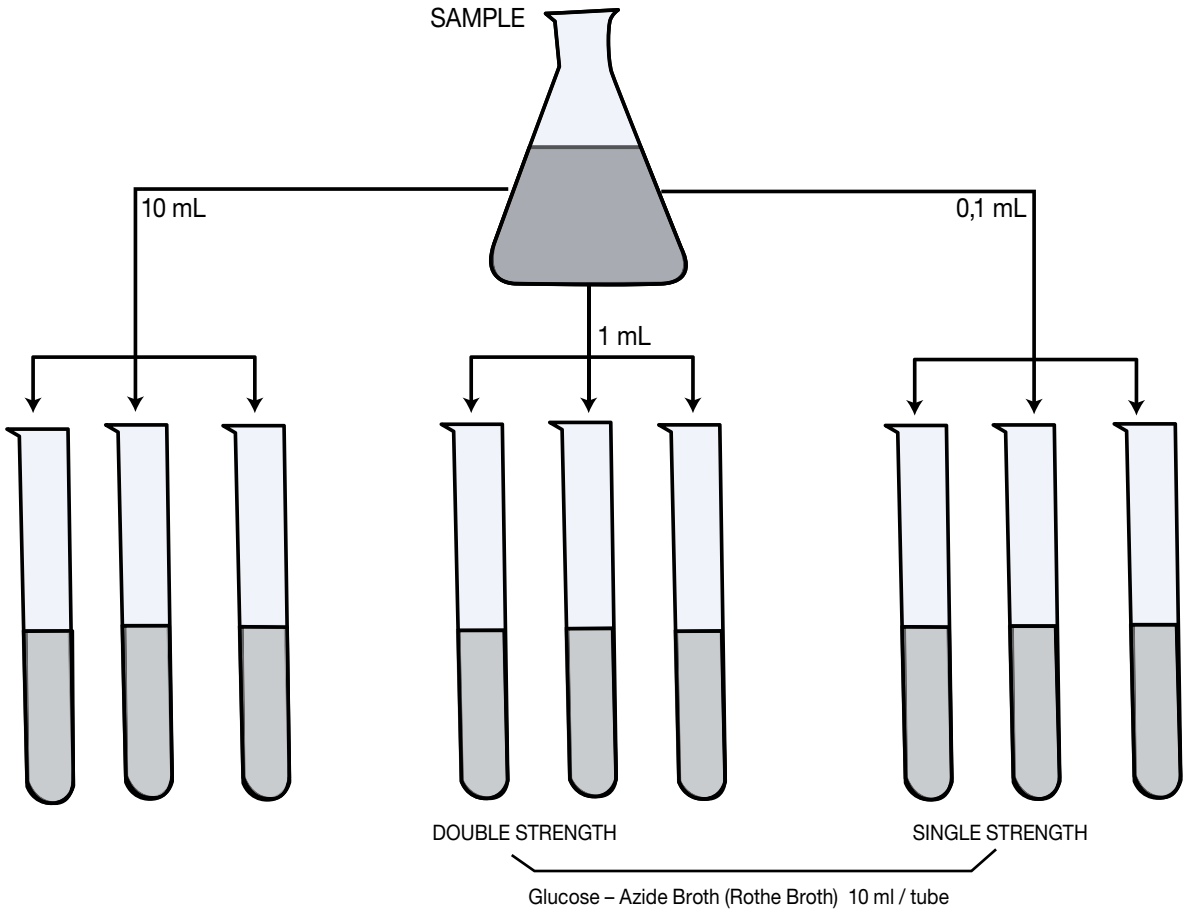
- To Estimate the MPN: See table 6.1.1

RESULTS: MPN of coliforms/100ml of sample

Using samples from the positive tubes above proceed to confirmation tests

Presumptive Colimetry

Inoculation of culture media



- Incubate for 48 h. at 37° Readings: Positive = Turbid
  Negative = No change
- Estimate the MPN using the corresponding tables

FROM THE POSITIVE TUBES CONFIRMATIVE TESTS ARE CARRIED OUT

Presumptive Streptometry

Large volumes can also be filtered through an appropriate pore **membrane filter** to retain microorganisms and then the filter may be used as the inoculum. To know if the filtered sample contains microorganisms, the membrane can be placed in a tube with a suitable liquid medium and incubated in appropriate conditions, with periodic observations. Any sign of growth is a presumptive test for the presence of viable microorganisms. If in addition quantification of this population is required, the membranes used to filter the different volumes are placed carefully, face up, on the surface of a suitable solid medium taking care not to trap air bubbles between the medium and membrane. After incubation, the microorganisms have formed visible colonies on the filter. Although double-strength medium is sometimes recommended for the incubation of membranes it is not necessary, since the permeability of the filter allows free access to nutrients. Incubation of the membranes can also be done on porous and inert pads impregnated with liquid medium.

When the concentration of microorganism to be isolated is much lower than the rest of the population before isolation on a particular medium, the sample is grown in an "**enrichment broth**". This liquid medium (broth) promotes the growth of some microorganisms over others, so that by the end of the incubation, the population of the microorganism to be isolated is much larger than that of accompanying flora. Isolation of the microorganism can now be carried out from this broth. Any medium that favours the growth of a type or group of microorganism by inhibiting the growth of the rest of the microbes in the inoculum is called a "**selective medium**". Selective media are very often also, **differential media**, i.e. containing indicator substances that easily distinguish the organism or group of organisms to be isolated.

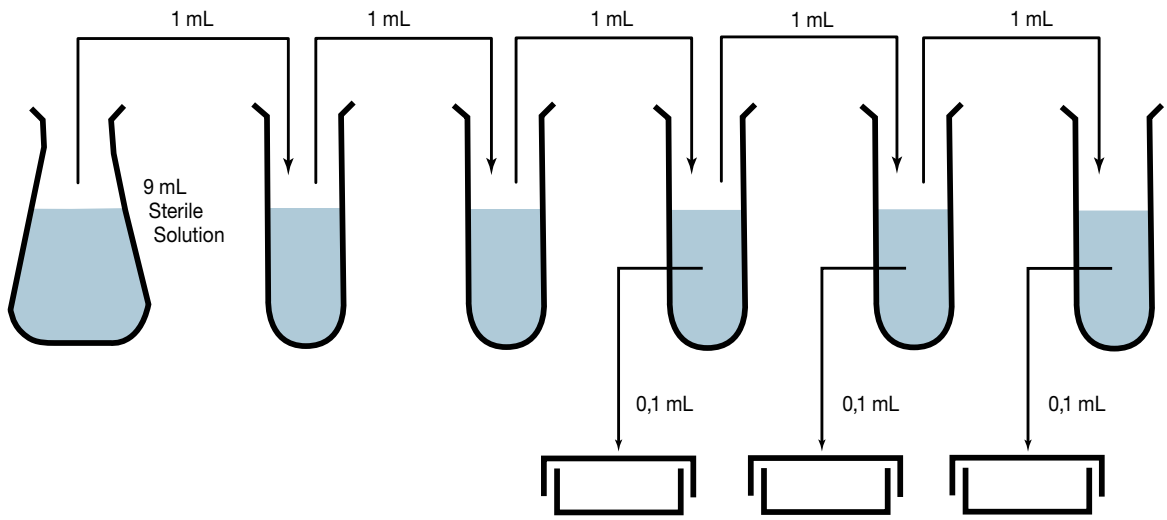
Dilution Techniques

Serial dilution method for counting

(Dilution Bank)

This is perhaps the most accurate method of isolating microorganisms as after plate inoculation and incubation we obtain,

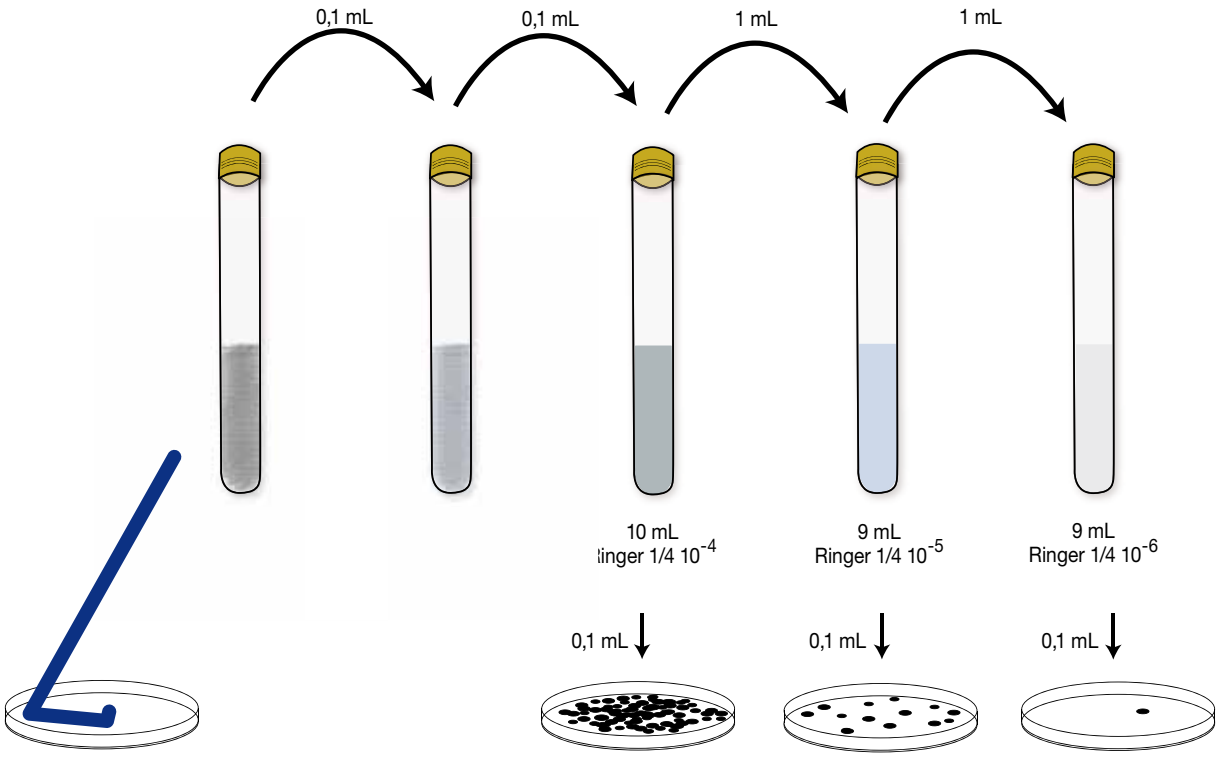
- Well separated colonies.
- Of the colonies that develop on the plate the different colonial morphology is easily distinguished.
- Being sufficiently separated colonies can be collected and seeded in separate tubes for further study (if the culture is pure).
- A colony plate count and therefore the cell count of the initial inoculum, at least of all the viable cells.

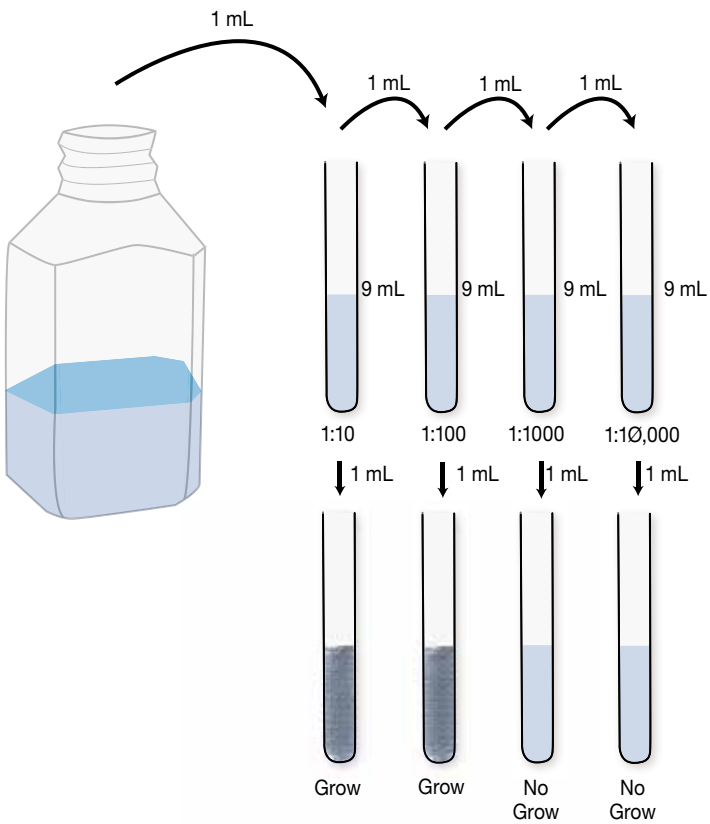


Method

1. All steps and procedures must be carried out aseptically.
2. Preparation of a sterile series of tubes with 9 mL of 1/4Ringer's in each. Add 1 mL of sample to the first tube, mixing thoroughly, until a truly homogeneous solution is obtained. Then take 1 mL from tube I and add to tube II. Homogenize and so on. A series of dilutions corresponding to 1:10, 1:100, 1:1000, etc. of the sample is obtained. However, the technician should prepare the serial dilutions as appropriate for the sample to be examined, as shown in the accompanying figures.
3. Take a 0.1 mL aliquot from the tube that seems most appropriate and spread it with a Drigalsky loop (L Rod) on a standard sterile tested agar plate. If the technician lacks experience a spread plate of each dilution is recommended. The spreading is carried out using a Drigalsky loop whilst continuously rotating the plate.
4. Incubate the plates at 30 ° C for 24 h. If a continuous film of growth has occurred within 24 h is likely that the surface of the medium was too wet when seeding. To avoid film growth the surface must be dry.

Dilution Techniques





Decimal Dilution Bank (Tenfold serial dilutions) and inoculation of liquid medium

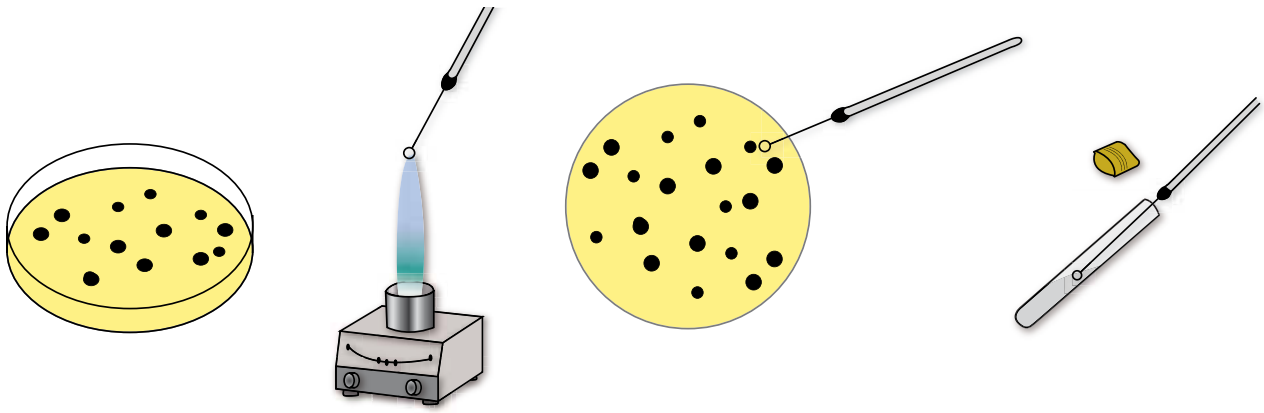
Pure Cultures

The term *culture* is used to describe microbial growth in general but when the term “pure culture” is used it refers to the progeny obtained from a single microbial cell and it would be more correct to call it, unicellular culture, axenic culture or clone.

From a practical standpoint, microorganisms in pure cultures can be maintained viable for longer, enabling their controlled exchange between laboratories and they can also be subcultured indefinitely for study.

Usually the pure culture is obtained by reseeding and isolation of a single discrete (isolated) colony on a solid medium, **assuming** that the discrete colony has developed from the multiplication of a single microorganism or (in the case where chains or clusters are formed) of a unit body. But it must be borne in mind that if the isolation is carried out on a selective medium or one containing a bacteriostatic agent that prevents growth but does not kill other microorganisms, the discrete colony may be contaminated with other microbes. In the above cases, repeated isolation and subculturing on media without inhibitors must be carried out.

Transferring a pure culture



1. Examine the plate and select the discrete single colony
2. Flame and then cool the needle
3. Touch the selected colony with the needle
4. Inoculate the slant drawing the needle on the surface. Flame the loop. Incubate the tube.

Obtaining a pure culture from an isolated colony

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Pure Cultures

Inocula

Inoculum is the name given to the microbial mass used to inoculate microbial culture media.

Normally the microbial mass comes from a pure culture, but also considered as inocula are portions/ aliquots of food stuffs, clinical specimens or other material allegedly contaminated and inoculated on a culture medium to verify the presence or absence of microorganisms.

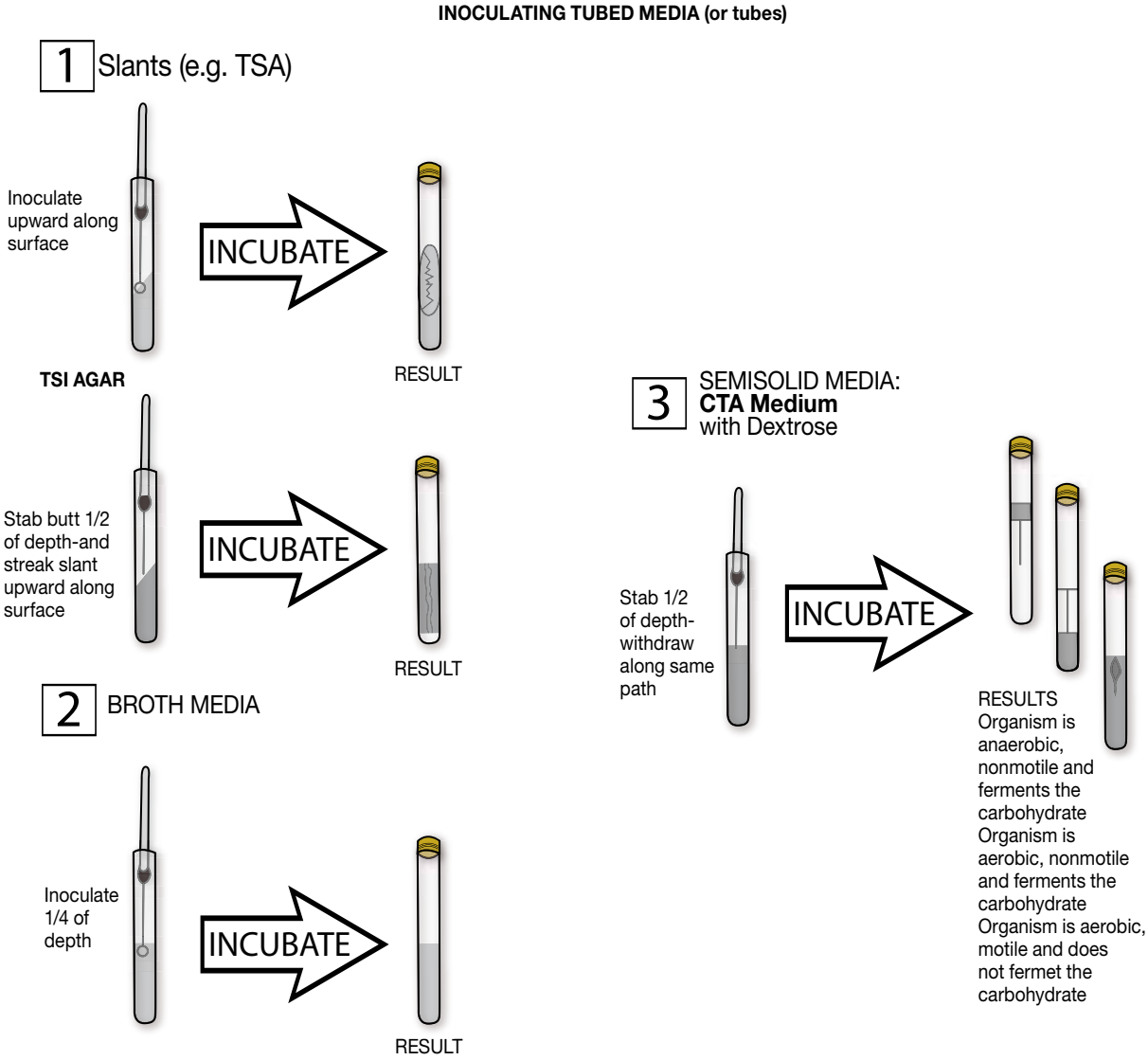


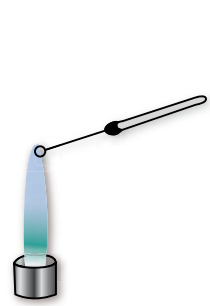
Figure text: 1. Slants (e.g. TSA)
Inoculate, upwards, the slant surface. Incubate. Result Slant (e.g. TSI) Stab the butt (at half of the depth) and streak the slant upward along the surface.

2. Broth (liquid media) (e.g. Thioglycollate broth without indicator)
Inoculate with the loop at ¼ depth. Incubate. Result.

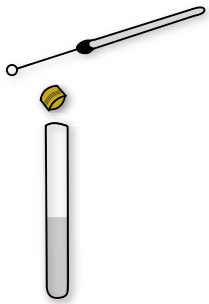
3. Semisolid media (e.g. CTA Medium with Dextrose)
Stab the butt at half of the depth, attempting to enter and exit from the same site. Incubate. Results: The organism is anaerobic, nonmotile and ferments the carbohydrate. The organism is aerobic, nonmotile and ferments the carbohydrate. The organism is aerobic motile and does not ferment the carbohydrate.

Pure Cultures

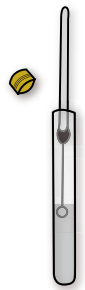
Inoculation methods



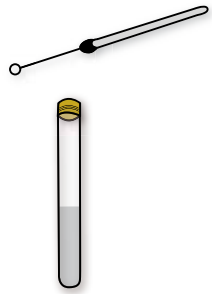
1. Flame loop-allow to cool



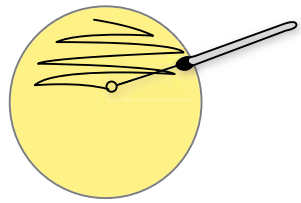
2. Remove cap from specimen tube



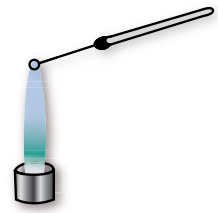
3. Take up a thin loopful of speciment



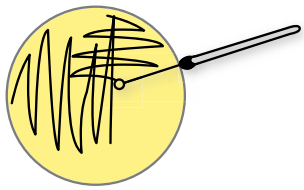
4. Replace cap on tube set aside



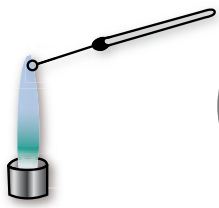
5. raise cover streak part of agar surface



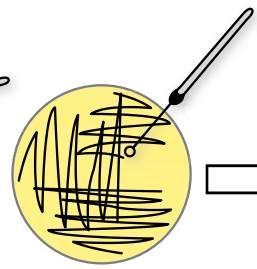
6. Flame loop-allow to cool



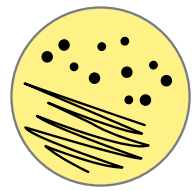
7. Streak a new section



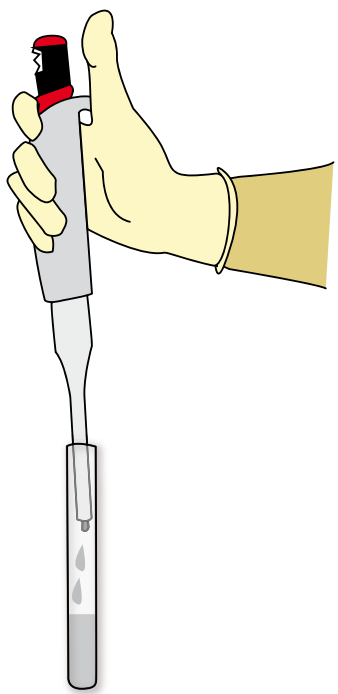
8. Flame loop allow to cool



9. Streak remaining area flame loop



10. Incubate



Inoculation of a liquid medium

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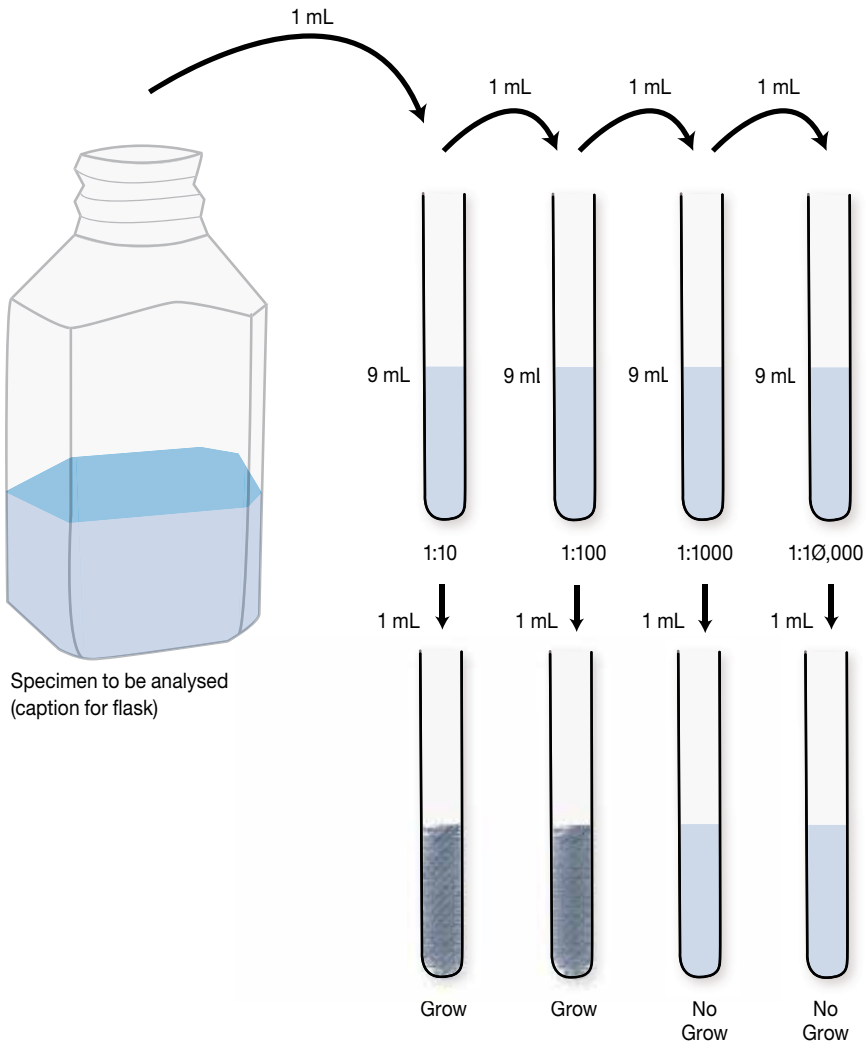
Pure Cultures



Inoculation of a bank of dilutions (10 fold serial dilution of a sample.)

Preparation of inocula

The preparation of inocula should be based on the origin of the microbial mass and the purpose of the sample.

Usually the media are inoculated from a pure culture obtained either by isolation from a sample or a standard culture from a collection or archive. In any case it should be a young culture or one with a guaranteed viability. The technician must decide in each case whether to use a subculture (less than 24hrs old) a refrigerated suspension or any other commercial preparations marketed for that purpose. In these latter cases the manufacturer's instructions should be followed.

Control of inocula

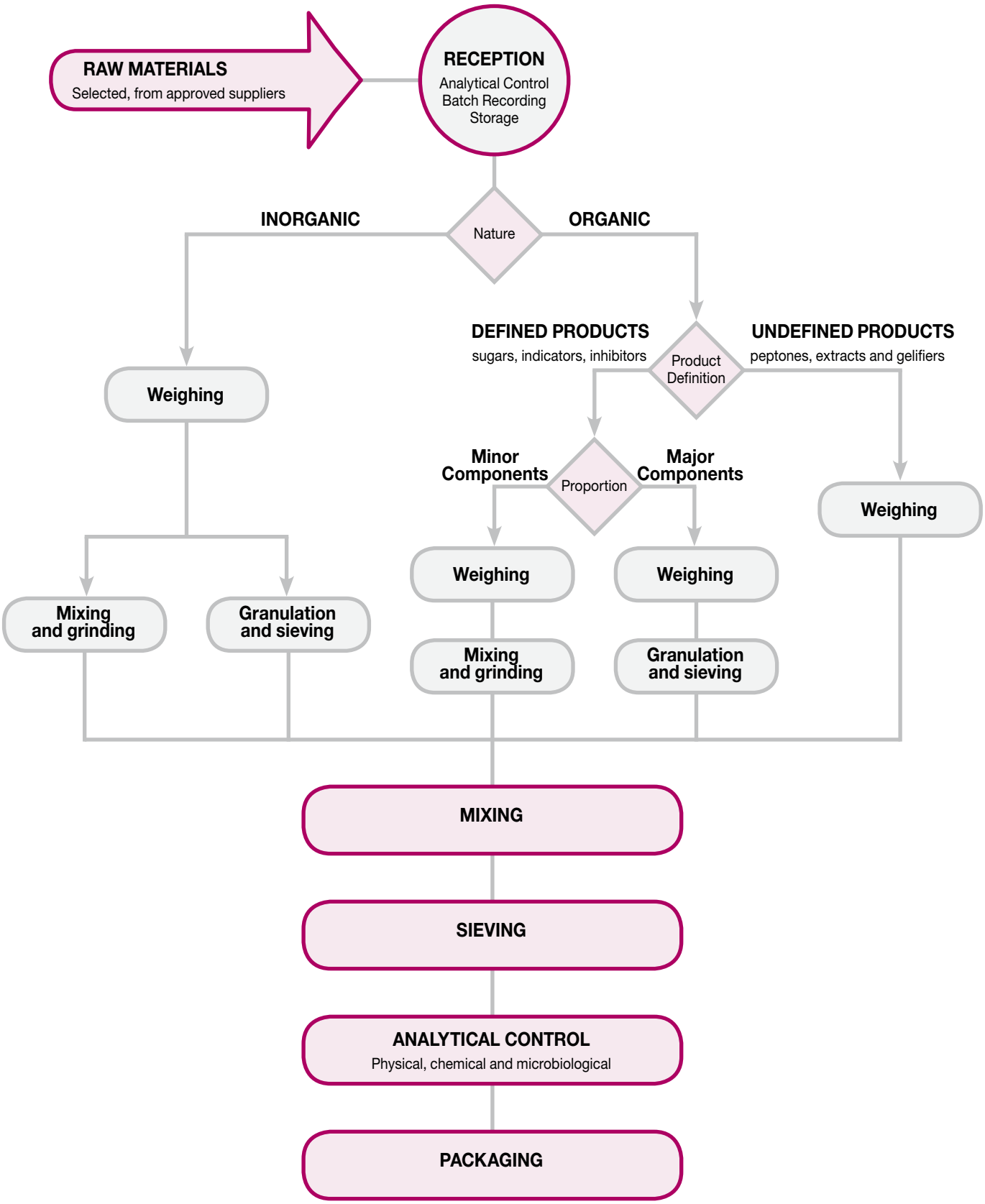
The quality control of seed stock should be carried out verifying the purity and identity of the pure culture in each case, verifying their macro and microscopic appearance and carrying out biochemical, genetic and immunological tests periodically.

Quantitatively the weights and sample volumes used in the dilution banks prior to inoculation should be verified as correct and as the norm.

Scharlau

Dehydrated Culture Media

Culture Media Manufacturing Process Flow Chart



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Acetamide Medium

Art. No. 03-428

WARNING

H: 3.6/2: H351

P: P281-P201-P202-P308+P313-P405-P501a

Specification

Liquid test medium use for the confirmation of *Pseudomonas aeruginosa* in water according to the ISO 16266:2006 standard.

Formula* in g/L

Acetamide	2,0000
Magnesium sulfate	0,2000
Sodium chloride	0,2000
Ferrous sulfate	0,0005
Mono-potassium phosphate	1,0000
Sodium molybdate	0,0050

Final pH 7,0 ± 0,5 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 3,4 g of powder in 1 L of distilled water. Heat only if necessary. Distribute in screw-capped tubes and sterilize in the autoclave at 121°C for 15 minutes. Prepared medium may be opalescent and with precipitate. To obtain a clear transparent medium without precipitation, avoid heating and sterilize the medium by filtration. The sterilized medium (with or without precipitates) remains active for 3 months if it is stored in the dark in a cool place.

Description

This nutrient solution uses acetamide as the sole carbon and nitrogen source, and therefore it only allows the growth of those microorganisms that are able to use acetamide. In water and in almost all food stuffs, these microorganisms are the non fermenting Gram negative bacillis, *Pseudomonas aeruginosa* is the only organism that can liberate ammonia by deamination of acetamide.

Some authors suggest the use of this nutrient solution as an enrichment medium prior to the use of isolation medium, will reduce false positives from heavily polluted samples. *Comamonas acidovorans, Achromobacter xylosoxidans and Alcaligenes faecalis (odorans)* can also deaminate acetamide, but can not growth on the plating medium that is selective for Pseudomonas.

Technique

To confirm potencial *Pseudomonas aeruginosa* colonies on the Cetrimide Agar (Art. No. 01-160 or 01-609) they must first be cultured on a non-selective medium to obtain pure cultures from which perform the confirmation tests.

Acetamide Medium is inoculated with a couple of colonies from the pure culture and is incubated at 36 ± 2°C for 22 ± 2 hours. Add 2 drops of Nessler's Reagent and examine the tubes for the production of ammonia, characterized by the production of a colour varying from yellow to brick red depending upon concentration of ammonia present.

References

- DIN Standard 3841. Deutsche Einheitsverfahren zür Wasser, Abwasser und Schlammuntersuchung Mikrobiologische Verfahren: Nachweiss von *Pseudomonas aeruginosa* (K8).
- EN 12780 Standard (2002) Water Quality - Detection and enumeration of *Pseudomonas aeruginosa* by membrane filtration. CEN. Brussels.
- ISO 16266 Standard (2006) Water Quality - Detection and enumeration of *Pseudomonas aeruginosa* - Method by membrane filtration.
- KELLY, N.M., C.T. KEANZ (1983) Acetamide Broth for Isolation of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J. Clin. Microbiol. 17.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Pure cultures using an inoculating needle

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Good	Green pigment. Acet (+)
Pseudomonas aeruginosa ATCC 15442	Good	Green pigment. Acet (+)
Pseudomonas aeruginosa ATCC 9027	Good	Green pigment (48 h). Acet (+)
Escherichia coli ATCC 25922	Inhibited	-

Left: *Pseudomonas aeruginosa* ATCC 27853
Centre: *Pseudomonas aeruginosa* ATCC 9027
Right: Uninoculated tube

Acetate Differential Agar

Art. No. 01-665

Specification

Solid medium used for the differentiation of *Shigella* species from *E. coli* according to ISO 21567:2004 standard.

Formula* in g/L

Sodium chloride	5,00
Sodium acetate	2,00
Mono-Ammonium phosphate	1,00
Bi-Potassium phosphate	1,00
Magnesium sulfate	0,20
Bromothymol blue	0,08
Agar	15,00

Final pH 6,7 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 24,3 g of powder in 1 L of distilled water and bring to the boil to dissolve. Dispense suitable volumes in tubes and sterilize in the autoclave at 121°C for 15 minutes. Solidify in slanted position to obtain a usable slope of 5 cm.

Description

Acetate Differential Agar is a chemically defined solid culture medium containing a mixture of mineral salts with an inorganic nitrogen source and sodium acetate a sole carbon source.

Organic acids as source of carbon without an organic nitrogen source in media is often used as a means of differentiating enterobacteria. The actual formulation of Acetate Differential Agar is a Simmons Citrate Agar (Art. No. 01-177) modification (Trabulsi & Ewing, 1962) in which the citrate is substituted by acetate. *Shigella* species are unable to use acetate and can not growth in this medium. Other bacteria, especially *E. coli* grow in 24-48 hours and the utilization of acetate produces alkaline metabolites that turn the pH indicator from green to blue.

Technique

The slope surface is inoculated with a straight streak of pure culture. Care must take to minimize the amount of culture medium transferred with the inoculum. Incubate for 48 hours at 37 ± 1ºC in aerobic conditions. Examine for growth: a positive result turns the green medium, blue. If no growth occurs incubate for and additional 48 hours and examine the growth again.

Shigella does not growth and no changes in the medium are found. *E. coli* produces blue colonies and turns the medium blue. Other species of bacteria can grow, but further testing would be required for full identification.

Limitations of the procedure

- Some biotypes of *Shigella flexneri* can use acetate as a sole carbon source.
- Some strains of *E. coli* use acetate slowly and can produce false negative results.
- Other bacteria of different genera to *Shigella* or *Escherichia* can use acetate as a sole carbon source.

References

- ATLAS, R.M. (1995) Handbook of Microbiological Media for the Examination of Food. CRC Press. Boca Raton Fla. USA.
- EDWARDS, P.R. y W.H. EWING (1955) Identification of Enterobacteriaceae. Burgess Pub. Co. Minneapolis.
- FDA (Food and Drug Adminstrations) Bacteriological Analitical Manual (1997) 8th ed. Revision A. AOAC Internacional. Gaithersburg. VA. USA.
- ISO 21567:2004 Standard. Microbiology of food and animal feeding stuffs - Horizontal Method for the detection of *Shigella spp*.
- MACFADDIN, J.F (1985) Media for the isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins. Baltimore. USA.
- TRABULSI, L.R. y W.H. EWING (1962) Sodium acetate medium for the differentiation of *Shigella* and *Escherichia* cultures. Public Health Lab. 20,137.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

(continues on the next page)

Acetate Differential Agar

Art. No. 01-665

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: Pure culture is inoculated by surface streaking

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good - very good	Blue medium
Escherichia coli ATCC 8739	Good - very good	Blue medium
Sjigella flexneri ATCC 12022	Inhibited to poor	Negative (Green medium)
Sjigella sonnei ATCC 25931	Inhibited to poor	Negative (Green medium)

Algae Agar

Art. No. 01-007

Specification

Solid culture medium for the isolation and cultivation of algae from soil, water and waste water.

Formula* in g/L

Sodium nitrate	1,000
Dipotassium phosphate	0,250
Magnesium sulfate	0,513
Ammonium chloride	0,050
Calcium chloride	0,058
Ferrous chloride	0,003
Agar	15,000

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 17 g of powder in 1 L of distilled water and bring to the boil stirring constantly. Distribute into appropriate containers and sterilize in the autoclave at 121°C for 15 min.

Description

The balanced nutrient composition of the medium provides all necessary nutrients for good growth of the algae, but it does not support the growth of fungi and bacteria. It is suitable medium for algaecide testing, however its main purpose is algae maintenance and cultivation or for the isolation of water contaminants.

Technique

For the maintenance of algal strains it is recommended incubating at room temperature, under a suitable light source (natural, fluorescent tube or incandescent lamp) until a good growth is obtained (within one to two weeks). In these conditions, and without gel dehydration, cultures can be maintained up to two months.

References

- ALLEN, (1952) Arch. Microbiol. 17:34.
- CLESCERI, L., A.E. GREENBERG, A.D. EATON (1998) Standard Methods for Examination of Water and Wastewater. APHA-AWWA-WEF. Washington, D.C.
- FITZGERALD (1962) Water and Sewage Works. 109:361.

Storage

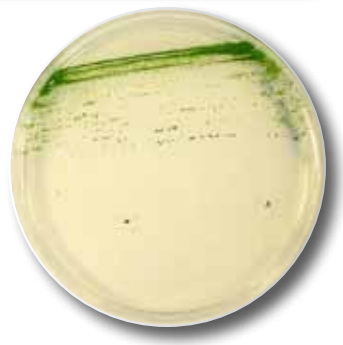
For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 20°C ± 2,0
Incubation time: 7 - 15 days
Inoculum: Streak isolation

Microorganism	Growth	Remarks
Chlorella ssp	Good - very good	Dark green at 10-15 days
Chlorella vulgaris	Good - very good	Dark green at 10-15 days

Chlorella ssp.



Chlorella vulgaris

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Algae Broth

Art. No. 02-007

DANGER

H: 2.14/2: H272-3.1.0/4: H302-3.3/2: H319

P: P221-P210-P220-P280-P305+P351+P338-P501a

Specification

Nutritive solution for algae and cyanobacteria, appropriate for water algicide biotesting.

Formula* in g/L

Sodium nitrate	1,000
Dipotassium phosphate	0,250
Magnesium sulfate	0,513
Ammonium chloride	0,050
Calcium chloride	0,058
Ferrous chloride	0,003

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 1,87 g of powder in 1 L of distilled water and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This liquid medium is suitable for algae and cyanobacteria cultivation, and is especially adapted for inoculum preparation and algicide biotesting, per Fitzgerald's technique. Due to the low energy source content, fungi and bacteria are inhibited.

Technique

Fitzgerald's procedure for the testing of algicide efficacy of chemical products:

a) Inoculum preparation

Prepare the Algae Broth and distribute 20 mL each into 50 mL capacity conical flasks. Sterilize and keep cool until usage.

Inoculate one of the conical flasks a couple of loops from *Chlorella emersonii* culture from slanted Algae agar (Art. No. 01-007) and incubate at room temperature until good growth is observed.

This culture can then be used as biotest inoculum, it can used for up to 30 days.

b) Biotest

1. Samples

Prepare 1 L of pure distilled water and 1 L of distilled water containing the inhibitor. Add 120 mg of sodium nitrate and 2,5 g of di-potassium phosphate to each sample.

2. Test technique

Prepare a double series of 50 mL capacity conical flasks and add 5, 12.5 and 25 mL respectively of water algicidal mixture, and then refill with pure water to get 25 mL in each conical flask.

Add only 25 mL of pure water in one or two conical flasks to use for control purposes. All the conical flasks are inoculated with the same volume of inoculum, the necessary amount to get an algae concentration about 300.000 cells/mL in each flask.

As a guide this concentration produces a slight greenish tinge. If necessary, adjust the inoculum concentration using counting or photocolorimetry methods as an aid.

Incubate the inoculated flasks at room temperature under a homogeneous and standardized light (i.e. 20 W fluorescent light).

Countings of all the flasks is carried out daily with a globule-cell count (Thoma, Neubauer type or similar).

The test is said to be over when the control conical flasks have an average concentration greater than $5x10^6$ cells/mL. The test flasks are then compared to them.

3. Interpretation

Inhibitor (algicide) concentration in the flasks with equal growth to the control is considered **non toxic** or **ineffective.** If algal concentration is maintained or remains the same as at the start of the experiment, the inhibitor is considered **algistatic**. Concentrations of inhibitor that have reduced the concentration of the starting population are considered as **algicidic**, with different effectivity ratios depending on the degree of reduction in this population.

References

- CLESCERI, L., A.E. GREENBERG, A.D. EATON (1998) Standard Methods for Examination of Water and Wastewater. APHA-AWWA-WEF. Washington. DC.
- D.C. ALLEN (1952) Arch. Microbiol. 17:34.
- FITZGERALD (1962) Water and Sewage Works. 109:361.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 20 ± 2,0°C
Incubation time: 7 - 15 days
Inoculum: 10 - 100 CFU

Microorganism	Growth	Remarks
Chlorella ssp	Good	Dark green at 10-15 days
Chlorella vulgaris	Good	Dark green at 10-15 days

A

Alkaline Saline Peptone Water

Art. No. 02-697

Also known as

ASPW

Specification

Liquid culture medium for the pre-enrichment and selective enrichment of potencially enteropathogenic *Vibrios*, in food samples, according to ISO/TS 21872-1 and 21872-2 : 2007 standards.

Formula* in g/L

Peptone	20,00
Sodium chloride	20,00

Final pH 8.5 at 25°C ± 0,2

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 40 g of the powder in a litre of distilled water, heating if required. Distribute into suitable containers and sterilize in an autoclave at 121°C for 15 minutes.

Technique

Technical specifications ISO 21872 in parts 1 and 2 describe this liquid culture medium for use in the pre-enrichment and selective enrichment of potentially enteropathogenic *Vibrios*, from food samples.

For pre-enrichment, the sample diluted in the medium is incubated at 37° for 6 ± 1 hours and from this culture the selective enrichment medium is inoculated and incubated at the same temperature for 18 ± 1 hours before proceeding to isolation on selective media.

If the presence of *V.parahaemolyticus* or *V.cholerae* is suspected then the pre-enrichment is carried out in the same manner only if the sample is a frozen food sample. With fresh foods the incubation must be carried out at 41,5°C, always for 6 ± 1 hours. Similarly the selective enrichment is carried out at 41,5°C for a period of 18 ± 1 h for these species of *Vibrio*, before proceeding to a selective medium for isolation.

References

- APHA AWWA WEF (1998) Standard Methods for the examination of water and wastewater. 20th ed. APHA. Washington, DC.
- ATLAS, R.M.& L.C. PARKS (1993) Handbook of Microbiological Media, CRC Press Inc., London.
- DOWNES, F.P. & K. ITO (2001). Compendium of Methods for the Microbiological Examination of Food. 4th Ed. APHA, Washington.
- ISO 21872-1 Technical Specification (2007) Microbiology of Food and animal feeding stuffs.- Horizontal method for the detection of potentially enteropathogenic *Vibrio spp*. - Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*.
- ISO 21872-2 Technical Specification (2007) Microbiology of Food and animal feeding stuffs.- Horizontal method for the detection of potentially enteropathogenic *Vibrio spp*. - Part 2: Detection of species other than *Vibrio parahaemolyticus*.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 3 - 6 h
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1/2) // Time: 0 and Time: 3 h to 6 h (35°C)

Microorganism	Growth	Remarks
Vibrio alginolyticus ATCC 17749	Good	Satisfactory recovery on TSA
Vibrio parahaemolyticus ATCC 17802	Good	Satisfactory recovery on TSA
Escherichia coli ATCC 25922	Poor	Satisfactory recovery on TSA

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Antibiotic Medium A pH 6.6 (Eur. Pharm.)

Art. No. 01-009

Also known as

Seed Agar; Medium A; Penassay Seed Agar; Penicillin Assay Seed Agar; Medium 1; AM1

Specification

Antibiotic Medium A at pH 6,6 is used in microbiological antibiotic assays using agar diffusion technique.

Formula* in g/L

Peptone	6,00
Casein peptone	4,00
Yeast extract	3,00
Meat extract	1,50
Dextrose	1,00
Agar	15,00

Final pH 6,6 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 30,5 g of powder in 1 litre of distilled water and bring to the boil stirring constantly. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Antibiotic Medium A at pH 6,6 is used as a maintenance culture media for the bacterial strains used in antibiotic assay. It is also used as a seed layer in the assay of bacitracin, cephalothin, cloxacillin, spiramycin, framycetin, josamycin, nafcillin, novobiocin, penicillin G and rifampicin.

Technique

The agar diffusion technique for antibiotic assays is performed according to the methodology recommended in the pharmacopoeia used in each country. Antibiotic Medium A at pH 6,6 by Scharlau Microbiology is suitable for use with paper discs, punched-holes or cylinder methodology because its gel strength is specially adjusted for all the techniques.

References

- ARRET, B.D., P.JOHNSON & A. KIRSCHBAUM (1971) Outline details for Microbiological Assays of Antibiotics: Second revision. J. Pharm. Sci. 60(11):1689-1694.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. §. 2.7.2 Microbiological Assay of Antibiotics. EDMH. Council of Europe. Strasbourg.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SANCHO, J:, J.GUINEA & R. PARÉS (1980) Microbiología Analítica Básica. Ed. JIMS. Barcelona.
- USP 33 - NF 28 (2011) <81> Antibiotics - Microbial Assays. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

(continues on the next page)

Antibiotic Medium A pH 6.6 (Eur. Pharm.)

Art. No. 01-009

Quality control

Incubation temperature: 30 - 35°C
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Micrococcus luteus ATCC 9341	Productivity > 0.70	-
Staphylococcus aureus ATCC 6538P	Productivity > 0.70	-
Staphylococcus epidermidis ATCC 12228	Productivity > 0.70	-

Staphylococcus aureus ATCC 6538P

Bacillus subtilis ATCC 6633

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Antibiotic Medium A pH 7.9 (Eur. Pharm.)

Art. No. 01-017

Also known as

Neomycin Assay Agar; Erythromycin Assay Agar; Medium C; Medium J

Specification

Antibiotic Medium A at pH 7,9 is used in microbiological antibiotic assays using agar diffusion technique.

Formula* in g/L

Peptone	6,00
Casein peptone	4,00
Yeast extract	3,00
Meat extract	1,50
Dextrose	1,00
Agar	15,00

Final pH 7,9 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 30,5 g of powder in 1 litre of distilled water and bring to the boil stirring constantly. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

The Antibiotic Medium A at pH 7,9 is used as seed layer or as the base layer in the assay of erythromycin, gentamicin, kanamycin, neomycin, netilmycin, paromomycin, sisomicin, streptomycin, tylosin and vancomycin.

Technique

The agar diffusion technique for antibiotic assays is performed according to the methodology recommended in the pharmacopoeia used in each country. Antibiotic Medium A at pH 7,9 by Scharlau Microbiology is suitable for use with paper discs, punched-holes or cylinder methodology as its gel strength is specially adjusted for all these techniques.

References

- ARRET, B.D., P.JOHNSON & A. KIRSCHBAUM (1971) Outline details for Microbiological Assays of Antibiotics: Second revision. J. Pharm. Sci. 60(11):1689-1694.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. §. 2.7.2 Microbiological Assay of Antibiotics. EDMH. Council of Europe. Strasbourg.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SANCHO, J:, J.GUINEA & R. PARÉS (1980) Microbiología Analítica Básica. Ed. JIMS. Barcelona.
- U.S. PHARMACOPOEIA 31 /NATIONAL FORMULARY 26 (2008) Biological Tests and Assays. {81} Antibiotic Microbial Assays. USP Convention Ltd. Rockville. MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 30 - 35°C
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Staphylococcus aureus ATCC 6538P	Productivity > 0.70	-
Staphylococcus epidermidis ATCC 12228	Productivity > 0.70	-
Micrococcus luteus ATCC 9341	Productivity > 0.70	-

Antibiotic Medium B (Eur. Pharm.)

Art. No. 01-016

Also known as

Antibiotic Medium 10, Medium 10

Specification

Antibiotic Medium B is used in the microbiological assays of Colistimethate and Polymyxin by the Agar Diffusion method.

Formula* in g/L

Casein Pancreatic Digest	17,00
Soybean meal Digest	3,00
Sodium chloride	5,00
Dextrose	2,50
Dipotassium phosphate	2,50
Agar	15,00

Final pH 7.3 ± 0.1 at 25°C

Reconstitution

Add 45 g of powder to 1 L of distilled water. Once dissolved, add 10 mL of Polysorbate 80 (Art. No. TW0080). Bring to the boil and dispense into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

Antibiotic Medium B is recommended by the European Pharmacopoeia and the USP for determining antibiotic potency by the microbiological assay techniques, specifically for colistimethate and polymyxin, in a single layer or double layer. For these assays seed cultures ATCC 4617 *Bordetella bronchiseptica* and ATCC 10536 *Escherichia coli* are recommended.

Technique

The diffusion method for the assay of antibiotics is carried out in accordance with the methodology in the current pharmacopoeias of each country. Scharlau Microbiology's Antibiotic Medium B can be used equally with impregnated paper discs, penicylinders and cut wells as the consistency of the gel is specifically adjusted to suit all of these methodologies.

References

- ARRET, B.D., P.JOHNSON & A. KIRSCHBAUM (1971) Outline details for Microbiological Assays of Antibiotics: Second revision. J. Pharm. Sci. 60(11):1689-1694
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. §. 2.7.2 Microbiological Assay of Antibiotics. EDMH. Council of Europe. Strasbourg.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SANCHO, J:, J.GUINEA & R. PARÉS. (1980) Microbiología Analítica Básica. Ed. JIMS. Barcelona.
- U.S. PHARMACOPOEIA 31 /NATIONAL FORMULARY 26 (2008) Biological Tests and Assays. {81} Antibiotic Microbial Assays. USP Convention Ltd. Rockville, MD.
- USP 33 - NF 28 (2011) <81> Antibiotics - Microbial Assays. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Escherichia coli ATCC 10536	Productivity > 0.70	-
Bordetella bronchiseptica ATCC 4617	Productivity > 0.70	-

Antibiotic Medium C (Eur. Pharm.)

Art. No. 02-011

Also known as

Antibiotic Assay Broth

Specification

Antibiotic Medium C may be used in the preparation of inoculum, serial dilutions or turbidimetric antibiotic assays.

Formula* in g/L

Peptone	6,00
Yeast extract	3,00
Meat extract	1,50
Sodium chloride	3,50
Dextrose	1,00
Monopotassium phosphate	1,32
Dipotassium phosphate	3,68

Final pH 7 ± 0,05 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 20 g of medium to 1 L of distilled water. Heat to boiling and dispense into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

Antibiotic Medium C is used in inoculum preparation of bacterial strains used in antibiotic assays. It is also used to perform assays of sodium colistimethate, framycetin sulfate, gentamicin sulfate, gramicidin, josamycin, josamycin propionate, kanamycin monosulphate, neomycin sulfate, sodium rifamycin, spiramycin, streptomycin sulfate, tylosin, tylosin tatrate, tyrothricin and vancomycin HCl.

Technique

The turbidimetric technique for antibiotic assays must be performed according to the methodology recommended in the appropriate pharmacopoeia.

References

- ARRET, B.D., P. JOHNSON & A. KIRSCHBAUM (1971) Outline Details for Microbiological Assays of Antibiotics: Second revision. J. Pharm. Sci. 60(11):1689-1694.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.7.2. Microbiological Assay of Antibiotics. EDQM. Council of Europe. Strasbourg.
- SANCHO, J., J. GUINEA & R. PARÉS (1980) Microbiología Analítica Básica. Ed. JIMS. Barcelona.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Klebsiella pneumoniae ATCC 10031	Good	-
Staphylococcus aureus ATCC 6538P	Good	-
Enterococcus hirae ATCC 10541	Good	-
E. coli ATCC 10536	Good	-

Antibiotic Medium E (Eur. Pharm.)

Art. No. 01-430

Specification

Antibiotic Medium E is used in the microbiological assays of Framycetin and Neomycin using the Agar Diffusion method.

Formula* in g/L

Peptone	5,00
Meat Extract	3,00
Disodium phosphate dodecahydrate	26,90
Agar	10,00

Final pH 7,9 ± 0,1 at 25°C

Reconstitution

Add 44,9g of powder to 1L of water. Boil and distribute in suitable containers. Sterilise in an autoclave at 121°C for 15 minutes.

Description

Antibiotic Medium E is recommended by the European Pharmacopoeia and the USP for determining antibiotic potency by microbiological assay technique, specifically for framycetin and neomycin, in a single layer or double layer. For these assays seed cultures ATCC 6633 *Bacillus subtilis* as well as NCTC 8241 *Bacillus pumilus* are recommended.

Technique

The diffusion method for the assay of antibiotics is carried out in accordance with the methodology in the cuurrent pharmacopoeias of each country. *Scharlau Microbiology's* Antibiotic Medium E can be used equally with impregnated paper discs, penicylinders and cut wells as the consistency of the gel is specifically adjusted to suit all of these methodologies.

References

- EUROPEAN PHARMACOPOEIA (2009) Supplement 6.3 §. 2.7.2 Microbiological Assay of Antibiotics. European Directorate for the Quality of Medicines & Health Care (EDQM). Council of Europe. Strasbourg.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.7.2. Microbiological Assay of Antibiotics. EDQM. Council of Europe. Strasbourg.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SANCHO, J:, J.GUINEA & R. PARÉS. (1980) Microbiología Analítica Básica. Ed. JIMS. Barcelona.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Quality control

Incubation temperature: 30 - 37°C
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Productivity > 0.70	-

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Antibiotic Medium F (Eur. Pharm.)

Art. No. 01-434

Also known as

Antibiotic Medium 19

Specification

Antibiotic Medium F is used in the titration of antifungal antibiotics by the agar diffusion method.

Formula* in g/L

Peptone	9,40
Yeast extract	4,70
Meat extract	2,40
Sodium chloride	10,00
Dextrose	10,00
Agar	23,50

Final pH 6.1 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Reconstitution

Add 60 g of powder to 1 L of distilled water. Bring to the boil and distribute into suitable containers. Autoclave at 121°C for 15 minutes.

Description

Antibiotic Medium F is recommended both for the maintenance of yeast strains used in titration assays of antifungal activity, (particularly amphotericin B and nystatin), as well as for the test itself, whether single-layer or double layer.

Technique

The antibiotic sensitivity assay agar diffusion method is performed according to the methodology described in the appropriate pharmacopoeia in each country. Scharlau Microbiology's Antibiotic Medium F can be used whether using impregnated paper discs, penicylinders or cut wells as the hardness of the gel is especially adjusted for any of these methodologies.

References

- ARRET, B.D., P.JOHNSON & A. KIRSHBAUM (1971) Outline details for Microbiological Assays of Antibiotics: Second revision. J. Pharm. Sci. 60(11):1689-1694.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.7.2. Microbiological Assay of Antibiotics. EDQM. Council of Europe. Strasbourg.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SANCHO, J:, J.GUINEA & R. PARÉS. (1980) Microbiología Analítica Básica. Ed. JIMS. Barcelona.
- USP 33 - NF 28 (2011) <81> Antibiotics - Microbial Assays. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Quality control

Incubation temperature: 30 - 37 °C
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Candida tropicalis ATCC 20956	Productivity > 0.70	-

APT Agar

Art. No. 01-026

Also known as

All Purpose Polysorbate Agar

Specification

Solid medium for general purpose use, especially designed for the cultivation of the heterofermentative lactic acid bacteria that cause greening of meat.

Formula* in g/L

Casein peptone	12,500
Yeast extract	7,500
Sodium chloride	5,000
Potassium phosphate	5,000
Sodium citrate	5,000
Dextrose	10,000
Magnesium sulfate	0,800
Manganous chloride	0,140
Iron sulfate	0,040
Thiamine HCL	0,001
Polysorbate 80	0,200
Agar	15,000

Final pH 6,7 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 61,2 g of powder in 1 L of distilled water and allow it to soak. Bring to the boil stirring constantly. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This general purpose medium (APT= All Purpose with Polysorbate 80), originally formulated by Evans and Niven, have been used successfully for the isolation and cultivation of lactic acid bacteria that, alter the quality and composition of food (especially meat), and require high levels of thiamine for growth. For this reason, the medium has been complemented with an increased amount of thiamine.

Both versions, solid and liquid, have demonstrated their efficacy in detecting lactobacilli that produce meat greening. Moreover, if the medium is supplemented with 5% fruit juice, as APHA states, it is converted into a growth media for many food bio modifiers.

Without the inclusion of an inhibitory agent in the formulation, the medium has no selective ability, and can support the growth of almost all types of microbes.

Technique

Detection Technique for bacteria causing greening in meat:

Products samples to be examined are crushed carefully in Tryptone Water (Art. No. 03-156). Using the same diluent, a dilution bank (dilution series) is prepared. From each dilution, APT Agar plates are inoculated using the pour plate technique (in triplicate), and, once set, they are incubated at 32°C for 48 hours. After incubation, colonies are counted using standard techniques, and different types are selected. Each type is inoculated in APT Broth, and is incubated at 32°C for 24 hours or longer if necessary. From these pure cultures, streak onto Frankfurt sausage slices, and incubate to verify greening capacity. Also include one without inoculation as a Control. Final identification is done by morphological and biochemical characteristics.

References

- EVANS, J.B. & C.F. NIVEN (1951) Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products J.Bact. 62:599.
- DEIBEL, R.H, J.B. EVANS & C.F. NIVEN (1957) Microbiological assay for the thiamine using *Lactobacillus viridescens*. J. Bact. 74:818-821.
- DOWNES, F.P., K. ITO (2002) Compendium of methods for the microbiological examination of food. 4th ed. APHA. Washington.

Storage

Keep tightly closed, away from bright light, in a cool dry place between 4°C and 30°C. The dehydrated medium is very hygroscopi.

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APT Agar
Art. No. 01-026

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 - 72 h
Inoculum: 10 - 100 CFU. Spiral Plate Method

Microorganism	Growth	Remarks
Lactobacillus lactis ATCC 19435	Good to very good	-
Lactobacillus fermentum ATCC 9338	Good to very good	-
Lactobacillus sakei ATCC 15521	Good to very good	-
Lactobacillus acidophilus ATCC 4356	Good to very good	-

Lactobacillus lactis ATCC 19435

Lactobacillus fermentum ATCC 9338

Asparagine Broth

Art. No. 02-271

Specification

Liquid medium for assay and enumeration of presumptive *Pseudomonas aeruginosa* in bottled water by MPN method.

Formula* in g/L

Asparagine	2,00
Dipotassium phosphate	1,00
Monopotassium phosphate	10,00
Magnesium sulfate	0,50

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 13,5 g of powder in 1 L of distilled water containing 8 mL of glycerol. Sterilize by filtration and distribute in tubes (10 mL/tube). To obtain broth of double strength, dissolve 27 g of powder in 1 L of distilled water containing 16 mL of glycerol.

Description

Asparagine medium is recommended for the microbiological analysis of bottled water. This is an excellent enrichment medium for *Pseudomonas aeruginosa*, since it is composed of a mineral base and the only carbon source is asparagine. It may also be used in the multiple tube technique in microbiological analysis of recreational waters and as a presumptive test medium for the differentiation of non fermentative Gram negative bacteria.

Technique

Some standards suggests viable enumeration by the MPN method with 5 tubes per series, inoculating 10 mL, 1 mL and 0,1 mL. All the tubes are incubated at 37°C for 48 hours. Growth, with or without pigmentation, is estimated as presumptive evidence of the presence of *Pseudomonas aeruginosa*. Enumeration is carried out with MPN tables for 5 tubes. Confirmation is performed sub-culturing a loop of each tube in Acetamide Medium (Art. No. 03-428).

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- PASCUAL ANDERSON, M.R. (1992) Microbiología Alimentaria. Díaz de Santos. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 1.000 - 10.000 CFU

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Inhibited	-
Escherichia coli ATCC 8739	Inhibited	-
Pseudomonas aeruginosa ATCC 15442	Good	Green
Pseudomonas aeruginosa ATCC 27853	Good	Green
Pseudomonas aeruginosa ATCC 9027	Good	Green

Azide Dextrose Broth (Rothe)

Art. No. 02-027

WARNING

H: 3.1.O/4: H302-4.1.C/3: H412

P: P273-P264-P270-P301+P312-P330-P501a

Specification

Medium for the detection and enumeration of enterococci in water.

Formula* in g/L

Meat peptone	10,00
Casein peptone	10,00
Dextrose	5,00
Sodium chloride	5,00
Dipotassium hydrogen phosphate	2,70
Potassium dihydrogen phosphate	2,70
Sodium azide	0,20

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 35,6 g in 1 L of distilled water. Heat if necessary to dissolve. Divide into 10 mL volumes and pour into tubes. Sterilize in the autoclave at 121°C for 15 minutes. For double strength medium, dissolve 71,2 g/L.

Description

Azide Dextrose Broth according to Rothe has been widely used since 1948 for the detection of faecal streptococci. It usually provides a higher rate of positive results than similar media. Its efficacy is due to the Sodium Azide, which is both selective for enterococci and inhibitive to the accompanying flora through interference of the electron transport chain. This medium is also used for the primary enrichment of food samples, particularly frozen vegetables.

Technique

Water Samples

Add 10 mL of water to be examined to each of three tubes containing 10 mL of double strength medium. Add 1 mL of sample to an additional three tubes containing 10 mL, of single strength medium. Then add 0.1 mL of water to each of three tubes containing 10 mL of single strength medium. Incubate at 37°C and examine after 24 and 48 h. All tubes which are turbid due to growth will be considered as presumptively positive and will have to be confirmed using EVA Broth (Art. No. 02-028). All tubes which are positive on this second testing should be considered for testing using the Most Probable Number (MPN) count method.

When considering other type of samples, dilute them in 1/4 Ringer's solution or peptone water and then inoculate the tubes as previously described.

In highly contaminated samples, dilutions should be carried out prior to inoculation.

References

- CLESCERI, L., A.E. GREENBERG & E.A. EATON (1998) Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF. 20th ed. Washington.
- DOWNES, F.C. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- GUINEA, SANCHO & PARÉS (1979) Análisis Microbiológico de Aguas: Aspectos Aplicados. Ed. Omega. Barcelona.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ROTHE (1948) Illinois State Health Department.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Azide Dextrose Broth (Rothe)

Art. No. 02-027

WARNING

H: 3.1.O/4; H302-4.1.C/3; H412

P: P273-P264-P270-P301+P312-P330-P501a

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Enterococcus faecalis ATCC 29212	Good to very good	-
Enterococcus faecalis ATCC 19433	Good to very good	-

Left: Uninoculated tube (Control)
Centre: *Enterococcus faecalis* ATCC 29212
Right: *Escherichia coli* ATCC 25922

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Bacillus cereus Agar

Art. No. 01-262

Also known as

Mannitol Egg Yolk Polymyxin Agar; MYP Agar

Specification

Selective solid medium, according to Mossel, for the isolation and identification of *Bacillus cereus* from food samples according to ISO standards.

Formula* in g/L

Peptone	10,000
Mannitol	10,000
Sodium chloride	10,000
Meat extract	1,000
Phenol red	0,025
Agar	15,000

Final pH 7,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 46 g of powder in 900 mL of distilled water. Sterilize in the autoclave at 121°C for 15 minutes. Let it cool to 50°C and then add 100 mL of Egg Yolk Sterile Emulsion (Art. No. 06-016) and 100 mg/L of Polymyxin (Art. No. 06-021CASE or 06-021-LYO). Homogenize well and distribute into plates. **Do not reheat or remelt** the complete medium.

Description

Mossel's formulation is developed to detect and enumerate *B. cereus* in any food stuff. It is both selective and differential for this microorganism. Polymyxin addition inhibits most of accompanying bacteria, but does not affect the growth of *B. cereus*. This bacterium does not ferment mannitol and thus there is no change in the colour of the indicator around the colonies. The lecithinase activity of *B. cereus* produces a halo or zone of white precipitate around the colonies.

A count of *B. cereus* over 100.000 cells/g of food sample is considered hazardous, since the accumulated phosphoril-choline may cause toxic symptoms in children. For this reason a viable enumeration must be performed to evaluate the real population of cells.

Technique

According to the authors, dehydrated or dry samples must be treated in the following way: 20 g of sample is mixed with 90 mL of Tryptone Water (Art. No. 03-156) for a minimum period of 1 hour, at room temperature. Afterwards, add an additional 90 mL of Tryptone Water and homogenize. If necessary dilute 1:10. Proceed to a 1/10 serial dilution bank using Tryptone water as the diluent if necessary. With a Drigalsky loop, spread aliquots of 0,1 mL over the surface of the agar plates and let the agar medium absorb the aliquots. Incubate the plates at 30°C for 18-24 hours to allow spore germination before giving definite results.

Suspected colonies have the following appearance: irregular borders, pink colour becoming purple in the centre, with a halo of white precipitate. Colonies with yellow halos must be discounted.

Confusion with other colonies of Gram positive bacilli is possible, and hence, confirmation tests must be carried out i.e. glucose fermentation, gelatine degradation and nitrate reduction.

Necessary supplements

Polymyxin B Sulfate Selective Supplement (Art. No. 06-021CASE / 06-021-LYO)

Vial Contents:

Polymyxin B sulfate	50000,00 IU
Mannitol (excipient)	100,00 mg

Distilled water (Solvent)

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Londres.
- CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD. (2003) Handbook of Culture Media for Food Microbiology. Elsevier Sci. B.V. Amsterdam. The Netherlands.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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Bacillus cereus Agar

Art. No. 01-262

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	Yellow colonies irregulars borders. Man -
Bacillus cereus var. mycoides ATCC 11778	Productivity > 0.70	Red colonies irregulars borders. Man +
Bacillus cereus ATCC 10876	Productivity > 0.70	Red colonies irregulars borders. Man +
Escherichia coli ATCC 25922	Inihibited	Selectivity

Bacillus cereus var. mycoides ATCC 11778

Uninoculate plate (Control)

Bacillus cereus ATCC 10876

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Bacillus cereus Selective Agar

Art. No. 01-487

Also known as

PEMBA (Polymyxin Egg yolk Manitol Blue Agar)

Specification

Selective solid medium for the enumeration of *Bacillus cereus* in food, according to ISO 21871 and NMKL 674 standards.

Formula* in g/L

Peptone	1,00
Mannitol	10,00
Sodium chloride	2,00
Magnesium sulfate	0,20
Disodium phosphate	2,50
Potassium phosphate	0,25
Brom thymol blue	0,12
Sodium pyruvate	10,00
Agar	14,00

Final pH 7,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 40 g of powder in 950 mL of distilled water. Allow it to soak and bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121ºC for 15 minutes. Let it cool to 50ºC and then add 50 mL/L of Egg Yolk Sterile Emulsion (Art. No. 06-016) and 2 vials/Litre of Polymyxin B Sulfate (Art. No. 06-021CASE or 06-021-LYO) to obtein a 100 U/mL concentration. Homogenize and pour into plates.

Description

Bacillus cereus Selective Agar is formulated according to the Food Analysis Nordic Committee (NMLK) standards. This standard uses this medium and Blood Agar Base (Art. No. 01-352) simultaneously for the detection and enumeration of *B. cereus* in any type of food. This medium can also be used to confirm presumptive colonies, in this instance Polymyxin may be omitted.

Technique

NMLK proposes the simultaneous use of *Bacillus cereus* Selective Agar and Blood Agar Base (Art. No. 01-352). Both media are inoculated by surface streaking with 0,1 mL aliquots which are spread with a Drigalsky loop. Both series of plates are incubated at 30ºC for 24 hours.

Typical *B. cereus* colonies in Blood Agar are big, irregular, dirty-white or grey with a surrounding halo of haemolysis. With *B.cereus* Selective Agar, colonies are blue, surrounded by a clear zone of egg yolk digestion (lecithinase positive).

If there is an equal amount of typical colonies on both the media, confirmative tests may not be necessary.

Necessary supplements

Polymyxin B Sulfate Selective Supplement (Art. No. 06-021CASE / 06-021-LYO)

Vial Contents:

Polymyxin B sulfate	50000,00 IU
Mannitol (excipient)	100,00 mg

Distilled water (Solvent)

References

- CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD (2003) Handbook of Culture Media for Food Microbiology. Elsevier Sci. B.V. Amsterdam. The Netherlands.
- FIL-IDF 181:1998 Provisional Int. Standard. Dried Milk Products. Enumeration of *Bacillus cereus*.- Most probable number technique.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 21871 Standard (2006) Microbiology of food and feeding stuffs.- Horizontal method for the determination of low numbers of presumptive *Bacillus cereus*.- Most probable number technique and detection method.
- NORDISK METODIK KOMITE FÖR LIVSMEDEL (1997) UDC 570.852.11 #674ntg.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Bacillus cereus Selective Agar

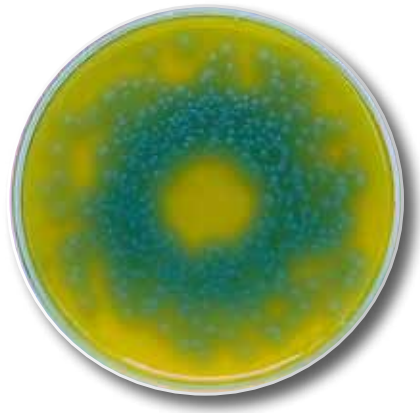
Art. No. 01-487

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Fair to good	-
Bacillus cereus var. mycoides ATCC 11778	Productivity > 0.70	White colonies w. precipitate. Green Medium
Bacillus cereus ATCC 10876	Productivity > 0.70	White colonies w. precipitate. Green Medium
Pseudomonas aeruginosa ATCC 27853	Inhibited	-

Uninoculated Plate (Control)



Bacillus cereus var. mycoides ATCC 11778

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Baird Parker Agar Base

Art. No. 01-030

Also known as

BP Agar; Egg Yolk Tellurite Glycine Pyruvate Agar; ETGP Agar

Specification

Solid selective culture medium for the screening of staphylococci from a variety of samples, according to pharmacopoeias and ISO standards.

Formula* in g/L

Tryptone	10,00
Sodium pyruvate	10,00
Glycine	12,00
Meat extract	5,00
Lithium chloride	5,00
Yeast extract	1,00
Agar	17,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 60 g in 950 mL of distilled water. Allow to soak and bring to the boil stirring constantly. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and add 50 mL of Egg Yolk Tellurite Sterile Emulsion (Art. No. 06-026). Homogenize and distribute into plates. Once prepared, the medium must not be reheated nor sterilized again.

Description

Baird Parker Agar Base is recommended for the detection and enumeration of staphylococci in food and other material, since it allows a good differentiation of coagulase-positive strains. The growth of the accompanying bacteria is usually suppressed by the high concentration in lithium, glycine and pyruvate. Lithium and glycine enhances the growth of staphylococci. Occasionally the medium may grow some *Bacillus species*, yeast and very rarely, *Proteus*. The growth of *Proteus species* can be suppressed by adding 50 mg/l of sulphamethazine.

The presence of tellurite and egg yolk, which must be added to the medium after sterilization, allows the differentiation of presumptive pathogenic staphylococcal colonies. There is a high correlation between the coagulase test and the presence of clear zones of lypolysis in this medium, which is due to the staphylococcal lecithinase. Studies show that almost 100% of coagulase-positive staphylococci are capable of reducing tellurite, which produces black colonies, whereas other staphylococci can not always do so.

When using sterile reagents other than Scharlau microbiology brand the prepare the medium by adding 50 mL sterile egg yolk and 10 mL of 1% potassium tellurite solution. Plates should be used on the same day of preparation or within 48 hours, to avoid the loss of definition in the precipitated zones. The medium base, without yolk or tellurite, is perfectly stable and therefore can be melted repeatedly.

Technique

The inoculation is carried out by spreading 0,5 mL of sample over each plate with a Drigalsky loop. After 18-24 hours of incubation at 35°C, select the colonies which are black, shiny and convex with regular margins surrounded by a clear zone. These can be presumptly identified as coagulase-positive *Staphylococcus aureus*.

Colonial appearance after 24 hours at 35°C:

- *Staphylococcus aureus*: Black, shiny, convex, regular margins, 1,0-1,5 mm diameter, surrounded by a clear zone of lipolysis 2-5 mm in width. Wide opaque zones of precipitate extending into the cleared medium may occur after 48 hours.
- Other species of *Staphylococcus*: Black, usually dull, with regular margins. Sometimes brown with zones of clearing but these present as wide opaque zones.
- *Micrococcus* spp: Brown, very small and without clearing zones.
- *Bacillus* spp: Various shades of brown, big. May produce clearing zones after 48 hours.
- Yeasts: White, big and smooth.

Egg yolk emulsion can be prepared by mixing a fresh egg yolk with an equivalent quantity (w/v) of saline solution. Sterilize by filtration and aseptically add to the medium. (This reagent is available, presterelized, from Scharlau microbiology Art. No. 06-016.).

The potassium tellurite solution is prepared by dissolving 3,5 g potassium tellurite in 100 mL distilled water. Sterilize by filtration. (This reagent is available presterilized from Scharlau microbiology Art. No. 06-011.)

Although these solutions can be mixed and added to the Baird Parker Agar Base forming the additive commonly known Egg Yolk Tellurite Sterile Emulsion (Art. No. 06-026 and 064-BA1018 ISO), they are also stable as the separate supplement and can be used in many other culture media.

References

- ATLAS R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Londres.
- BAIRD-PARKER, A.C. (1962) An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bact. 25:12.
- COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
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Baird Parker Agar Base

Art. No. 01-030

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- FIL-IDF 60:2001 Standard. Lait et produits à base de lait - Detection des staphylocoques à coagulase positive - Technique du nombre le plus probable. Brussels.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 22718:2006 Standard. Cosmetics - Detection of *Staphylococcus aureus*.
- USP 31 - NF 26 (2008) <61> Microbial Limit Tests. US Phamacopoeial Conv. Inc. Rockville. MD. USA.
- ZANGERL, P. & H. ASPERGER (2003) Media used in the detection and enumeration of *Staphylococcus aureus*. In Handbook

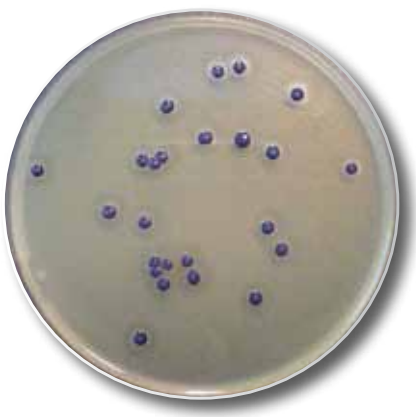
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

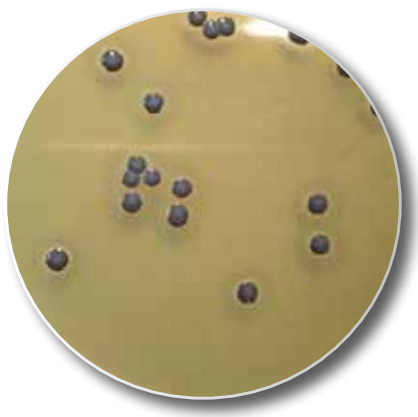
Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited	Selectivity
Escherichia coli ATCC 8739	Inhibited	Selectivity
Staphylococcus aureus ATCC 25923	Productivity > 0.50	Black colonies; Lecithinase (+)
Staphylococcus aureus ATCC 6538	Productivity > 0.50	Black colonies; Lecithinase (+)
Staphylococcus epidermidis ATCC 12228	Productivity > 0.50	Black colonies; Lecithinase (+)



Staphylococcus aureus ATCC 25923



Staphylococcus aureus ATCC 25923
(Lecitinase Halos)

Staphylococcus aureus ATCC 6538

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BAT Agar

Art. No. 01-675

Specification

Solid medium for the detection and isolation of *Alicyclobacillus*, in fruit juices and other acidic food, according to IFU standard Method No. 12.

Formula* in g/L

Yeast extract	2,00000
Dextrose	5,00000
Potassium hydrogen phosphate	3,00000
Calcium chloride	0,25000
Magnesium sulfate	0,50000
Ammonium sulfate	0,20000
Zinc sulfate	0,00018
Copper sulfate	0,00016
Manganese sulfate	0,00015
Cobalt chloride	0,00018
Boric acid	0,00010
Sodium molybdate	0,00030
Agar	20,00000

Final pH 4,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 31 g of BAT Agar in 1 L of distilled water and bring to the boil to dissolve. Distribute in suitable containers and sterilise in the autoclave at 121°C for 15 minutes. Cool to 45-50°C and adjust the pH to 4,0 ± 0,2 by adding 1N H_2SO_4. Mix well to homogenise and pour into sterile Petri dishes. Avoid heating or remelting the medium after the pH adjustment.

Description

Since the early 1980's, when spoilage of fruit juices by acid dependent thermotolerant spore-forming bacteria was recognized (Cerny *et al.,* 1984) members of the genus *Alicyclobacillus* have been identified as food spoilage organisms of major significance to the fruit juice industry (Baumgart & Menje, 2000). Spoilage is generally manifested as the formation of off flavours and odours from compounds such as guaiacol and the halogenated phenols. The economic impact of such incidents can be very high, nevertheless, to date, no human risk to be associated with the consumption of juices and other food products containing *Alicyclobacillus* bacteria.

An acidic environment is required to grow alicyclobacilli and BAT (*Bacillus AcidoTerrestris*) media supports the growth of all currently known species of *Alicyclobacillus* (*A. acidocaldarius, A. acidoterrestris, A. cycloheptanicus* and *A. hesperidium*). These media comply with the Standard IFU Method for the detection of organisms that taint fruit juices (No. 12).

The low pH-value of the media, in combination with the high incubation temperature inhibits the growth of contaminating microbiota.

K Agar (Art. No. 01-674) when incubated at 45°C supports the growth of predominantly *A. acidoterrestris* and limited growth of other species of the genus. Therefore, K Agar (Art. No. 01-674) can be used to detect predominantly *A. acidoterrestris* strains.

Technique

The IFU Standard describes three methods of detection depending on the sample composition and the time elapsed since processing:

1. Raw materials (including processed water): A heat shock treatment is required followed by direct plating (optional), filtration or enrichment in liquid medium, of the heated material.
2. End products: sampled directly after (heat) processing where an additional heat shock is unnecessary: Pre-incubation of the sample in liquid medium is required.
3. End products taken from the market: Pre-incubation of the sample, and heat shock treatment are optional. However if spoilage is suspected and no alicyclobacilli detected after direct plating, a heath shock and enrichment is recommended.

In all methodology incubation for 3-5 days at 45 ± 1°C is recommended. Count all colonies growing on the BAT Agar as presumptive alicyclobacilli. Confirm these colonies by further testing.

References

- BAUMGART, J. (2003) Media for detection and enumeration of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* in foods. In Handbook of Culture Media for Food Microbiology. J.E.L. Corry et al. (Eds.) Elsevier Sci B.V. Amsterdam.
- BAUMGART, J. & S. MENJE (2000) The impact of *Alicyclobacillus acidoterrestris* on the quality of juices and soft drinks. Fruit Processing 7:251-254.
- CERNY, G., W. HENNLICH & K. PORALLA (1984) Fruchsaftverdeb durch Bazillen: Isolierung und Charakterisierung des Verdebserregers. Z. Lebens. Unter Forsch. 179:224-227.
- IFU STANDARDS (2004) Method No. 12 on the detection of taint producing *Alicyclobacillus* in fruit juices. Revision march 2007.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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BAT Agar

Art. No. 01-675

Quality control

Incubation temperature: 45°C ± 2,0
Incubation time: 72 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibited	-
Bacillus cereus var. mycoides ATCC 11778	Inhibited	-
Alicyclobacillus acidoterrestris ATCC 49025	Productivity > 0.70	-
Alicyclobacillus acidocalcarius ATCC 27009	Productivity > 0.70	-

BAT Broth

Art. No. 02-675

Specification

Liquid medium used for the enrichment of *Alicyclobacillus*, from fruit juices and other acidic foods, according to IFU standard Method No. 12.

Formula* in g/L

Yeast extract	2,00000
Dextrose	5,00000
Potassium hydrogen phosphate	3,00000
Calcium chloride	0,25000
Magnesium sulfate	0,50000
Ammonium sulfate	0,20000
Zinc sulfate	0,00018
Copper sulfate	0,00016
Manganese sulfate	0,00015
Cobalt chloride	0,00018
Boric acid	0,00010
Sodium molybdate	0,00030

Final pH 4,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 11 g in 1 L of distilled water and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C and adjust the pH to 4,0 ± 0,2 by adding 1N H_2SO_4. Mix well to homogenize and aseptically distribute into sterile tubes. Avoid overheating the medium after the pH adjustment.

Description

Since the early 1980s, when spoilage of fruit juices by acid dependent thermotolerant spore-forming bacteria was recognized (Cerny *et al.,* 1984) members of the genus *Alicyclobacillus* have been identified as food spoilage organisms of major significance to the fruit juice industry (Baumgart & Menje, 2000). Spoilage is generally manifested as the formation of off flavours and odours from compounds such as guaiacol and the halogenated phenols. The economic impact of such incidents can be very high, nevertheless, to date, no human risk is known to be associated with the consumption of juices and other food products containing *Alicyclobacillus* bacteria.

An acidic environment is required to grow alicyclobacilli and BAT (*Bacillus AcidoTerrestris*) media supports the growth of all current known species of *Alicyclobacillus* (*A. acidocaldarius, A. acidoterrestris, A. cycloheptanicus* and *A. hesperidium*). These media comply with the Standard IFU Method for the detection of organisms that taint fruit juices.

The low pH-value of the media, in combination with the high incubation temperature inhibits the growth of contaminating microbiota.

K Agar (Art. No. 01-674) when incubated at 45°C supports the growth of predominantly *A. acidoterrestris* and the limited growth of other species of the genus. Therefore, K Agar (Art. No. 01-674) can be used to detect predominantly *A. acidoterrestris* strains.

Technique

The IFU Standard describes three methods of detection depending on the sample composition and the time elapsed since processing:

1. Raw materials (including processed water): A heat shock treatment is prescribed followed by direct plating (optional), filtration or enrichment in liquid medium of the heated material.
2. End products: sampled directly after (heat) processing where an additional heat shock is unnecessary: Pre-incubation of the sample in liquid medium is required.
3. End products taken from the market: Pre-incubation of the sample, and heat shock treatment are optional. However if spoilage is suspected and no alicyclobacilli detected after direct plating, a heath shock and enrichment is recommended.

In all the methodologies incubation for 2-4 days at 45 ± 1°C is recommended for the enrichment step.

References

- BAUMGART, J. (2003) Media for detection and enumeration of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* in foods. In "Handbook of Culture media for food Microbiology". J.E.L. Corry et al. (Eds.) Elsevier Sci B.V. Amsterdam.
- BAUMGART, J. & S. MENJE (2000) The impact of *Alicyclobacillus acidoterrestris* on the quality of juices and soft drinks. Fruit Processing 7:251-254.
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- IFU Standards (2004) Method No. 12 on the detection of taint producing *Alicyclobacillus* in fruit juices. Revision march 2007.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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BAT Broth

Art. No. 02-675

Quality control

Incubation temperature: 45°C ± 2,0
Incubation time: 72 h - 5 days
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibited	-
Bacillus cereus var. mycoides ATCC 11778	Inhibited	-
Alicyclobacillus acidoterrestris ATCC 49025	Good	-
Alicyclobacillus acidocalcarius ATCC 27009	Good	-

Beerens Cosmetic Diluent

Art. No. 02-257

Specification

Diluent used to neutralize preservative systems in routine examination of cosmetic products.

Formula* in g/L

Lecithin	3,00
Sodium thiosulfate	5,00
L-Histidine HCl	1,00
Peptone	1,00
Sodium chloride	8,50
Dipotassium phosphate	1,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 19,5 g of powder in 1 L of distilled water containing 30 mL of Polysorbate 80 (Art. No. TW0080).
Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Let cool to 50°C and shake gently to dissolve the polysorbate.

Description

Cosmetic Beeren's Diluent has all the necessary compounds to neutralize most of the chemical agents included in cosmetic products to maintain and preserve it free of microorganisms.
It complies with the EU recommendation that states that before any microbiological examination, a treatment to remove all growth inhibitor systems in cosmetics must be performed.
However, this standard also declares that later dilutions must be performed in less aggressive media, that may be considered as an enrichment and revitalization system, and suggests the use of Letheen Broth (Art. No. 02-236) or Letheen Modified Broth (Art. No. 02-237).

References

- BEERENS, H., RAMONS, C., LEMAIRE, D. (1976) Rev. Inst. Pasteur. Lyon. 9:127.
- BRIGIDI, P., MATTEUZZI, D. (1982) Il Farmaco Ed. Pr. 37:8:260.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	-
Enterococcus faecalis ATCC 19433	Good	-
Bacillus subtilis ATCC 6633	Good	-
Escherichia coli ATCC 8739	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Yersinia enterocolitica ATCC 9610	Good	-

Bile Esculin Modified Agar

Art. No. 01-265

Also known as

Bile Esculin Medium; BEM; Bile Esculin Agar

Specification

Solid culture medium for identification of probiotic streptococci in food samples.

Formula* in g/L

Meat extract	3,00
Peptone	5,00
Bile Salts	20,00
Ferric citrate	0,50
Esculin	1,00
Agar	15,00

Final pH 7,0 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 44,5 g of powder in 1 L of distilled water and let soak. Bring to the boil and distribute into containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium formulation is based on the modification by Facklam and Moody of the original formulation by Swan to verify the esculin hydrolysing capacity of streptococci and their resistance to bile salts which inhibit Gram positive bacteria.

In fact, this medium can be used as a substitute KAA Confirmative Agar (Art. No. 01-263), but as it does not have the same selectivity. It is used as a substrate to verify the two assays simultaneously in the biochemical tests that identify enterococci.

Technique

The assay is performed by inoculating the surface of a slant with a pure culture of the organism to be verified. After the 24 hours incubation at 35°C, it may produce translucent colonies, surrounded by black haloes or zones, due to esculin hydrolysis. Resistance to bile salts is indicated by the growth.

References

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- DEIBEL, R.H. y HARTMAN, P.A. (1976) The Enterococci, en Compendium of Methods for the Microbiological Examination of Foods. APHA.
- DOWNES, F.P. y K. ITO (2001) Compendium of methods for the Microbiological Examination of Foods. APHA. Washington.
- FACKLAM, R.R. y MOODY, M.D. (1970) Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. 20:245.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Rev. A. APHA Internacional Gaithersburg. VA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LEUCHNER, R.G.K., J. BEW, K.J. DOMIG y W. KNEIFEL (2002) A collarative study of a method for enumeration of probiotic enterococci in animal feed. J. Appl. Microbiol. 93:781-786.
- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- SWAN, A. (1954) The use of bile-esculin medium and Maxted's technique of Lancefield grouping in the identification of streptococci. J. Clin. Pathol. 7:160.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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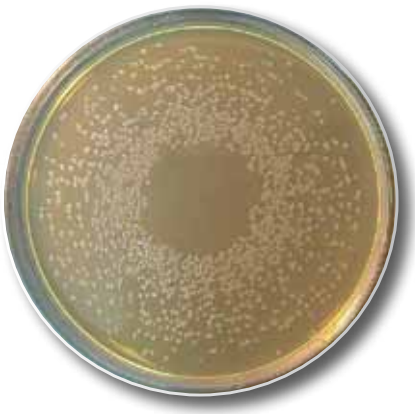
Bile Esculin Modified Agar

Art. No. 01-265

Quality control

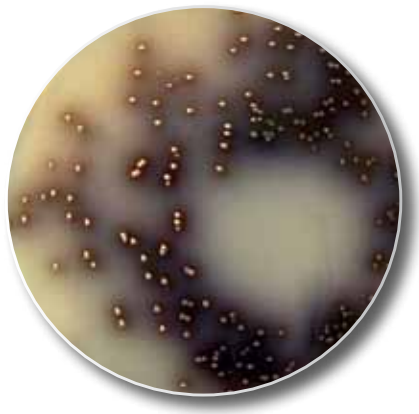
Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	White colonies (Esculin -)
Enterococcus faecalis ATCC 29212	Productivity > 0.70	Brown to black colonies (Esculin +)
Enterococcus faecalis ATCC 19433	Productivity > 0.70	Brown to black colonies (Esculin +)



Escherichia coli ATCC 25922

Enterococcus faecalis ATCC 29212



Enterococcus faecalis ATCC 29212
" Detail"

Bile Esculin Azide Agar

Art. No. 01-592

WARNING

H: 4.1.C/3: H412

P: P273-P501a

Specification

Solid medium for the confirmation and enumeration of enterococci in water by the membrane filtration method according to ISO 7899-2.

Formula* in g/L

Tryptone	17,00
Peptone	3,00
Yeast extract	5,00
Bile	10,00
Sodium chloride	5,00
Esculin	1,00
Ammonium ferric citrate	0,50
Sodium azide	0,15
Agar	15,00

Final pH 7,20 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 56.6 g of powder in 1 L of distilled water and bring to the boil. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 50-60°C and pour plates to 3-5 mm thickness. These plates can be stored at 2-8°C for up to two weeks.

Description

Bile Esculin Azide Medium is a modification of the classical Bile Esculin proposed by Isenberg, Goldberg and Sampson in 1970, but with a reduction in the amount of bile and the addition of sodium azide. Brodsky and Schieman showed that this medium, also known as Pfizer Enterococci Selective Medium gave the best results using the membrane filtration technique.

The actual formulation according to the ISO Standard 7899-2:2000 is used for the second step in the confirmation and enumeration of enterococci in water by the membrane filtration method. The colonies previously selected in the Slanetz Bartley Agar (Art. No. 01-579 + 06-023) must be confirmed by a short incubation on Bile Esculin Azide Medium for verification of esculin hydrolysis in a selective environment.

Technique

After an incubation of 24-48 hours on Slanetz Bartley Agar (Art. No. 01-579 + 06-023), the membrane filter showing typical colonies is transferred, with sterile forceps in an upright position, to a pre-warmed plate of Bile Esculin Azide Agar. After two hours of incubation at 44 ± 0.5°C the membrane filter is inspected. All the typical colonies that show brown to black colour in the surrounding medium are considered positive and therefore intestinal enterococci.

A heterogeneous distribution of the colonies or the presence of abundant and different microorganisms can interfere with the differentiation of positive colonies.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla.
- BRODSKY M.H. & D.A. SCHIEMANN (1976) Evaluation of Pfizer Selective *Enterococcus* and KF media for recovery of fecal streptococci from water by membrane filtration. Appl. Environ. Microbiol. 31 :695-699.
- ISENBERG, H.D., D. GOLDBERG & J. SAMPSON (1970) Laboratory studies with a selective enterococcus medium. Appl. Microbiol. 20:433.
- ISO Standard 7899-2 (2000) Water Quality. Detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60 % RH).

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Bile Esculin Azide Agar

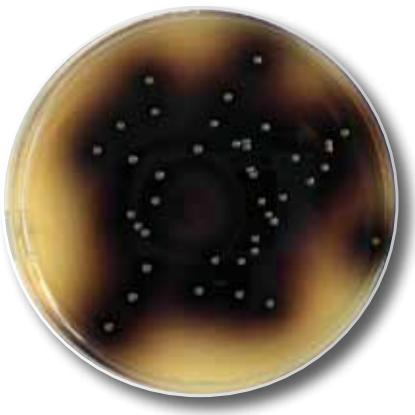
Art. No. 01-592

WARNING
H: 4.1.C/3; H412
P: P273-P501a

Quality control

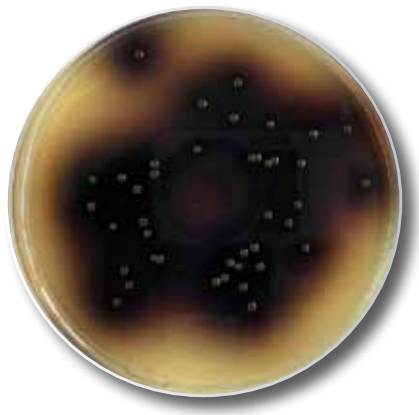
Incubation temperature: 35°C ± 2.0
Incubation time: 48 h
Inoculum: 10-100 CFU (Productivity) / 1.000-10.000 CFU (Selectivity). Spiral Plate Method (or Membrane Filter Method)

Microorganism	Growth	Remarks
Bacillus cereus var. mycoides ATCC 11778	Inhibited	Selectivity
Escherichia coli ATCC 25922	Inhibited	Selectivity
Enterococcus faecalis ATCC 29212	Productivity > 0.70	Black medium. E (+)
Enterococcus faecalis ATCC 19433	Productivity > 0.70	Black medium. E (+)



Enterococcus faecalis ATCC 29212

Uninoculated Plate (Control)



Enterococcus faecalis ATCC 19433

Blood Agar Base

Art. No. 01-352

CE IVD

Specification

Nutrient rich medium suitable for the isolation of pathogenic microorganisms from clinical specimens.

Formula* in g/L

Meat extract	10,00
Tryptone	10,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 40 g of powder in 950 mL of distilled water. Heat to the boiling point and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. Let it cool to 45-50°C and then add defibrinated blood in a proportion of about 5% or to the desired enrichment level.

Description

Blood Agar Base may be used for the cultivation of non fastidious microorganisms, since it has a balanced nutrient base.
For fastidious microorganisms, it is advisable to add special enrichment supplements, such as ascitic liquid, egg yolk, etc...
This medium, with the addition of blood, is suitable for studies in haemolytic activity, but for the isolation of pathogens Blood Agar Base Columbia type (Art. No. 01-034) is recommended.

References

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, London.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Blood Agar Base

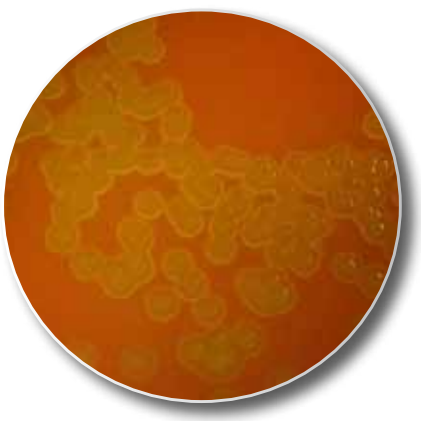
Art. No. 01-352

CE IVD

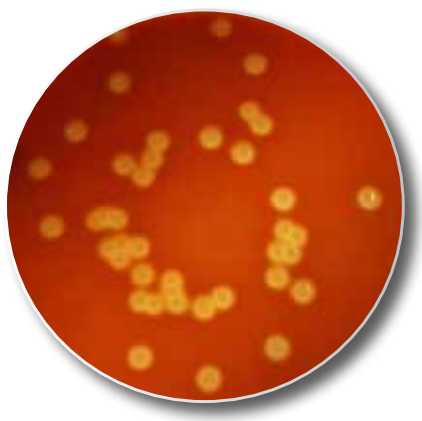
Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	β - haemolysis
Staphylococcus aureus ATCC 6538	Productivity > 0.70	β - haemolysis
Enterococcus faecalis ATCC 19433	Productivity > 0.70	γ - haemolysis
Escherichia coli ATCC 8739	Productivity > 0.70	γ - haemolysis
Streptococcus pyogenes ATCC 19615	Productivity > 0.70	β - haemolysis
Streptococcus pneumoniae ATCC 49619	Productivity > 0.70	α - haemolysis



Streptococcus pneumoniae ATCC 49619



Staphylococcus aureus ATCC 25923

Blood Agar Base No. 2

Art. No. 01-505

CE IVD

Specification

Nutrient rich medium suitable for the isolation of pathogenic microorganisms from clinical specimens.

Formula* in g/L

Proteose peptone	15,00
Liver extract	2,50
Yeast extract	5,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 42,5 g in 950 mL of distilled water and bring to boil. Distribute into flasks and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C and aseptically add 5% of sterile defibrinated blood. Mix gently and pour into plates.
Note: Blood and medium should be mixed in a big flask to ensure proper blood oxidation and mixing.

Description

Blood Agar Base No. 2 allows maximum recovery of weak organisms without altering or interfering in their haemolytic reactions. Compared to other Blood Agar bases, Blood Agar Base No. 2 has an equal or higher stimulatory growth capacity, however it is specially formulated to promote pigment production in chromogenic bacteria. The formulation according to ISO standard 7932 (2003) differs from other authors in its final pH value.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla.
- CASMAN, E. (1947) A non-infusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Path. 17:281-289.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the Microbiological Examination of Foods. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Rev. A. APHA International. Gaithersburg, VA.
- ISO 7932 Standard (2003) Microbiology of food and animal feeding stuffs. Horizontal Methods for the enumeration of presumptive *Bacillus cereus*. Colony count technique at 30°C.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	β - haemolysis
Staphylococcus aureus ATCC 6538	Productivity > 0.70	β - haemolysis
Streptococcus pyogenes ATCC 19615	Productivity > 0.70	β - haemolysis
Streptococcus pneumoniae ATCC 49619	Productivity > 0.70	α – haemolysis
Neisseria meningitidis ATCC 13090	Productivity > 0.70	γ – haemolysis

Blood Agar Base (Columbia)

Art. No. 01-034

CE IVD

Also known as

CA; C Agar; Columbia Blood Agar; CB Agar

Specification

Nutrient rich medium suitable for the isolation of pathogenic microorganisms from clinical specimens.

Formula* in g/L

Casein peptone	12,00
Meat peptone	11,00
Starch	1,50
Sodium chloride	5,00
Agar	15,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 44,5 g of powder to 950 mL of distilled water and bring it to the boil. Distribute into suitable containers and sterilize at 121°C for 15 minutes. To obtain Blood Agar cool to 45-50°C and aseptically add sterile defibrinated blood a proportion of 5%.

Description

Blood Agar Base contains a balanced mixture of meat and casein peptones, making it suitable for preparing selective media and as a diagnostic medium with the addition of blood or inhibitors. The base formulation, without additives, is also an excellent general culture medium. Generally, Blood Agar base contains a casein peptone, that aids large colony formation, or a meat peptone, that provides for well defined haemolysis haloes or zones. Blood Agar Base is prepared according to the Columbia University formulation, and meets the two conditions mentioned above. Some applications for this base are:

- Agar base without either enrichment or inhibitors: This medium supports growth of normal microorganisms such as enterobacteria and more fastidous organisms such as *Pasteurella, Brucella* and *Clostridium perfringens*.
- *Clostridium* Selective Agar base: Should a selective *clostridium* medium be desired, add 240 mg/L sodium Azide and 180 mg/L neomycin before sterilization.
- Blood Agar: Aseptically add to the sterile medium 5% sterile defibrinated Horse/Sheep's blood when cooled to 45°C.
  The medium is now enriched and allows the determination of typical haemolytic reactions necessary for the identification of enterococci, streptococci, staphylococci and other microorganisms.
- Selective Gram positive cocci Blood Agar: As above add blood and simultaneously, also add 10 mg/L of colistin and 15 mg/L of nalidixic acid, to obtain an excellent selective medium for Gram positive cocci.

Note: Some authors recommend Blood Agar Base as the maintenance medium for *Campylobacter.*

References

- ATLAS, RM & LC PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- CASMAN, E. (1947) A non-infusion blood agar base for neiseriae, pneumococci and streptococci. Am. J. Clin. Path. 17:281-289.
- ELLNER, PD, CJ STOESSEL, E. DRAKEFORD, & F. VASI (1966) A new culture medium for medical bacteriology. Amer.J.Clin.Path 45:502-504.
- ISENBERG H.D. (1992) Clinical Microbiology Procedures Handbook. ASM Washington. DC. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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B

Blood Agar Base (Columbia)

Art. No. 01-034

CE IVD

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	β - haemolysis
Staphylococcus aureus ATCC 6538	Productivity > 0.70	β - haemolysis
Escherichia coli ATCC 8739	Productivity > 0.70	γ - haemolysis
Enterococcus faecalis ATCC 19433	Productivity > 0.70	γ - haemolysis
Streptococcus pneumoniae ATCC 49619	Productivity > 0.70	α - haemolysis
Streptococcus pyogenes ATCC 19615	Productivity > 0.70	β - haemolysis

Enterococcus faecalis ATCC 19433

Staphylococcus aureus ATCC 6538
48hr 35ºC ± 2.0

Streptococcus pneumoniae ATCC 49619

www.scharlab.com
scharlab@scharlab.com

Bolton Enrichment Broth Base

Art. No. 02-688

Specification

Liquid culture medium used for the enrichment of *Campylobacter* from food samples according to the ISO standard 10272-1:2006.

Formula* in g/L

Meat peptone	10,00
Lactalbumin hydrolysate	5,00
Yeast extract	5,00
Sodium chloride	5,00
Sodium pyruvate	0,50
Sodium metabisulfite	0,50
Sodium carbonate	0,60
α-Ketoglutaric acid	1,00
Haemin	0,01

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 13,8 g of the powder in 500 mL of distilled water, heating if necessary. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 47-50° C, add 25 mL of lysed horse blood aseptically, and the content of one vial of *Campylobacter* Bolton Selective Supplement (Art. No. 06-131-LYO). Mix throughly. Dispense the complete medium into suitable containers.

Note: If the enrichment broth has been prepared in advance, it should be kept for no more than 4 hours at ambient temperature or in the dark at 3 ± 2°C for not more than 7 days.

Description

Bolton Broth Base is intended for the enrichment of *Campylobacter* from food samples. Food processing and preservation injure *Campylobacter* cells and resuscitation steps by a double incubation in Bolton Broth encourages them to multiply and grow.

The meat peptone and lactalbumin hydrolysate supply the carbon and nitrogen for growth. Sodium chloride provides osmotic balance and the sodium carbonate neutralizes the acidity generated by the microbial growth. Yeast extract and ketoglutaric acid act as growth factors. Inclusion of sodium metabisulfite, sodium pyruvate and haemin neutralises toxic compounds that may form in the culture medium due to the action of oxygen action and avoid the need for a microaerobic atmosphere. Lysed blood is necessary to neutralize trimethoprim antagonists present in the medium.

The selectivity of the enrichment step is optimized with the Selective Supplement (Art. No. 06-131-LYO): Vancomycin is active against Gram positive microorganisms. Cephoperazone is predominantly active against Gram negative bacteria. Trimethoprim acts against a wide variety of Gram positive and Gram negative cells and cycloheximide or amphotericin B are efficient fungicides.

Technique

Introduce a quantity (mass or volume) into nine times its volume of Bolton Selective Enrichment Broth so as to obtain a test sample/medium ratio of 1:10 (w/v or v/v) and homogenize.

Bolton Selective Enrichment Broth does not require incubation in a microaerobic environment, but must be used in screw topped containers which are filled leaving a headspace of less than 20 mm, and have tightly closing caps.

Incubate the initial suspension at 37°C for 4-6 hours, then at 41,5°C for 44 ± 4 hours.

For the isolation and identification techniques, please, refer to ISO or BAM (Bacteriological Analytical Manual) methods.

Necessary supplements

Campylobacter Bolton Selective Supplement (Art. No. 06-131-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Vancomycin	10,00 mg
Cefoperazone	10,00 mg
Trimethoprim	10,00 mg
Amphotericin B sulfate	5,00 mg

Distilled water (Solvent)

References

- BAYLIS, C.L., (editor) (2007) Manual of Microbiological Methods for the Food and Drinks Industry. 5th ed. Method 3.3.1:2007. CCFRA . Chipping Campden. UK.
- BOLTON, F.J. (2000) Methods for isolation of *campylobacters* from humans, animals, food and water. In "The increasing incidence of human campylobacteriosis" Report and Proceedings of a WHO Consultation of Experts. Copenhagen Denmark 21-25 November 2000, WHO/CDS/CSRAPH 2001. p. 87-93.
- BOLTON, F.J., D. COATES, P.M. HINCHCLIFFE & L. ROBERTSON (1983) Comparison of selective media for isolation of *Campylobacter jejuni/coli*. J. Clin. Pathol. 36:78-83.
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- DOYLE, M.P. & D.J. ROMAN (1982) Recovery of *Campylobacter jejuni* and *C. coli* from inoculated foods by selective enrichment. Appl. Environm. Microbiol. 43:1343-1353.

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Bolton Enrichment Broth Base

Art. No. 02-688

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- HUNT, J.M., C. ABEYTA & T. TRAN (1998) *Campylobacter*. In: FDA BAM 8th ed. (revision A) 7.01-7.027 AOAC International. Gaithersburg. MD. USA.
- ISO 10272-1 Standard (2006) Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of *Campylobacter spp*. - Part 1: Detection method.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- STERN, N.J., J.E. LINE & H.C. CHEN (2001) *Campylobacter* in "Compendium of methods for the Microbiological Examination of Foods" 4th ed. F.P. Downes & K. Ito (Eds.) APHA, Washington. DC. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 41,5°C ± 0,5
Incubation time: 44 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity); according to ISO 11133-1/2

Microorganism	Growth	Remarks
Campylobacter jejuni ATCC 29428	Good	-
Escherichia coli ATCC 25922	Inhibited	-
Staphylococcus aureus ATCC 25923	Inhibited	-

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Brain Heart Infusion Agar (BHI Agar)

Art. No. 01-599

Specification

General purpose solid medium for fastidious pathogenic microorganisms.

Formula* in g/L

Brain extract	12,50
Heart extract	5,00
Proteose peptone	10,00
Sodium chloride	5,00
Di-sodium phosphate	2,50
Dextrose	2,00
Agar	15,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 52 g of powder in 1 L of distilled water and bring to the boil. Distribute in tubes or flasks and sterilize in the autoclave at 121°C for 15 minutes.

Description

Brain Heart Infusion is used for the cultivation of fastidious bacteria (streptococci, pnemococci, meningococci, etc.) and is also recommended for the cultivation of pathogenic fungi.

Growth of the accompanying bacterial microbiota can be almost completely suppressed by adding 20 iu penicillin and 40 µg streptomycin per mL culture medium.

If this medium is to be used for the selective isolation of fastidious fungi (especially of *Histoplasma capsulatum* and *Blastomyces*) add 10% sterile defibrinated blood and for mixed infected samples also add 0,05 µg/mL cycloheximide and 0,5 µg/mL chloramphenicol.

This medium is not suitable for obtaining characteristic haemolytic reactions even after addition of blood because of its glucose content.

References

- AJELLO,L., L.K. GEORG, W. KAPLAN & L. KAUFMAN (1966) Laboratory Manual for Medical Mycology. (CDC) US DHEW, Center fpr Disease Control. Atlanta.
- APHA-AWWA-AWPC (1998) Standard methods for the examination of water and wastewater. 20th ed. Washington. DC. USA.
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- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. VA. USA.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ROSENOW, E.C. (1919) Studies on elective localization. Focal infection with special reference to oral sepsis. J. Dental Res. 1:205-249.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Brain Heart Infusion Agar (BHI Agar)

Art. No. 01-599

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Staphylococcus aureus ATCC 6538	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Escherichia coli ATCC 8739	Productivity > 0.70	-
Streptococcus pyogenes ATCC 19615	Productivity > 0.70	-
Streptococcus pneumoniae ATCC 49619	Productivity > 0.70	-

Streptococcus pyogenes ATCC 19615

Staphylococcus aureus ATCC 25923

Enterococcus faecalis ATCC 19433

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Brain Heart Infusion Broth (BHI Broth)

Art. No. 02-599

CE IVD

Specification

Nutrient rich medium suitable for the isolation of pathogenic microorganisms from clinical specimens.

Formula* in g/L

Brain extract	12,50
Heart extract	5,00
Peptone	10,00
Dextrose	2,00
Sodium chloride	5,00
Di-sodium phosphate	2,50

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 37 g of powder in 1 L of distilled water, heating if necessary. Distribute into containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Brain Heart Infusion is used for the cultivation of fastidious bacteria (streptococci, pnemococci, meningococci, etc.) and is also recommended for the cultivation of pathogenic fungi. Growth of the accompanying bacterial microbiota can be almost completely suppressed by adding 20 IU penicillin and 40 µg streptomycin per mL of culture medium.

If this medium is to be used for the selective isolation of fastidious fungi (especially of *Histoplasma capsulatum and Blastomyces*) from mixed infected samples add 10% sterile defibrinated blood and also add 0,05 µg/mL of cycloheximide and 0,5 µg/mL of chloramphenicol.

This medium is not suitable for obtaining characteristic haemolytic reactions even after addition of blood because of its glucose content.

References

- AJELLO,L., L.K. GEORG, W. KAPLAN & L. KAUFMAN (1966) Laboratory Manual for Medical Mycology. (CDC) US DHEW, Centre for Disease Control. Atlanta.
- APHA-AWWA-AWPC (1998) Standard methods for the examination of water and wastewater. 20th ed. Washington. DC. USA.
- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Culture Media. CRC Press. Londres.
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- DIN 10163 Norme. Mikrobiologische Untersuchung von Fleisch und Fleischerzeugnissen. Bestimmung Koagulase-positiver Staphylokokken. Referenzverfahren.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. DC. USA.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. VA. USA.
- HAYDEN, R.L. (1923) Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. 32:828 -849.
- HOWELL, A. (1948) The efficiency of methods for the isolation of *Histoplasma capsulatum*. Public Health Reports. 63:173-178.
- ISO Standard 5944 (2001) Milk and milk based products - Detection of coagulase positive staphylococci - MPN Technique.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ROSENOW, E.C. (1919) Studies on elective localization. Focal infection with special reference to oral sepsis. J. Dental Res. 1:205-249.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Brain Heart Infusion Broth (BHI Broth)

Art. No. 02-599

CE IVD

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Staphylococcus aureus ATCC 6538	Good	-
Enterococcus faecalis ATCC 19433	Good	-
Escherichia coli ATCC 8739	Good	-
Streptococcus pyogenes ATCC 19615	Good	-
Streptococcus pneumoniae ATCC 49619	Good	-

Left: Uninoculated Tube (Control)
Centre: *Staphylococcus aureus* ATCC 25923
Right: *Escherichia coli* ATCC 8739

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Brilliant Green Agar (BGA)

Art. No. 01-203

Also known as

Brilliant Green Phenol Red Lactose Agar; BPLA

Specification

Medium for *Salmonella* isolation, according to the European Pharmacopoeia.

Formula* in g/L

Meat peptone	5,0000
Casein peptone	5,0000
Sodium chloride	5,0000
Yeast extract	3,0000
Lactose	10,0000
Sucrose	10,0000
Phenol red	0,0800
Brilliant green	0,0125
Agar	15,0000

Final pH 7,0 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 53 g of powder in 1 L of distilled water and bring to the boil stirring constantly. Dispense into containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

BGA is a differential selective medium, able to detect the presence of enteropathogenic bacteria in different samples. This medium is a modification to Kauffman's original formulation, and it complies with the WMO, Eur. Pharm., USP and APHA specifications.

Since it has a high brilliant green concentration, it inhibits the growth of most bacteria, except *Salmonella*. However, *S. typhi* and *S. paratyphi* are also inhibited. Therefore, when their presence or *Shigella* is suspected, it is recommended to use other media in parallel, such as Deoxycholate Lactose Agar (Art. No. 01-057), MacConkey Agar (Art. No. 01-118), *Salmonella-Shigella Agar* (Art. No. 01-555), Xylose Lysine Deoxycholate Agar (Art. No. 1-552) or Endo Agar Base (Art. No. 01-589), which are less inhibitory.

The presence of lactose and sucrose allows a good differentiation between *Salmonella*, which produce pink or colourless colonies with a red halo or zone, and the companion microbiota, which produce smaller and green yellowish colonies with a yellow halo, due to acid created by lactose and/or sucrose fermentation.

Osborn and Stokes suggested the addition of 0,08 g/L of sulfadiazine or 1 g/L of sulfapyridine in order to make this medium more selective for *Salmonella* and therefore making the medium more suitable for the testing of food and eggs and their derivatives.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media CRC Press. BocaRaton. Fla. USA.
- CLESCERI, L:S., A.E. GREENBERG & A.D. EATON (Eds) (1998) Standard Methods for the Examination of Water and Wastewater 20th ed. APHA-AWWA-WEF Washington DC. USA.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods.4th ed. APHA. Washington DC.
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- MURRAY, P.R., E.J. BARON, J.H. JORGENSEN, M.A. PFALLER & R.H. YOLKEN (Eds) (2003) Manual of Clinical Mcrobiology 8th ed. ASM Press. Washington DC, USA.
- OSBORN, W.W. and STOKES, J.L. (1955) The determination of Salmonellae in Foods. Ottawa: Food and Drug Laboratoires. 1962.
- US FDA (Food and Drug Adminstrations) (1998). Bacteriological Analytical Manual. 8th ed. AOAC International. Gaithersburg, MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Brilliant Green Agar (BGA)

Art. No. 01-203

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Total inhibition	-
Escherichia coli ATCC 25922	Partial inibition	Green-Yellow colonies w. yellow halo
Salmonella typhimurium ATCC 14028	Productivity > 0.70	Pink to red colonies and red medium
Salmonella abony NCTC 6017	Productivity > 0.70	Pink to red colonies and red medium
Salmonella enteritidis ATCC 13076	Productivity > 0.70	Pink to red colonies and red medium

Salmonella typhimurium ATCC 14028

Salmonella abony NCTC 6017

Escherichia coli ATCC 25922

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Brilliant Green Bile 2% Broth

Art. No. 02-041

Also known as

BGBLB

Specification

Liquid medium used for the detection of coliforms in water, as recommended by APHA and ISO standards.

Formula* in g/L

Bile	20,000
Lactose	10,000
Peptone	10,000
Brilliant green	0,013

Final pH 7,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 40 g of powder in 1 L of distilled water and bring to the boil. Distribute into containers containing Durham tubes and sterilize in the autoclave at 115ºC for 15 minutes.

Description

Brilliant Green Bile 2% Broth has been widely used as a medium for the assay of presumptive coliforms in food, milk and water, using the Most Probable Number Technique. This broth offers some advantages over other similar broths as its balanced composition of Bile and Brilliant Green effectively suppresses the growth of Gram positive bacteria.

It is recommended by the APHA for colimetry of water, milk and food. British and Australian methodology use the broth as an intermediate stage between presumptive and confirmative colimetry. Other authors suggest it as an optimal base for the Eijkman testing of gas production at 44ºC, for the identification of *E. coli.*

This medium can be used as presumptive broth for *E. coli* (by fluorescent reaction) if prior to sterilization MUG (4-Methylumbelliferyl-β-D-Glucuronide) (Art. No. 06-102CASE or 06-102-LYO) is added.

References

- APHA (1971) Standard Methods for the Examination of Water and Wastewatter.13th ed. Washington.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4rd ed. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analitical Manual. 8th ed. Rev. A. AOAC Intl. Gaithersburg. MD. USA.
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- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- PASCUAL ANDERSON, MªRª (1992) Microbiología Alimentaria. Díaz de Santos. S.A. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Brilliant Green Bile 2% Broth

Art. No. 02-041

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Poor to good	Gas (-)
Salmonella typhimurium ATCC 14028	Good to very good	Gas (-)
Escherichia coli ATCC 8739	Good to very good	Gas (+)
Escherichia coli ATCC 25922	Good to very good	Gas (+)
Citrobacter freundii ATCC 43864	Good to very good	Gas (+)
Enterococcus faecalis ATCC 29212	Total to partial inhibition	Selectivity

Left: Uninoculated tube (Control)
Centre: *Salmonella typhimurium* ATCC 14028
Right: *Escherichia coli* ATCC 25922

" Detail"

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Brilliant Green Modified Agar (BGA Modified)

Art. No. 01-309

Specification

Solid culture medium for the selective isolation of *salmonellae* in food (except *S. typhi*) according ISO & IDF standards.

Formula* in g/L

Peptone	10,000
Meat extract	5,000
Yeast extract	3,000
Lactose	10,000
Sucrose	10,000
Disodium phosphate	1,000
Sodium phosphate	0,600
Phenol red	0,090
Brilliant green	0,005
Agar	15,000

Final pH 6,9 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 54,7 g of powder in 1 L of distilled water. Let it soak and heat to boiling point stirring constantly. Distribute in plates. Do not autoclave.

Description

In this modification of the classical medium for *Salmonella*, the concentration of brilliant green has been reduced to obtain a less inhibitory medium. At the same time, the nutrient basis has been enriched to enhance the recovery of those microorganisms that are stressed during the food production process.

This formulation was subsequently adopted by the ISO and DIN official method for detecting *Salmonella* in meat.

Technique

A prior enrichment in Tetrathionate Broth Base (Art. No. 02-033) is recommended. Inoculate on the surface of the plate medium in order to obtain individual colonies. Incubate at 35-37°C for 18-24 hours.

Salmonella colonies (except *S.typhi*) are red, pinkish or white, and they are always surrounded by a red halo or zone, which demostrates non-lactose or sucrose fermentation. Colonies of lactose and/or sucrose fermenting bacteria produce yellow-green colonies surrounded by a yellow halo. Sometimes, *Proteus or* Pseudomonas may appear, and they produce red pointed colonies.

In very polluted samples, the addition of 1 g/L of sodium sulfacetamide and 250 mg/L of sodium mandelate is recommended.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DIN 10160 Norme. Untersuchung von fleisch und fleischerzengnissen. Nachweis von Salmonellen. Referenzverfahren.
- DIN 10181 Norme. Mikrobiologische Milchuntersuchung Nachweis von Salmonellen. Referenzverfahren.
- FIL-IDF 93 Standard (2001) Milk and Milk products - Detection of *Salmonella spp*.
- ISO 6340 Standard (1995) Water Quality.Detection of *Salmonella*.
- ISO 6785 Standard (2001) Milk and Milk products.- Detection of *Salmonella spp* .
- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Brilliant Green Modified Agar (BGA Modified)

Art. No. 01-309

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: Pre-enrichment in Art. No. 02-602 Selenite Cystine Broth (according to standard ISO 6340)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Total inhibition	-
Escherichia coli ATCC 25922	Partial inhibition	Green colonies / Yellow medium
Salmonella enteritidis ATCC 13076	Good to very good	Pink-red colonies / Red medium
Salmonella abony NCTC 6017	Good to very good	Pink-red colonies / Red medium
Salmonella typhimurium ATCC 14028	Good to very good	Pink-red colonies / Red medium

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Buffered Peptone Water

Art. No. 02-277

Also known as

Tryptone Buffered Peptone

Specification

Dilution and non-selective pre-enrichment liquid medium according to ISO standards 6579, 6785, 6887 and 8261.

Formula* in g/L

Casein Peptone	10,00
Sodium chloride	5,00
Di-Sodium phosphate anhydrous	3,50 (*1)
Potassium phosphate	1,50

Final pH 7,0 ± 0,2 at 25ºC

(*1) Equivalent to 9,0 g of disodium hydrogen phosphate dodecahydrate.

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20 g of powder in 1 L of distilled water. Distribute into suitable containers and sterilize in the autoclave at 121ºC for 15 minutes.

Description

This formulation of Buffered Peptone Water has the advantages of the two classical diluents used for food samples: it has the property of revitalization of the peptone water and the pH change absorbing capacity of the phosphate buffer.

The composition of this diluent is made according to the specification of the ISO Standard 6579 for the detection of *Salmonella* in foods and other ISO Standards (6785, 6887 and 8261).

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. Londres.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 6579 (2002) Microbiology and animal feeding stuffs. Horizontal methods for the detection of *Salmonella spp.*
- ISO 6785 (2001) Milk and milk products. Detection of *Salmonella spp*.
- ISO 6887-1 (1999) Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
- ISO 6887-2 (2003) Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2: Specific rules for the preparation of meat and meat products.
- ISO 6887-3 (2003) Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 3: Specific rules for the preparation of fish and fishery products.
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- ISO/DIS 6887-5(2009) Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 5: Specific rules for the preparation of milk and milk products.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Buffered Peptone Water

Art. No. 02-277

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2) // Time: 0 and 45 minutes (20 - 25°C)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	Satisfactory recovery on TSA
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory recovery on TSA
Bacillus subtilis ATCC 6633	Good	Satisfactory recovery on TSA
Escherichia coli ATCC 8739	Good	Satisfactory recovery on TSA
Salmonella typhimurium ATCC 14028	Good	Satisfactory recovery on TSA
Candida albicans ATCC 10231	Good	Satisfactory recovery on SAB

Left: *Staphylococcus aureus* ATCC 25923
Centre: *Salmonella typhimurium* ATCC 14028
Right: ***Pseudomonas aeruginosa*** ATCC 9027

Buffered Peptone Water (Eur. Pharm.)

Art. No. 02-494

Also known as

Buffered Sodium Chloride Peptone Solution pH 7,0

Specification

Diluent for the Homogenization of samples for the microbiological examination according to the European Pharmacopeial Harmonised Method and ISO standard.

Formula* in g/L

Peptone	1,00
Sodium chloride	4,30
Disodium phosphate	7,23
Potassium phosphate	3,56

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 16,1 g of powder in 1 L of distilled water, heating if necessary. Add 1 to 10 mL of Polysorbate 80 (Art. No. TW0080) or Polysorbate 20 depending on the type of food or product to be diluted. Homogenize and distribute into containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This solution is recommended by the European Pharmacopoeia to dilute samples for microbiological examination. The quantity of emulsifying agent used will depend on the amount of fat in the sample being examined.

References

- COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- ISO 21149:2006 Cosmetics - Enumeration and detection of aerobic mesophilic bacteria.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2) // Time: 0 and 45 minutes (20 - 25°C)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Satisfactory
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory
Candida albicans ATCC 10231	Good	No significant reduccion
Escherichia coli ATCC 8739	Good	Satisfactory
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Bacillus subtillis ATCC 6633	Good	Satisfactory

Cary-Blair Transport Medium

Art. No. 03-643

Specification

Semisolid non-nutritive medium used for transporting and preserving microbiological specimens.

Formula* in g/L

Sodium chloride	5,00
Sodium thioglycollate	1,50
Disodium phosphate	1,10
Calcium chloride	0,09
Agar	5,60

Final pH 8,4 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 13,3 g of the powder in 1 L of distilled water and boil until is completely dissolved. Distribute into suitable containers and sterilize in flowing steam for 15 minutes. Store the prepared medium at room temperature. **Do not refrigerate.**

Description

Transport media are a chemically defined, semisolid, non-nutritive, buffered media that provide a reduced environment to maintain the viability of microorganisms without promoting growth.
Cary & Blair modified Stuart's Transport Medium for gonococci producing a formulation more adapted to the transport of faecal samples. The basic modification was the substitution of glycerophosphate for an inorganic phosphate that prevents bacterial overgrowth. Raising the pH to 8,4 and the suppressive effect of methylene blue favours the viability of *Shigella*, *Salmonella* and *Vibrio sps*.

Technique

Use sterile cotton tipped swabs on wooden sticks to collect the specimen, insert the swabs into the upper third of the medium in the transport container. Cut the protruding swab stick and tightly close the container. Label the container and send it to the laboratory with minimum delay. The culture and analysis of the sample must be performed within 24 hours of collection.

Precautions and Limitations of the Procedure

Optimal growth and typical morphology can only be expected following direct inoculation and appropriate cultivation.

- Prior to use, the medium should not be incubated to check the sterility.
- The sterility of the medium can be verified using sterile control samples (uninoculated swabs). This medium must not be employed subsequently.
- The medium can maintain the viability of several microorganisms for transport purposes only. It should not be used as a storage or enrichment medium.
- The results obtained from this medium are dependent on the quality of the specimen and on the time elapsed from collection until analysis in the laboratory. The viability of the cells will diminish over time and some overgrowth of accompanying microbiota can occur during prolonged periods of transit.
- Survival of bacteria in a transport medium depends up on the formulation and on many other factors including media type, the number of organisms in the specimen, the temperature and duration of transport. Inoculation of appropriate culture media should be carried out within 24 hours.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Ratón. Fla. USA.
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- CARY, S.G., M.S. MATTHEW, M.H. FUSILLO & C. HARKINS (1965) Survival of *Shigella* and *Salmonella* in a new transport medium. Am. J. Clin. Path. 43:294-296.
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- MCFADDIN, J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol 1. William& Wilkins. Baltimore. USA.
- MOFFET, M., J.L. YOUNG & R.D. STUART (1948) Centralized gonococcus culture for dispersed clinics; the value of a new transport medium for gonococci and trichomonas. Brit. Med. J. 2:421-424.
- STUART, R.D., S.R. TOSHACH & T.M. PATSULA (1954) The problem of transport of specimens for culture gonococci. Can. J. public Health 45:73-83.
- STUART, R.D. (1959) Transport medium for specimens in public health bacteriology. Public Health Reports 74:431-438.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Cary-Blair Transport Medium

Art. No. 03-643

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Inoculate sterile swabs with suspensions of McFarland 0,5 (20 - 25°C for 24 h)

Microorganism	Growth	Remarks
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Shigella sonnei ATCC 9290	Good	Satisfactory
Klebsiella pneumoniae ATCC 10031	Good	Satisfactory
Streptococcus pneumoniae ATCC 49619	Good	Satisfactory

Casein Lecithin Polysorbate Broth Base

Art. No. 02-539

Specification

Liquid medium used to dilute and neutralize samples of pharmaceutical, cosmetic, raw material or end products for the purpose of microbial enumeration.

Formula* in g/L

Casein peptone.....20,00
Soya lecithin.....5,00
Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 25 g of powder in 960 mL of distilled water pre-warmed to 50°C. Add 40 mL of Polysorbate 20, homogenize and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is produced according to the formulation of the U.S. Pharmacopoeia. In Section <61> "Microbial Limit Tests" it is an alternative system to neutralize preservatives and disinfectants before proceeding with the enumeration process, especially by the membrane filtration method.

References

- US PHARMACOPOEIA (2002) <61> Microbial Limits Tests. 25th ed. US Pharmacopeial Conv. Inc. Rockville. MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. (Productivity) at 0, 45 minutes and 3 h. (20 - 25°C)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Satisfactory
Enterococcus faecalis ATCC 19433	Good	Satisfactory
Bacillus subtilis ATCC 6633	Good	Satisfactory
Escherichia coli ATCC 8739	Good	Satisfactory
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Candida albicans ATCC 10231	Good	No significant reduction

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Cetrimide Agar (Pseudomonas Selective Agar) (Eur. Pharm.)

Art. No. 01-160

Also known as

Pseudosel; Pseudomonas Selective Medium; Pseudomonas Selective Agar Base

Specification

Solid culture medium for selective isolation of *Pseudomonas aeruginosa* according to the Pharmacopeial Harmonised Method and the ISO 22717 standard.

Formula* in g/L

Gelatin peptone	20,00
Magnesium chloride	1,40
Potassium sulfate	10,00
Cetyltrimethyl-ammonium bromide	0,30
Agar	13,60

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 45,3 g of powder in 1 L of distilled water and add 10 mL of glycerol. Bring to the boil and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

The Cetrimide Agar is based on the resistance of *P. aeruginosa* strains to Quaternary Ammonium Compounds (QAC's). With Cetyltrimethyl-Ammonium Bromide a growth at concentrations of 1g/L has been archieved, but has been very poor and slow.

An inhibitor concentration of 0,3-0,5 g/L does not seem to affect the viability of pyogenic species. But it does inhibit the accompanying bacteria, both Gram positive and Gram negative organisms. Other species of Pseudomonas which may develop at lower inhibitory concentrations are also inhibited.

Although *P. aeruginosa* prevails over any other fastidious bacteria after a 48 hour incubation at 35°C, an initial incubation at 42°C for 48 hours followed by an incubation at 35°C for 48 hours is recommended. Using this method almost complete inhibition of other microorganisms is obtained.

References

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Boca Raton,Fla.
- BROWN, V.I. & J.L. LOWBURY (1965) Use of an improved Cetrimide Agar Medium and of culture methods for *Pseudomonas aeruginosa*. J. Clin. Path. 18.752.
- COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Rev. A. AOAC International. Gaitherburg. VA.
- ISO 22717:2006 Standard. Cosmetics - Detection of *Pseudomonas aeruginosa.*
- LOWBURY,E.J.L. & A.G. COLLINS (1955) The use of a new cetrimide product in a selective medium for *Pseudomonas aeruginosa* J. Clin. Path. 8.47.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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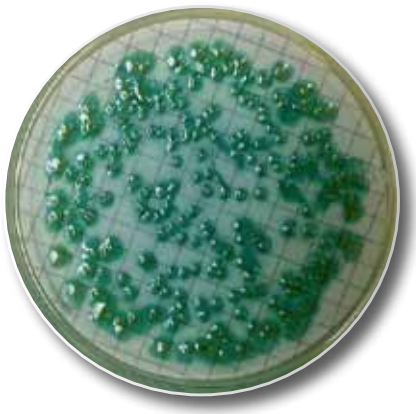
Cetrimide Agar (Pseudomonas Selective Agar) (Eur. Pharm.)

Art. No. 01-160

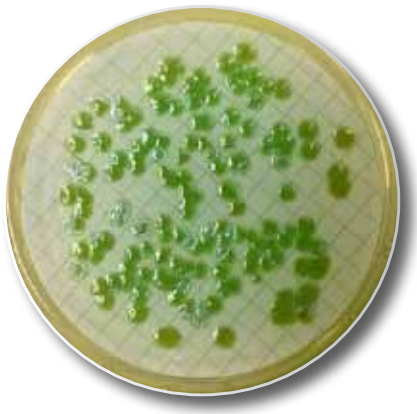
Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method

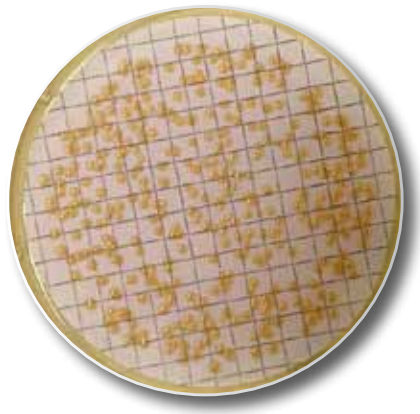
Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Inhibited	Selectivity
Staphylococcus aureus ATCC 6538	Inhibited	Selectivity
Pseudomonas aeruginosa ATCC 9027	Productivity > 0.50	Green-Yellowish to dark green
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.50	Green-Yellowish to dark green
Pseudomonas aeruginosa ATCC 15442	Productivity > 0.50	Green-Yellowish to green



Pseudomonas aeruginosa ATCC 15442



Pseudomonas aeruginosa ATCC 9027



Pseudomonas aeruginosa ATCC 27853

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Chapman TTC Agar (Tergitol® 7 Agar)

Art. No. 01-053

Also known as

T7 Agar

Specification

Medium for the detection of coliforms by membrane filtration in water analyses according to ISO 9308-1:2000 standard.

Formula* in g/L

Meat peptone	10,00
Meat extract	5,00
Lactose	20,00
Yeast extract	6,00
Bromothymol blue	0,05
Sodium heptadecyl sulfate	0,10
Agar	15,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 56,2 g in 1 L of distilled water and bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C. Add 2-3 mL/L of filtered sterile 1% aqueous 2, 3, 5-triphenyltetrazolium chloride (TTC) (Art. No. 06-023) and pour plates. **Do not reheat.**

Description

This medium is formulated for the presumptive identification of coliforms in drinking water, by membrane filtration according to ISO 9308-1:2000.
Due to the instability of the triphenyltetrazolium it is provided in a separate container, sterilized and ready to use (Art. No. 06-023).
Poured plates can be stored refrigerated for up to 8 days without losing their effectiveness. They should not be used if any signs of dehydration or drying appear.

Technique

While using the membrane filter technique for the presumptive identification of coliforms in water, it should be kept in mind that the minimum volume to be filtered depends on the type of water being tested. If necessary dilute with sterile phosphate buffer in order to obtain the number of colonies on the membrane appropriate for counting.
For every water sample two volumes must be filtered over two different membranes and incubated on Chapman TTC Agar at 35°C and 44°C respectively.

After 48 hours typical colonies have the appearance as follow:

- *Escherichia coli, Citrobacter spp.*: Yellow with a centred orange nucleus under the membrane filter (MF).
- *Klebsiella spp.*: Brick red or yellow without a nucleus. The medium under the (MF) is yellow.
- *Enterobacter spp.*: Dark yellow or brick red with an orange nucleus. The medium is also yellow.
- Non lactose-fermenters: Violet or indigo colonies. The medium turns blue.

Most coliforms can not grow on this medium when incubated at 44°C, except *E. coli* which forms a colony with a characteristic appearance.
Results are always expressed per 100 mL sample including any applied dilutions. Estimation is done by taking typical colonies which have grown at 35°C as faecal coliforms, together with those grown at 44°C as *E. coli.*
Nevertheless, according to legislation and despite the medium's selectivity, results can only be considered as presumptive and all coliform colonies have to be confirmed by following the criteria below:
Typical appearance in EMB Agar (Art. No. 01-068) or Endo Agar Base (Art. No. 01-589) and characteristic reactions in Kligler Iron Agar (Art. No. 01-103).
For the confirmation of faecal *E. coli*, the following characteristics are used for verification: a motile, Gram negative bacillus and lactose fermenter with acid and gas production, which gives negative results on the citrate test and indol production positive.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- CHAPMAN G.H. (1951) A culture medium for detecting and confirming *E. coli* in ten hours. Am. J. Publ. Hlth 41:1381-1386.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. APHA.Washington.
- GUINEA, SANCHO,PARES (1979) Análisis Microbiológico de Aguas. Ed. Omega. Barcelona.
- ISO 9308-1:2000 Standard. Water Quality - Detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method.
- SPECK, M (Ed.) (1982) Compendium of Methods for the Microbiological Examination of Foods. 2nd ed. APHA.Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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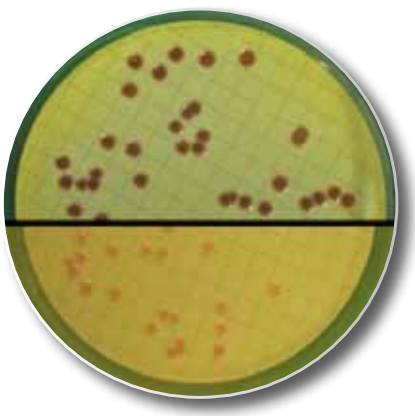
Chapman TTC Agar (Tergitol® 7 Agar)

Art. No. 01-053

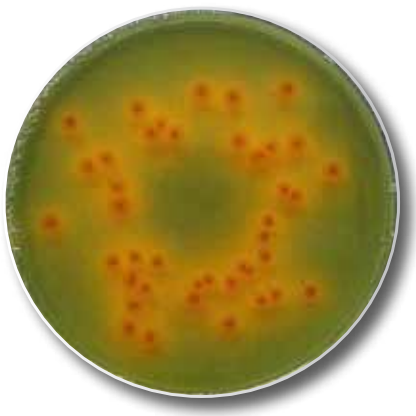
Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Partial to total inhibition	Selectivity
Escherichia coli ATCC 8739	Productivity > 0.70	Yellow-orange colonies under the MF
Escherichia coli ATCC 11775	Productivity > 0.70	Yellow-orange colonies under the MF
Escherichia coli ATCC 25922	Productivity > 0.70	Yellow-orange colonies under the MF
Salmonella abony NCTC 6017	Productivity > 0.70	Violet or dark red colonies under the MF
Salmonella typhimurium ATCC 14028	Productivity > 0.70	Violet or dark red colonies under the MF
Staphylococcus aureus ATCC 25923	Total inhibition	Selectivity



Salmonella typhimurium ATCC 14028
on the membrane filter
Escherichia coli ATCC 8739



Escherichia coli ATCC 25922

Salmonella typhimurium ATCC 14028

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Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base

Art. No. 01-685

Also known as

mCCDA

Specification

Selective plating medium used for the detection and enumeration of *Campylobacter spp* according to the ISO 10272-1:2006 standard.

Formula* in g/L

Meat extract	10,00
Peptone	10,00
Sodium chloride	5,00
Bacteriological charcoal	4,00
Casein hydrolysate	3,00
Sodium Deoxycholate	1,00
Iron (II) sulfate	0,25
Sodium pyruvate	0,25
Agar	15,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 24,2 g of powder in 500 ml of distilled water and bring to the boil to dissolve. Sterilize in an autoclave at 121°C for 15 minutes. Cool to 47-50°C and aseptically add one vial of the *Campylobacter* CCDA Selective Supplement (Art. No. 06-133-LYO). Mix carefully and pour into sterile Petri dishes.

Note: If the plates are prepared in advance, they should be kept for not more than 4 hours at ambient temperature or for no more than 7 days in the dark at 3 ± 2°C.

Description

CCD Modified Agar is formulated according to the ISO Standard 10272-1:2006 and is intended to detect and enumerate *Campylobacter spp* from food and animal feeding stuffs.

After determining that *campylobacter* species grow best on solidified Nutrient Broth No. 2 compared to other media workers (1983) carried out a systematic survey of alternatives to blood for neutralizing oxygen toxicity. A combination of 0,4% charcoal, 0,25% ferrous sulfate and 0,25% sodium pyruvate proved best.

A further study surveyed the suppressive effect of several inhibitors on the undesirable microbiota showing deoxycholate and cefazolin as the most effective inhibitory agents. Later, in 1984, Hutchinson and Bolton replaced cefazolin (10 mg/L) with cefoperazone (32 mg/L). This allowed fewer contaminants to grow, and permitted the modified medium (modified CCD Agar or mCCDA) to be used at 37°C. However amphotericin B was needed to prevent overgrowth by yeast able to grow at 37°C but not at 42°C.

In 1993 Aspinall *et al.* developed a modification of mCCDA designed for use at 37°C to isolate *C. upsaliensis* as well as the other thermophilic *campylobacter* species. This medium contains 8 mg/L cefoperazone and 4 mg/L teicoplanin replacing 32 mg/L cefoperazone in mCCDA. Teicoplanin has an antimicrobial spectrum similar to that of vancomycin, active mainly against gram-positive bacteria. By comparison with mCCDA the final formulation of this medium, called CAT Agar, isolated the same numbers of *Campylobacter spp* other than *C. upsaliensis* from faeces and is superior to mCCDA for *C. upsaliensis* with slightly higher growth of competing microflora.

Technique

Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet, until the agar surface is free of visible moisture (maximum 30 minutes).

Using the culture obtained from enrichment broth (Bolton Broth, Art. No. 02-688), inoculate the mCCDA with a sterile loop. Incubate plates at 41,5°C in a microaerobic atmosphere (approximately 5% O_2, 10% CO_2 and 85% N_2 or H_2), for 44 ± 4 hours.

- *Campylobacter jejuni* strains produce grey, moist flat and occasionally spreading growth which may be accompanied with a green hue and/or a metallic sheen.
- *Campylobacter coli* strains tend to be creamy-grey in colour, moist and often produce a more discrete type of colony.
- *Campylobacter lari* strains are more varied and produce both types of colonial morphology
- Occasionally contaminating organisms may grow on this medium. These include cefoperazone-resistant Pseudomonas *spp* Enterobacteriaceae, and some streptococci and yeasts.

Necessary supplements

Campylobacter CCDA Selective Suplement (Art. No. 06-133-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Amphotericin B	5,00 mg
Cefoperazone	16,00 mg

Distilled water (Solvent)

References

- ASPINALL, S.T., D.R.A. WAREING, P.G. HAYWARD & D.N. HUTCHINSON (1993) Selective medium for thermophilic *campylobacters* including *Campylobacter upsaliensis*. J. Clin. Pathol. 46:829-831
- BAYLIS, C.L., (editor) (2007) Manual of Microbiological Methods for the Food and Drinks Industry. 5th Edition Method 3.3.1:2007. CCFRA . Chipping Campden. U.K.

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Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base

Art. No. 01-685

- BOLTON, F.J. (2000) Methods for isolation of *campylobacters* from humans, animals, food and water. In "The increasing incidence of human campylobacteriosis" Report and Proceedings of a WHO Consultation of Experts. Copenhagen Denmark 21-25 November 2000, WHO/CDS/CSRAPH 2001. p. 87-93
- BOLTON, F.J., D. COATES, (1983) Development of a blood-free *campylobacter* medium: screening tests on basal media and supplements, and the ability of selected supplements to facilitate aerotolerance. J. Appl. Bacteriol. 54:115-125
- BOLTON, F.J., D. COATES & D.N. HUTCHINSON (1984) The ability of *Campylobacter* media supplements to neutralize photochemically induced toxicity and hydrogen peroxide. J. Appl. Bacteriol. 56:151-157.
- CORRY, J.E.L., H. IBRAHIM ATABAY, S.J. FORSYTHE & L.P. MANSFIELD (2003) Culture Media for the isolation of *campylobacters*, helicobacters and arcobacters. In Handbook of Culture Media for Food Microbiologists. J.E.L. Corry *et al* (Eds.) Elsevier Science B.V. Amsterdam.
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- HUNT, J.M., C. ABEYTA & T. TRAN (1998) *Campylobacter.* In FDA BAM 8th Edition (revision A) 7.01-7.027 AOAC International. Gaithersburg, Md, USA.
- HUTCHINSON, D.N. & F.J. BOLTON (1984) Improved blood-free selective medium for the isolation of *Campylobacter jejuni* from faecal specimens. J. Clin Pathol. 37:956-957.
- ISO 10272-1 Standard (2006) Microbiology of food and animal feeding stuffs - Horizontal Method for detection and enumeration of *Campylobacter spp.* - Part 1: Detection method.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- STERN, N.J., J.E. LINE & H.C. CHEN (2001) *Campylobacter* In "Compendium of methods for the Microbiological Examination of Foods" 4th Ed. F.P. Downes & K. Ito (Eds.) APHA, Washington DC. USA

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 42°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 100-1000 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 1113-1/2)

Microorganism	Growth	Remarks
Campylobacter jejuni ATCC 29428	Productivity > 0.70	Under microaerophilic atmosphere
Escherichia coli ATCC 25922	Inhibited	Under microaerophilic atmosphere

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Chloramphenicol Glucose Agar (CGA)

Art. No. 01-366

DANGER
H: 3.6/1A; H350
P: P281-P201-P202-P308+P313-P405-P501a

Also known as

Yeast Extract-Glucose-Chloramphenicol Agar; YGC Agar; Yeast Extract-Dextrose-Chloramphenicol Agar; YDC Agar.

Specification

Solid and selective medium for the isolation and enumeration of fungi in milk and dairy products according to ISO 7954 and FIL-IDF 94B standards.

Formula* in g/L

Dextrose	20,00
Yeast extract	5,00
Chloramphenicol	0,10
Agar	15,00

Final pH 6,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 40 g of powder in 1 L of distilled water and let it soak. Bring to the boil and distribute into containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is recommended by the Federation International Laitière-International Dairy Federation (FIL-IDF) for the isolation and enumeration of fungi (moulds and yeast) in milk and dairy products. This medium has also been adopted by the DIN and ISO standards.

This medium's selectivity is due to the bactericidal action of chloramphenicol which, due to its thermostable it, may be sterilized with the medium in the autoclave. Also due to the pH being neutral, the medium is able to be re-melted several times without affecting its stability, selectivity and efficacy. Re-melting and overheating may make the medium darker.

Technique

Generally a stab inoculation method or pour plate method is used to inoculate the medium. Incubation is at 22-25°C for 4 to 5 days.

References

- DIN Standard 10186. Mikrobiologische Milch Untersuchung. Bestimmung der Anzahl von Hefen und Schimmelpilzen. Referenzverfahren.
- FIL-IDF 94B Standard (1991) Enumeration of yeast and moulds. Colony Count Technique at 25°C.
- ISO 7954 Standard (1987) General guidance for enumeration of yeast and moulds - Colony count at 25°C.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 25°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus cereus var. mycoides ATCC 11778	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	Black sporulation at 5 days
Candida albicans ATCC 10231	Productivity > 0.70	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-

Candida albicans ATCC 10231

Christensen's Citrate Agar

Art. No. 01-664

Specification

Differential solid medium to verify the use of citrate as a sole carbon source in the presence of organic nitrogen, according to the ISO 21567:2004 standard.

Formula* in g/L

Sodium chloride	5,000
Sodium citrate	3,000
Potassium phosphate	1,000
Yeast extract	0,500
Ferric ammonium citrate	0,400
Dextrose	0,200
Cysteine HCl	0,100
Potassium thiosulfate	0,080
Phenol red	0,012
Agar	15,000

Final pH 6,9 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 25,3 g of the powder in 1 L of distilled water and bring to the boil. Dispense suitable volumes into tubes and sterilize in the autoclave at 121°C for 15 min. Solidify in a slanted position to obtain a slope of 4-5 cm and a butt of 2-3 cm.

Description

This medium was described in 1949 by Christensen to verify citrate utilization and H_2S production to differentiate coliforms and enteric pathogens. In 1955 Edwards and Ewing recommended the medium to test the use of citrate as sole carbon source in the presence of organic nitrogen among the Enterobacteriaceae.

Costin, in 1965 eliminated thiosulfate and ferric ammonium citrate in the formulation because the H_2S production was not in accordance with other culture media.

The sole carbon source is sodium citrate because dextrose is present in too low a concentration. Cysteine and yeast extracts supply growth factors. Sodium chloride and potassium phosphate maintains osmotic pressure and pH. Ferric ammonium citrate and thiosulfate are the substrates that demonstrate H_2S production and phenol red is an indicator of pH that turns from light yellow to red when the medium becomes alkalines.

Technique

If only utilization of citrate is being tested, according to the ISO 21567:2004 standard, the slope surface must be inoculated with a very light inoculum from a pure culture. Care must be taken to minimize the quantity of the medium transferred with the inoculum.

If the production of H_2S is being tested then the butt should be stab inoculated.

The tubes are incubated in aerobic conditions at 37 ± 1°C for 24-48 hours. Growth can be detected by the change in colour of the medium to red due to the production of alkaline substances. H_2S production is detected by a blackening in the butt around the growth.

If there is no growth in the first two days, incubate the tubes for another 2 days and examine again. *Shigella* does not grow in this medium.

Limitations of the procedure

- An excess of inoculum can give false positive results.
- The transfer of medium with the inoculum can produce ambiguous results. Some authors recommend dilution or washing of the inoculum with saline solution to minimize the supply of carbon source other than citrate.
- Some citrate-positive microorganisms grow slowly and may require longer incubations of 4 or more days to turn the phenol red.
- Some results can be ambiguous (e.g. *Providencia*) and in these cases re-inoculating a second tube of citrate incubating at room temperature for a week is recommended.

References

- ATLAS, R.M. (1995) Handbook of microbiological media for the examination of food. CRC Press. Boca Raton. Fla. USA.
- CHRISTENSEN, W.B. (1949) Hydrogen sulfide production and citrate utilization in the differentiation of enteric pathogens and coliform bacteria. Res. Bull. Weld County Health Dept. Greeley. CO. 1, 3.
- COSTIN, I.D. (1965) Bemerkungen zur praxis der biochemischen identifizierung der darmbakterien in der routinearbeit. Zentralb. Bakteriol. I Abt. 198(5), 385.
- EDWARDS, P.R. & W.H. EWING (1955) Identification of Enterobacteriaceae. Burgess Pub. Co. Minneapolis.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological analytical manual. 8th ed, revision A. AOAC Intl. Gaithersburg. Va. USA.
- ISO 21567 (2004) Standard. Microbiology of food and animal feeding stuffs - Horizontal detection of *Shigella spp.*
- MaCFADDIN, J.F (1985) Media for the isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins. Baltimore. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Christensen's Citrate Agar

Art. No. 01-664

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: Pure culture is inoculated by surface streaking

Microorganism	Growth	Remarks
Salmonella typhimurium ATCC 14028	Good - very good	Blue medium. H_2S +
Pseudomonas aeruginosa ATCC 27853	Good - very good	Blue medium
Escherichia coli ATCC 25922	Inhibited	-
Escherichia coli ATCC 8739	Inhibited	-
Shigella flexneri ATCC 12022	Inhibited	-

Chromogenic Coliform Agar Base

Art. No. 01-695

Also known as

CCA; ACC

Specification

Solid, selective and differential culture medium for the detection and enumeration of total coliform and *E. coli* in water samples by the membrane-filtration technique.

Formula* in g/L

Peptone	3,00
Sodium chloride	5,00
Disodium phosphate	2,70
Monosodium phosphate	2,20
Tryptophan	1,00
Sodium pyruvate	1,00
Tergitol® 7	0,15
Sorbitol	1,00
Salmon®-Gal	0,20
X-Glucuronide	0,20
Agar	13,00

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 29,45 g of powder in 1 L of distilled water and bring to the boil until fully dissolved. **Do not autoclave nor overheat**. Cool until to 45-50°C and add the contents of one vial of CV Selective Supplement for Coliforms (Art. No. 06-140-LYO). Mix well and distribute into Petri dishes avoiding bubble formation. The finished plates remain effectives for at least one month if stored in the dark between 2-8°C.

Description

The combined action of peptone, pyruvate and sorbitol allow rapid colony growth in this phosphate buffered medium, which also permits simple recovery of sublethal thermally injured coliforms. Sodium chloride provides the correct osmotic environment necessary for growth.

The selectivity is attained, partially, by the Tergitol® 7, which inhibits the growth of gram positive bacteria and some gram negative without effecting the coliform bacteria. Selectivity is enhanced by the cefsulodin and Vancomycin that which acts against pseudomonads and Gram negative oxidase positive bacteria enterococci and other gram positive bacteria.

The colonial differentiation is due to the chromogenic mixture, composed of two enzyme substrates: 6-chloro-3-indoxyl-β-D-galacto-pyranoside (Salmon®- GAL) and 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (X - Glucuronide).

The first one is cleaved by the characteristic enzyme found in coliforms, β-D-galactosidase and gives a salmon-red colour to the coliform colonies. The second chromogenic substance is cleaved by the β-D-glucuronidase enzyme characteristic of *E. coli* and turns the colonies of these bacteria a blue colour.

E. coli has the two enzymes and cleaves both chromogenic substances giving dark blue to violet colonies. Total coliforms are the sum of *E. coli* colonies plus salmon-red colonies.

Other gram negative bacteria produce colourless colonies except some that possess glucuronidase activity (but not galactosidase) and they produce light blue to turquoise colonies.

To confirm the *E. coli* colonies in this medium a small amount of tryptophane is included verifying indol production: coat the blue-violet colonies with a drop of Kovacs Reagent (Art. No. RE0007). If the reagent turns a cherry-red colour in a few seconds this confirms the production of indol and hence the presence of *E. coli.*

When the Chromogenic Agar for Coliform is used with the membrane filter method, the colour and growth of the colonies can be modified by the characteristics of the membrane filter. It is advisable to perform validation of the membrane filter type used.

The Spanish Health Ministry (Ministerio de Sanidad y Consumo) has officially adopted this medium as an alternative methodology for the microbiological analysis of water for human consumption, giving a new definition for *Escherichia coli* ("Enterobacteriaceae that express the β-D-galactosidase and the β-D-glucuronidase enzymes simultaneously") and coliform bacteria: "Enterobacteriaceae that express the β-D-galactosidase enzyme".

Technique

The water sample is filtered trough a membrane filter of 0,45 µm of pore diameter validated according to the ISO Standard 7704:1985. The membrane is then placed on the surface of the CCA medium avoiding entrapment of air bubbles between the membrane and agar surface.

The petri dish with the membrane is incubated for 18-24 h at 36 ± 2°C. If in 18 h there is growth of red or colourless colonies, extend the incubation until 24 h to include late reactions of β-galactosidase or β-glucuronidase.

Count β-galactosidase positive colonies and β-glucuronidase negative colonies (all colonies coloured from salmon-rose to red) as Coliform bacteria not-*E. coli*.

Count β-galactosidase positive colonies and β-glucuronidase positive colonies (all colonies coloured from deep blue to violet) as *E. coli*.

Total Coliform count is obtained by the addition of the salmon-rose to red colonies plus the deep blue to violet colonies.

Calculate the concentration of Coliform bacteria and *E. coli* in 100 mL from the initial volume of water filtered and the number of characteristic colonies counted on the membrane. The results are expressed as Colony Forming Units per millilitre (CFU/mL).

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Chromogenic Coliforms Agar Base

Art. No. 01-695

Necessary supplements

Coliform CV Selective Supplement for (Art. No. 06-140-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Cefsulodin.....2,50 mg

Vancomycin.....2,50 mg

Distilled water (Solvent)

References

- ADAMS, M.., R.GRUBB, S.M. HAMER & A. CLIFFORD (1990) Colorimetric enumeration of *Escherichia coli* based on β-glucuronidase activity. Appl. Environ. Microbiol. 56:2021.
- ISO 7704 Standard (1985) Water Quality - Evaluation of membrane filters used for micropbiological analyses.
- KILIAN, M. & P. BÜLOW (1976) Rapid Diagnostic of Enterobacteriaceae. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B 84:245-251.
- MANAFI, M & W. KNEIFEL (1989) A combined chromogenic-fluorogenic medium for the simultaneous detection of total coliform and *E. coli* in water. Zentralbl. Hyg. 189:225-234.
- MINISTERIO DE SANIDAD Y CONSUMO (2009) Orden SCO/778/2009 de 17 de marzo sobre métodos alternativos para el análisis microbiológico del agua de consumo humano. BOE. n.º 78 de 31-04-2009. Sección I, Págs. 30417-30420. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

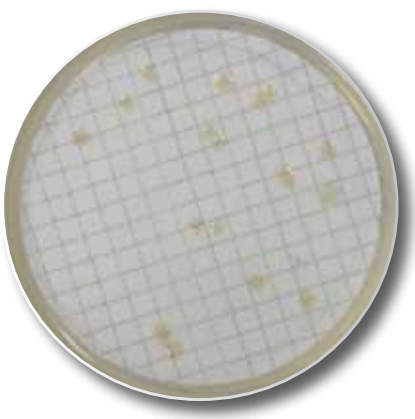
Quality control

Incubation temperature: 36°C ± 2,0

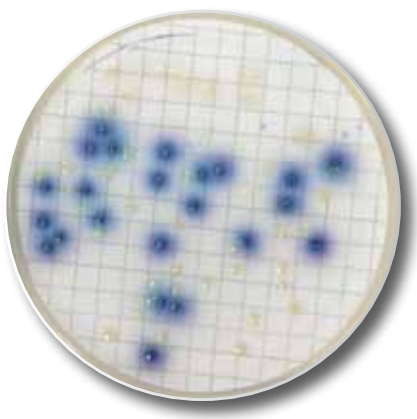
Incubation time: 18 - 24 h

Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method.

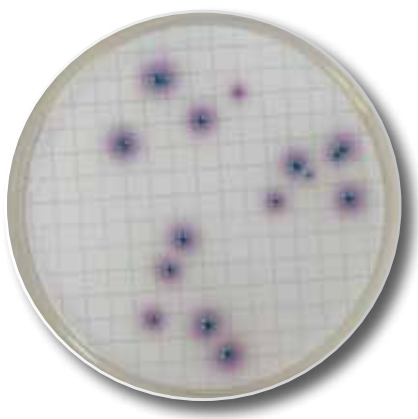
Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Inhibited	-
Escherichia coli ATCC 25922	Productivity > 0.70	Blue-violet colonies. Indol (+)
Escherichia coli ATCC 11775	Productivity > 0.70	Blue-violet colonies. Indol (+)
Salmonella enterica ATCC 13076	Productivity > 0.70	Colourless colonies. Indol (-)
Salmonella typhimurium ATCC 14028	Productivity > 0.70	Colourless colonies. Indol (-)
C.freundii ATCC 8090	Productivity > 0.70	Salmon to red colonies. Indol (-)
Enterococcus faecalis ATCC 29212	Inhibited	-



Salmonella typhimurium ATCC 14028



Salmonella typhimurium ATCC 14028
Escherichia coli ATCC 11775



Escherichia coli ATCC 25922

Chromogenic Colinstant Agar

Art. No. 01-618

Specification

Solid selective and differential medium for the detection of total coliforms and *E. coli* in water and food samples.

Formula* in g/L

Tryptone	10,00
Yeast extract	3,00
Meat extract	5,00
Bile salts No. 3	1,50
Di-sodium phosphate	2,70
Sodium phosphate	2,20
Chromogenic mixture	0,40
Agar	13,00

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 37,8 g of powder in 1 L of distilled water. Bring to the boil and distribute into suitable containers.
Sterilize in the autoclave at 121°C for 15 minutes. If the medium is used on the same day of preparation sterilization can be omitted, but the boiling must be maintained for 2-3 minutes.
Note: This is a low selectivity medium. If high numbers of accompanying microbiota, especially Pseudomonas and Aeromonas are expected, the selectivity of the medium can be improved by the addition of the contents of one vial of Coliform CV Selective Supplement (Art. No. 06-140-LYO) to 500mL of the medium cooled at 45-50°C.

Description

The selectivity of Colinstant Chromogenic Agar is due to the surfactant action of bile salts No. 3 that inhibits the growth of almost all Gram positive bacteria. Other inhibitory agents must be added if a greater selectivity is desireds.
The chromogenic mixture is mainly composed of two substances: 6-chloro-3-indolyl-β-D-galacto-pyranoside and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. The first is cleaved by the characteristic enzyme of coliforms, β-D-galactosidase and gives a salmon to red colour to the coliform colonies. The second chromogenic substance is cleaved by the β-Dglucuronidase enzyme characteristic of *E. coli* and turns the colonies a blue colour.
E. coli has both enzymes and cleaves both chromogenic substances which produces a dark blue to violet colonies. Total coliforms are the sum of *E. coli* colonies plus the salmon-red colonies. Other Gram negative bacteria produce colourless colonies except for some that possess glucuronidase activity (but not galactosidase) and produces light blue to turquoise colonies.

To confirm the *E. coli* colonies it is recommended to verify by the production of indol: coat the blue-violet colonies with a drop of Kovacs' Reagent (Art. No. RE0007). If the reagent turns a cherry-red colour in a few seconds this is a positive production of indol which confirms the presence of *E. coli*.
If the Colinstant Chromogenic Agar is used with the membrane filter method, the colour and growth of the colonies may be effected. It is advisable to perform validation of the membrane filter used.

Necessary supplements

Coliform CV Selective Supplement for (Art. No. 06-140-LYO)
Vial Contents:
Necessary amount for 500 mL of complete medium.

Cefsulodin	2,50 mg
Vancomycin	2,50 mg

Distilled water (Solvent)

References

- ADAMS, M., R.GRUBB, S.M. HAMER & A. CLIFFORD (1990) Colorimetric enumeration of *Escherichia coli* based on -glucuronidase activity. Appl. Environ. Microbiol. 56:2021.
- KILIAN, M. & P. BÜLOW (1976) Rapid Diagnostic of Enterobacteriaceae. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B 84:245:251.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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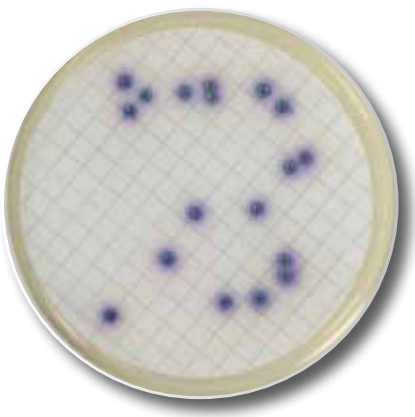
Chromogenic Colinstant Agar

Art. No. 01-618

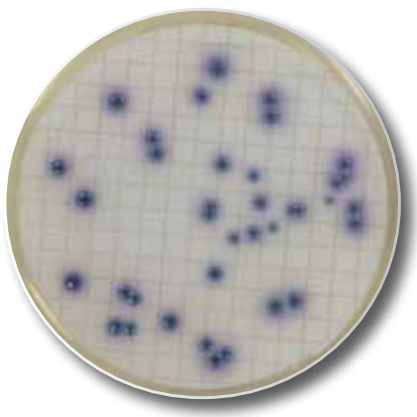
Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method or Spiral Plate Method.

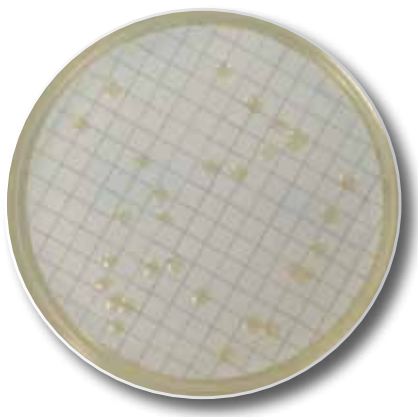
Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Productivity > 0.70	Colonies: Blue-violet
Escherichia coli ATCC 11775	Productivity > 0.70	Colonies: Blue-violet
Salmonella enterica ATCC 13076	Productivity > 0.70	Colonies: Colourless
Salmonella typhimurium ATCC 14028	Productivity > 0.70	Colonies: Colourless
C.freundii ATCC 8090	Productivity > 0.70	Colonies: Salmon to red
Enterococcus faecalis ATCC 29212	Inhibited	Selectivity



Escherichia coli ATCC 25922



Escherichia coli ATCC 11775



Salmonella typhimurium ATCC 14028

C

CLED Agar

Art. No. 01-047

CE IVD

Also known as

Cystine Lactose Electrolyte Deficient Agar; Brolacin Agar

Specification

Cystine, lactose, electrolyte deficient medium, recommended for the isolation and identification of urinary pathogenic bacteria.

Formula* in g/L

Peptone	4,000
Trypsic peptone	4,000
Meat extract	3,000
Lactose	10,000
L-Cystine	0,128
Bromothymol blue	0,020
Agar	15,000

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 36 g of powder to 1 L of distilled water and bring to the boil. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This general purpose medium has been recommended for bacteriological analysis. The current formulation is a modification of the original reported by Sandys, that achieves excellent colony differentiation without inhibitors. This fact, and also the careful selection of nutritive components, makes this medium a substrate able to support growth of most urinary pathogenic bacteria.

Presence of lactose as a fermentable sugar allows classic differentiation and, at the same time, lack of electrolytes suppresses swarming waves by members of the *Proteus* and occasionally *Shigella* species.

Typical colony characteristics after 18 hours of incubation:

- *Escherichia coli*: Yellowish colonies, opaque, with a core, 1,25 mm in diameter. Non fermentative strains give rise to blue colonies.
- *Klebsiella spp.*: mucoid colonies of variable colour, from yellow to blue-white.
- *Salmonella spp.*: Plain and blue colonies.
- *Enterococcus faecalis*: Yellow colonies. 0,5 mm diameter.
- *Staphylococcus aureus*: Convex yellow colonies. 0,75 mm diameter.
- Coagulase negative staphylococci: White or light yellow colonies, with haloes and the same size as those of enterococci.
- *Proteus spp.*: Blue, translucent and smaller than *E.coli*.
- *Pseudomonas aeruginosa*: Plain, matt and wrinkled colonies with green colour and irregular border.
- *Corynebacteria*: Pointed and grey colonies.
- *Lactobacilli*: Matt colonies, similar to corynebacteria.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BARON, E.J., L.R. PETERSON & S.M. FINEGOLD (1994) Bailey & Scott's Diagnostic Microbiology. 9th ed. Mosby-Year Book Inc. St Lous. MO. USA.
- ISENBERG, H.D. (1992) Clinical Microbiology Procedures Handbook. ASM Washington. DC. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MACKEY, J.P. & G.H. SANDYS (1966) Diagnosis of urinary tract infections. Brit. Med. J. 3:1.173.
- MURRAY, P.R., E.J. BARON, M.A. PFALLER, F.C. TENOVER & R.H. YOLKEN (1995) Manual of Clinical Microbiology 6th ed. ASM Washington. DC. USA.
- SANDYS, G H. (1960) A new method of preventive swarming of *Proteus sp.* J. Med. Lab. Tech. 17:224.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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CLED Agar

Art. No. 01-047

C

CE IVD

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Productivity > 0.70	Opaque yellowish colonies
Salmonella typhimurium ATCC 14028	Productivity > 0.70	Blue colonies
Staphylococcus aureus ATCC 25923	Productivity > 0.70	Opaque yellowish colonies
Proteus vulgaris ATCC 6380	Productivity > 0.70	Blue colonies without swarming waves
Proteus mirabilis ATCC 12453	Productivity > 0.70	Blue colonies without swarming waves
Proteus mirabilis ATCC 43071	Productivity > 0.70	Blue colonies without swarming waves
Proteus mirabilis ATCC 29906	Productivity > 0.70	Blue colonies with moderate swarming

Escherichia coli ATCC 25922

Proteus mirabilis ATCC 12453

Proteus mirabilis ATCC 43071

Clostridium perfringens, Selective Agar (SPS Agar)

Art. No. 01-050

Also known as

Sulfite Polymyxin Sulfadiazine Agar; Perfringens Selective Agar

Specification

Solid medium for the detection of *Clostridium perfringens* in food.

Formula* in g/L

Sodium sulfite	0,50
Polymyxin (B) sulfate	0,01
Sodium sulfadiazine	0,12
Casein peptone	15,00
Yeast extract	10,00
Ferric citrate	0,50
Sodium thioglycolate	0,10
Polysorbate 80	0,05
Agar	15,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 41,3 g of powder in 1 L of distilled water and bring to the boil. Distribute into tubes or screw-cap containers and sterilize in the autoclave at 121°C for 15 minutes. Cool the sterilized medium quickly by placing it in the refrigerator or in cold water.

Description

SPS Agar (Sulfite Polymyxin Sulfadiazine) is a modification of the original Wilson & Blair medium for the detection of clostridia. The present medium betters the formulation of Mossel and also the later modification of Angelotti et al. It achieves a higher selectivity for *C. perfringens* with the addition of Sulfadiazine and Polymyxin.

The nutritional substrates constituted by the tryptone and the yeast extract are complemented by the polysorbate, which also allows the recovery of the most delicate cells. The anaerobic conditions are improved by the thioglycolate, which permits the use of the medium on the plates without the Miller-Prichett tubes, used by Mossel and Wilson-Blair.

The differential system consists of sodium sulfite and ferric citrate which allows the detection of sulphite reducing organisms, which form black colonies due to ferrous sulfide precipitate.

Technique

The usual technique for the use of this medium is as follows:

The samples to be examined are ground or homogenized with a vortex in a stomacher and then a decimal dilution bank is prepared. A sample aliquot from each one of the dilutions is placed in a Petri dish. The medium, molten and cooled to 50°C, is now poured in the dishes and allowed to solidify. The dishes are incubated in an anaerobic system at 35°C for 24-36 hours.

90% of the black colonies which are formed can usually be attributed to *Clostridium perfringens*.

Since the medium is not extremely selective, it is advisable to verify black colonies by checking that they are Gram positive sporulated non-motile organisms incapable of reducing nitrates to nitrites.

Most clostridia are sulfite reducers. Among them are *C. perfringens* and *C. botulinum* which along with *C. bifermentans* are the species most frequently involved in food poisoning.

References

- ANGELOTTI, HALL, FOSTER y LEWIS (1962) Quantisation of *Clostridium perfringens* in foods. Appl. Microbiol., 10:193.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed., APHA. Washington.
- F.D.A. (1998) Bacteriological Analytical Manual. 8th ed. Rev. A., AOAC International. Gaithersburg. MD.
- MOSSEL, D.A.A. (1959) Enumeration of sulfite-reducing bacteria occurring in foods. J. Sci. Food Agric. 19:662.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Clostridium perfringens, Selective Agar (SPS Agar)

Art. No. 01-050

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 100-1000 CFU. Incubate in anaerobic conditions.

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Total inhibition	-
Staphylococcus aureus ATCC 25923	Total / Partial inhibition	-
Escherichia coli ATCC 25922	Total inhibition	-
Clostridium perfringens ATCC 13124	Good - very good	Black colonies SH_2 (+)
Clostridium sporogenes ATCC 19404	Good - very good	Black colonies SH_2 (+)
Clostridium perfringens ATCC 10543	Good - very good	Black colonies SH_2 (+)

Clostridium perfringens ATCC 13124
on a Membrane filter

Left: Uninoculated tube(Control)
Centre: *Clostridium perfringens* ATCC 13124
Right: *Clostridium perfringens* ATCC 10543

Clostridium perfringens ATCC 13124
in a double layer (anaerobiosis)

CN Selective Agar Base

Art. No. 01-609

Also known as

CN Pseudomonas Agar; CN Medium; Cetrimide-Nalidixic Acid Medium

Specification

Selective solid medium used for the detection of *Pseudomonas aeruginosa* according to the EN 12780-2002 and ISO 16266 standard.

Formula* in g/L

Gelatin peptone	16,00
Casein peptone	10,00
Potassium sulfate	10,00
Magnesium chloride	1,40
Cetyltrimethyl-ammonium bromide	0,20
Agar	15,00

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 52.6 g of powder to 1 L of distilled water with 10 mL of glycerol. Heat until completely dissolved. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 min. Cool to 45-50°C and to each 500 mL of medium add a vial of the Nalidixic Acid Selective Supplement (Art. No. 06-124CASE or 06-124-LYO). Mix well and pour into Petri dishes.

Do not allow the medium to remain in the molten state for more than 4 hours. Do not re-melt. The finished plates can be used without losing efficacy. For up to one month if they are refrigerated and kept in the dark.

Description

The CN Selective Medium for Pseudomonas was progressively developed from the basic medium of King, Ward and Raney for the enhanced production of pigments. Browne and Lowbury added cetrimide as a selective agent and Goto and Enomoto improved the selectivity by adding nalidixic acid. The presence of both inhibitors eliminates the contaminating microbiota from heavily polluted specimens and was adopted by the CEN (Centre Europeen de Normalisation) in its EN Standard 12780 for the detection of *P. aeruginosa* by membrane filtration of water.

Technique

A volume of the sample is passed through a filter membrane of 0,45 µm pore and the membrane is then placed on the surface of the CN medium. The plates are incubated at 36 ± 2°C for a period of 44 ± 4 hours with a partial examination at 22 ± 2 hours.

All colonies producing a green or blue (pyocyanin) pigmentation in this period may be considered *Pseudomonas aeruginosa* and do not require further conformational testing.

All colonies that produce fluorescence under the Wood's light (without pyocyanin production) are considered presumptive *P. aeruginosa* but must be confirmed on Acetamide Medium (Art. No. 03-428).

All colonies producing a brown-reddish pigment and have no fluorescence or pyocyanine are also considered presumptive *P. aeruginosa* and must be confirmed by the oxidase test and by typical growth on Acetamide Medium (Art. No. 03-428) and King B Agar (F Agar Art. No. 01-029).

Necessary supplements

Nalidixic Acid Selective Suplement (Art. No. 06-124CASE / 06-124-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Nalidixic acid, sodium salt ..... 7,50 mg

Distilled water (Solvent)

References

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- EN 12780 Standard (2002) Water Quality. Detection and enumeration of *P. aeruginosa* by membrane filtration.
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- ROBIN, T. & J.M. JANDA (1984) Enhanced recovery of *P. aeruginosa* from diverse clinical specimens on a new selective agar. Diag. Microbiol. Infect Dis. 2:207.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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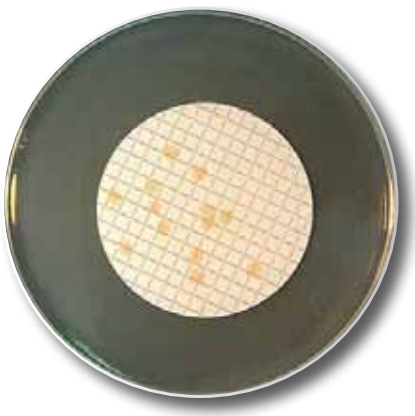
CN Selective Agar Base

Art. No. 01-609

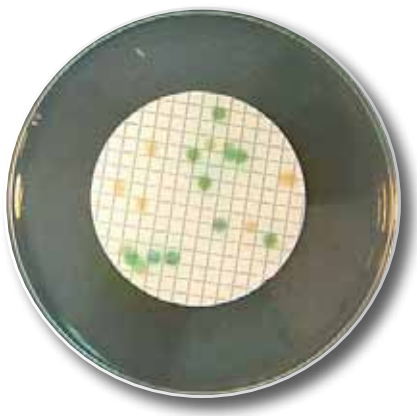
Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method

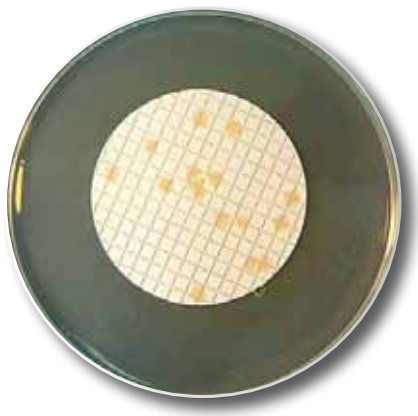
Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Inhibited	-
Staphylococcus aureus ATCC 6538	Inhibited	-
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.50	-
Pseudomonas aeruginosa ATCC 15442	Productivity > 0.50	-
Pseudomonas aeruginosa ATCC 9027	Productivity > 0.50	-



Pseudomonas aeruginosa ATCC 27853



Pseudomonas aeruginosa ATCC 15442
Pseudomonas aeruginosa ATCC 27853



Pseudomonas aeruginosa ATCC 9027

Columbia Agar (Eur. Pharm)

Art. No. 01-680

Specification

Highly nutritious general purpose medium used for the isolation and cultivation of fastidious and nonfastidious microorganisms from clinical and non-clinical materials according to the Pharmacopeial Harmonized Method.

Formula* in g/L

Casein peptone	10,00
Meat peptone	5,00
Heart peptone	3,00
Yeast extract	5,00
Maize starch	1,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 44 g of powder in 1 L of distilled water. Bring to the boil stirring constantly. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Where necessary, cool to 45-50°C and add supplements or inhibitors as required. Mix well and pour into Petri dishes.

Description

In 1966, Ellner *et al*. of Columbia University described a new culture medium for medical bacteriology that could be used with or without the addition of blood to obtain abundant growth and characteristic colonies. Since then a lot of modifications of this medium have been made to serve several purposes.

The present formulation complies with the description as per the Harmonized Method of the European Pharmacopoeia 6th ed. for the microbiological examination of non-sterile products. In the Test for Clostridia, Columbia Agar is used to verify the identity of the colonies sub-cultured from Reinforced Clostridial Medium (Art. No. 03-289) in anaerobic conditions. The European Pharmacopoeia Methodology recommends the sterile addition of gentamicin sulfate equivalent to 20 mg/L of gentamicin base where ever necessary, before pouring the plates.

References

- ELLNER, P.D., C.J. STOESSEL, E. DRAKENFORD & F. VASSI (1966) A new culture medium for medical bacteriology. Am. J. Clin. Pathol. 45:502-504.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- MACFADDIN, J. F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol I. William & Wilkins. Baltimore. USA.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH)

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Productivity > 0.70	-
Escherichia coli ATCC 8739	Productivity > 0.70	-
Clostridium sporogenes ATCC 19404	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-

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Columbia CNA Agar Base

Art. No. 01-703

Specification

Solid medium used, with the addition of blood for the selective isolation of Gram-positive cocci, from clinical samples.

Formula* in g/L

Peptone Mixture	20,000
Meat extract	3,000
Starch	1,000
Sodium chloride	5,000
Nalidixic acid sodium salt	0,015
Colistin sulfate	0,010
Agar	15,000

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Reconstitution

Add 44.0 g of powder in 500 mL of distilled water and heat to boiling point. Sterilize for 15 minutes at 121 ° C. Cool to 45-50 ° C and aseptically add defibrinated blood in a proportion of 5%. Mix gently and pour plates, avoiding the formation of bubbles.

Description

Columbia CNA Agar Base was first described in 1966 by Ellner et al. to selectively isolate Gram-positive cocci and fungi in urine cultures, it was found that the addition of colistin and nalidixic acid significantly suppressed the growth of Gram negative bacteria. It contains a balanced mixture of peptones which, together with the meat extract, are a very good source of carbon, nitrogen and vitamins. The starch promotes the growth of *Neisseria* and the added blood enhances the haemolytic reaction of some streptococci. Sodium chloride maintains the osmotic balance and agar acts as a gelling agent. Blood is an additional source of growth factors and the basic constituent for the determination of haemolytic reactions.

Colistin solubilises the cell membrane of Gram negative bacteria and is especially effective against Pseudomonas. Nalidixic acid blocks DNA replication, especially in Enterobacteriaceae, and also in other Gram negatives. The combination of these two antibiotics is very effective in suppressing the growth of Enterobacteriaceae and members of the genus Pseudomonas, allowing the yeasts, staphylococci, streptococci and enterococci to grow more freely.

Some Gram negative bacteria such as Gardnerella vaginalis and Bacteroides species grow well in this environment, and some tiny colonies of *Proteus* may also grow. Some strains of streptococci, despite the nutritional richness of the environment, can grow poorly and may fail to grow.

Technique

Inoculate the samples directly on the surface of agar, streaking to obtain isolated colonies. Some stab inoculations should also be carried out to deposit Beta-haemolytic streptococci deep in the medium as this subsurface growth allows manifestation of both oxygen-stable and oxygen-labile streptolysin activity, giving clear haemolytic reactions.

The plates are incubated in (aerobic, anaerobic or 5-10% CO_2 enriched atmosphere) according to laboratory protocol, for each sample type.

After incubation for 18 to 24 h at 35°C the plates are examined for growth and, subsequently, for haemolytic reactions:

- Alpha-haemolysis (α) is the reduction of haemoglobin to methaemoglobin in the medium surrounding the colony, producing a green halo.
- Beta-haemolysis (β) is the total lysis of the blood erythrocytes producing a clear zone around the colony.
- Gamma-haemolysis (γ) is indicated by no haemolysis: No change in the environment.
- Alpha-prime-haemolysis (α´) presents as a zone of complete lysis next to the colony surrounded by an area of partial lysis.

The haemolytic effect of streptococci depends on many factors. Ruoff (1995) noted that incubation in atmospheres enriched in (5-10%) CO_2 optimized the action of beta-haemolytic streptococci and some strains of streptococci, (Lancefield group D) behave differently depending on the animal origin of the blood used in the medium: In Blood Agar with horse, human, or rabbit blood, beta-haemolytic action is manifested and with sheep blood alpha-haemolytic action is best observed.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- BARON, E.J., L.R. PETERSON & S.M. FINEGOLD (1994) Bailey & Scott's Diagnostic Microbiology. 9th ed. Mosby-Year Book Inc. St Louis, MO. USA.
- CASMAN, E. (1947) A non-infusion blood agar base for neiseriae, pneumococci and streptococci. Am. J. Clin. Path. 17:281-289.
- ELLNER, P.D., C.J. STOESSEL, E. DRAKEFORD, & F. VASI (1966) A new culture medium for medical bacteriology. Amer.J.Clin.Path 45:502-504.
- ESTEVEZ, E.G. (1984) Bacteriological Plate Media: review of mechanisms of action. Lab. Med. 15:258-262.
- ISENBERG H.D. (1992). Clinical Microbiology Procedures Handbook. Vol 1 ASM Washington DC, USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

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Columbia CNA Agar Base

Art. No. 01-703

· RUOFF, K.L. (1995) Streptococcus p. 299-305. En Manual of Clinical Microbiology 6th ed. Por Murray, Baron, Pfaller, Tenover y Yolker (editors) ASM. Washington DC. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	β-haemolysis
Streptococcus pyogenes ATCC 19615	Good	β-haemolysis
Streptococcus pneumoniae ATCC 49619	Good	α-haemolysis
Proteus mirabilis ATCC 12453	Inhibited	-

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m-CP Agar Base

Art. No. 01-513

Specification

Solid medium for the enumeration and isolation of *Clostridium perfringens* in water according to the European Directive 12767/97.

Formula* in g/L

Tryptose	30,00
Yeast Extract	20,00
Sucrose	5,00
L-Cysteine	1,00
Magnesium sulfate · $7H_2O$	0,10
Bromocresol purple	0,04
Agar	15,00

Final pH 7,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 35,5 g of powder in 500 mL of distilled water and bring to the boil. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C and aseptically add 1 vial of Selective Supplement m-CP (Art. No. 06-125-LYO). Mix well avoiding bubble formation and pour into Petri dishes.

Description

The m-CP Agar Base is a solid medium for counting and isolating vegetative cells and spores of *Clostridium perfringens* by the membrane filtration method. Its use is compulsory in determining the quality of water for human consumption according to the European Union by Directive 12767 (12-07-1997) of the European Council.

Technique

A suitable volume of water is filtered through a membrane filter of 47 mm diameter and 0,45 µm pore. The membrane is then placed on the surface of freshly prepared m-CP medium and incubated in an anaerobic atmosphere at 44 ± 1°C for 21 ± 3 hours. Expose the growth obtained to ammonium hydroxide vapours for 20-30 seconds. Count as *Clostridium perfringens* all the opaque yellow colonies that turn pink or red after ammonium hydroxide exposure. Express the results as CFU/mL.

Necessary supplements

m-CP Selective Suplement (Art. No. 06-125-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

D-Cycloserine	200,00 mg
Polymyxin B sulfate	12,50 mg
3-Indoxyl-β-D-glucopyranoside	30,00 mg
Phenolphthalein bi-phosphate	50,00 mg
Iron III chloride	45,00 mg

Distilled water (Solvent)

References

- European Council (1997) Directive 12767/97 on the quality of water destined for human consumption. EC Bull. 16-12-1997.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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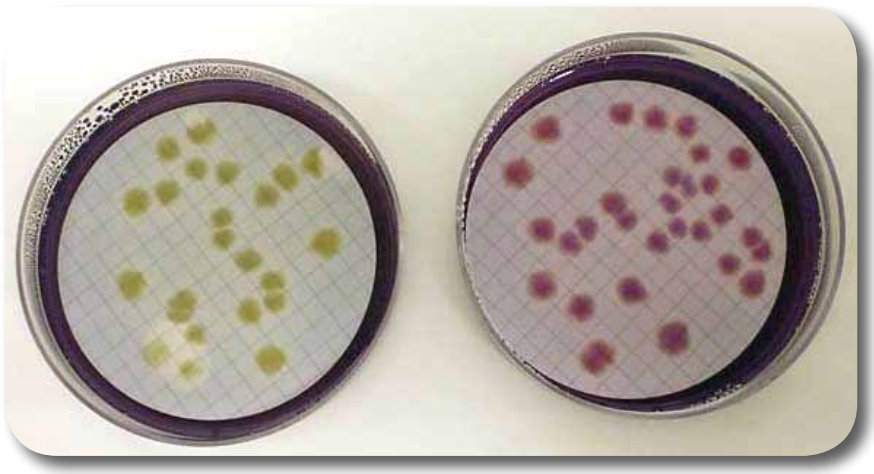
m-CP Agar Base

Art. No. 01-513

Quality control

Incubation temperature: 44 ± 0,1°C
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method. Anaerobic condition

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Inhibited	-
Clostridium perfringens ATCC 12917	Good	Yellow colonies turn to pink-red
Clostridium perfringens ATCC 13124	Good	Yellow colonies turn to pink-red



Clostridium perfringens ATCC 13124
Left: (w/o ammonia solution 32%) yellow
Right: (w. ammonia Solution 32%) red-violet

D/E Neutralizing Agar

Art. No. 01-610

WARNING

H: 3.4.S/1: H317

P: P261-P280-P321-P363-P333+P313-P501a

Also known as

Dey-Engley Neutralizing Agar

Specification

Solid culture medium for the neutralization and testing of antiseptics and disinfectants.

Formula* in g/L

Tryptone	5,00
Yeast extract	2,50
Dextrose	10,00
Lecithin	7,00
Sodium thioglycolate	1,00
Sodium thiosulfate	6,00
Sodium sulfite	2,50
Polysobate 80	5,00
Bromocresol Purple	0,02
Agar	15,00

Final pH 7,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 54 g of powder in 1 L of distilled water and bring to the boil. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. The appearance of precipitates is normal and does not affect results.

Description

Dey & Engley developed this medium in 1983 to recover chemically damaged staphylococci. At present its use is generally for testing by the contact plate method (Contact Plates), the efficiency of antiseptics and disinfectants on impervious surfaces. The present formulation incorporates neutralizing substances for almost all the active products used as antiseptics and disinfectants. Lecithin neutralizes quaternary ammonium compounds (QAC's); Polysorbate acts on phenolics and formalin; thioglycolate neutralizes the organic-mercurial compounds; thiosulfate-sulfite inactivates halogen-compounds and; lecithin + polysorbate neutralizes ethanol and other alcoholic compounds.

Technique

When the contact plates are filled in the laboratory, be careful with the meniscus of the agar: It should rise above the rim of the plate to give a slightly convex surface to make proper contact with the surface to be sampled.

For sampling, remove the cover of the contact plate and carefully press the agar surface to the surface being sampled. Make certain that the entire agar meniscus contacts the surface. Replace the cover and incubate in an inverted position under the time and temperature conditions for the microorganisms in question. Express the results as "colonies per contact plate" or "colonies per cm^2".

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Culture Media. CRC Press. Boca Ratón. Fla.
- DEY, B.P. & F.B. ENGLEY (1983) Methodology for recovery of chemically treated *Staphilococcus aureus* with neutralizing medium. Appl. Environm. Microbiol. 453:1533-1537.
- EVANCHO, G.M., W.H. SVEUM, LL. J. MOBERG & J.F. FRANK (2001) Microbiological Monitoring of the Food Processing Environment. In Downes & Ito (Eds) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC.
- HICKEY, P.J., C.E. BECKELHEIMER, & T. PARROW (1992) Microbiological tests for equipment, containers, water and air. In R.T. Marshall (Ed.) Standard Methods for the examination of Dairy Products. 16th ed. APHA. Washington.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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D/E Neutralizing Agar

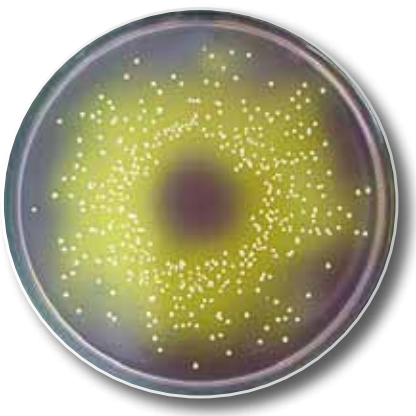
Art. No. 01-610

WARNING
H: 3.4.S/1: H317
P: P261-P280-P321-P363-P333+P313-P501a

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-



Staphylococcus aureus ATCC 25923

Bacillus subtilis ATCC 6633

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D/E Neutralizing Broth

Art. No. 02-610

WARNING

H: 3.4.S/1: H317

P: P261-P280-P321-P363-P333+P313-P501a

Specification

Liquid medium for enrichment cultures in cosmetics, according to ISO standards.

Formula* in g/L

Tryptone	5,00
Yeast extract	2,50
Dextrose	10,00
Lecithin	7,00
Sodium thiosulfate	6,00
Sodium sulfite	2,50
Sodium thioglycolate	1,00
Polysorbate 80	5,00
Bromocresol purple	0,02

Final pH 7,6 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 39 g of powder in 1 L of distilled water and bring to the boil. Distribute in suitable containers and sterilize in the autoclave at 121ºC for 15 minutes. The appearance of precipitates is normal and does not affect the results.

Description

Initially, Dey & Engley developed this medium in 1983 to recover chemically damaged staphylococci. At present its use has expanded to verify several aspects in the application of disinfectants and preservatives.

The present formulation incorporates neutralizing substances for almost all the active products used as preservatives in cosmetic production. Lecithin neutralizes quaternary ammonium compounds (*QAC's*); polysorbate acts on phenolics and formalin; thioglycolate neutralizes the organic-mercurial compounds; thiosulfate-sulfite inactivates halogen-compounds and lecithin + polysorbate neutralizes ethanol and other alcoholic compounds.

The ISO Standards give this medium as an alternative for culture enrichment in the detection of aerobic mesophilic bacteria (ISO 21149:2005), in the detection of *Escherichia coli* (ISO 21150:2006) and for verifying the presence of *Pseudomonas aeruginosa* (ISO 22717:2006) and *Staphylococcus aureus* (ISO 22718:2006).

Technique

When the product is water-soluble, a suitable sample (1 g or 1 mL) is transferred to 9 mL of D/E Neutralizing Broth. If the product is not water-soluble it must first be dissolved with Polysorbate 80 or another emulsifier product.

The D/E Neutralizing Broth with the sample is then incubated at 32,5ºC ± 2,5ºC for at least 20 hours and no more than 72 hours before subculturing on a suitable solid medium for isolation of colonies.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Culture Media. CRC Press. Boca Ratón. Fla.
- DEY, B.P. & F.B. ENGLEY (1983) Methodology for recovery of chemically treated *Staphilococcus aureus* with neutralizing medium. Appl. Environm. Microbiol. 453:1533-1537.
- HICKEY, P.J., C.E. BECKELHEIMER & T. PARROW (1992) Microbiological tests for equipment, containers, water and air. In R.T. Marshall (Ed.) Standard Methods for the Examination of Dairy Products. 16th ed. APHA. Washington.
- ISO Standard 21149:2005. Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria.
- ISO Standard 21150:2006. Cosmetics - Microbiology - Detection of *Escherichia coli.*
- ISO Standard 22717:2006. Cosmetics - Microbiology - Detection of *Pseudomonas aeruginosa.*
- ISO Standard 22718:2006. Cosmetics - Microbiology - Detection of *Staphylococcus aureus.*
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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D/E Neutralizing Broth

Art. No. 02-610

WARNING

H: 3.4.S/1: H317

P: P261-P280-P321-P363-P333+P313-P501a

Quality control

Incubation temperature: 30 - 35°C

Incubation time: 24 - 72 h

Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	Yellow medium
Enterococcus faecalis ATCC 19433	Good	-
Bacillus subtilis ATCC 6633	Good	Violet medium
Escherichia coli ATCC 25922	Good	Yellow medium
Salmonella typhimurium ATCC 14028	Good	Yellow medium
Pseudomonas aeruginosa ATCC 27853	Good	Violet medium

Decarboxylase Lysine Broth (Taylor)

Art. No. 02-336

Specification

Liquid medium to differentiate enteric bacteria using L-Lysine decarboxylation assays according to ISO and IDF standards.

Formula* in g/L

Yeast extract	3,000
Dextrose	1,000
Bromocresol purple	0,016
L-Lysine	5,000

Final pH 6,8 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 9 g of powder in 1 L of distilled water. Distribute into thin tubes in volumes of 2 or 5 mL per tube. Sterilize in the autoclave at 121ºC for 10 minutes.

Description

The capacity to decarboxylate some amino acids has been widely employed in the classification of Enterobacteriaceae.

Taylor's formulation, including lysine, has been recently included in several standards for the identification of *Salmonella*. This modification shows an improved performance, in comparison to Falkow's formulation.

Technique

It is advisable to use a vaseline seal to avoid spontaneous oxidation. The use of glucose in anaerobic conditions produces an acidification of the medium; causing the indicator to turn yellow.

If the organism can decarboxylase the amino acid alkaline bioproducts will be formed turning the medium grey and finally violet. The observations of these biochemical tests are performed after an incubation period of 24 hours at 37°C.

References

- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. APHA. Washington.
- FIL-IDF Standard 93 (2001) Detection of *Salmonella spp*.
- ISO 6785 Standard (2001) Milk and milk products. Detection of *Salmonella spp*.
- ISO 21567 Standard (2004) Food and feeding stuffs - Horizontal method for the detection of *Shigella spp*.
- TAYLOR, W. I. (1961) Isolation of Salmonellae from Food Supplies. V. Determination of the Method of Choice for Enumeration of *Salmonella*. Appl. Microbiol. 9:487-490.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 1.000-10.000 CFU

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	L-Lys (+) Purple medium
Shigella flexneri ATCC 12022	Good	L-Lys (-) Yellow medium
Proteus hauseri ATCC 13315	Good	L-Lys (-) Yellow medium

Deoxycholate Lactose Agar

Art. No. 01-057

Also known as

DCL Agar

Specification

Differential solid medium for the isolation of enterobacteria according to APHA.

Formula* in g/L

Peptone	10,00
Lactose	10,00
Sodium chloride	5,00
Sodium citrate	2,00
Sodium deoxycholate	0,50
Neutral red	0,03
Agar	15,00

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 42,5 g of powder in 1 L of distilled water and bring to the boil. **Do not autoclave** and pour into sterile Petri plates. The medium loses its efficiency if overheated and so avoid autoclaving and/or re-melting.

Description

The Deoxycholate-Lactose Agar is very close to the classical Deoxycholate Agar, differing only in the amount of Deoxycholate and in its reduced inhibitory capacity. The present formulation is made according to the recommendation of APHA and AOAC.

The inhibition of Gram positive microorganisms is due primarily to its content of sodium deoxycholate, although citrate is also an active inhibitor. Differentiation of enteric bacilli is achieved by lactose fermentation. Organisms that ferment lactose, produce acid that, in presence of neutral red indicator, produce pink colonies that may also be surrounded by a zone of precipitated deoxycholate. Non-Lactose-fermenting bacteria form colourless colonies that are surrounded by a clear orange-yellow zone.

Technique

Inoculate the specimen as soon as possible directly onto the surface of the medium. Incubate the plates at 35 ± 2°C for 18-24 hours. Plates can be incubated for an additional 24 hours if lactose-fermentation is not observed.

References

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla.
- GREENBERG, A.E., L.S. CLESCERI & A.D. EATON (1995) Standard Methods for the examination of Water and Wastewater. 19th ed. APHA-AWWA-WEF. Washington. DC.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- VANDERZANT,C. & SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of food. 3rd ed. APHA. Washington. DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Deoxycholate Lactose Agar

Art. No. 01-057

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Selectivity
Escherichia coli ATCC 25922	Productivity > 0.50	Pink colonies with a precipitation zone
Proteus mirabilis ATCC 43071	Productivity > 0.50	Colourless colonies w/o precipitation
Salmonella abony NCTC 6017	Productivity > 0.50	Colourless colonies w/o precipitation
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Colourless colonies w/o precipitation
Shigella sonnei ATCC 9290	Productivity > 0.50	Colourless colonies w/o precipitation
Shigella flexneri ATCC 12022	Productivity > 0.50	Colourless colonies w/o precipitation

Escherichia coli ATCC 25922

Dextrose Tryptone Purple Bromocresol Agar

Art. No. 01-556

Specification

Solid medium for the cultivation of "flat-sour" canned food spoiling microorganisms.

Formula* in g/L

Tryptone	10,00
Dextrose	5,00
Bromocresol purple	0,04
Agar	15,00

Final pH 6,9 ± 0,2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 30 g of powder in 1 L of distilled water and bring to the boil. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium was adopted in 1933 by the National Canners Association for the detection of microorganisms causing "flat-sour" spoilage in canned foods.

Later it was used for the detection and enumeration of all microorganisms related to acid spoilage of foods, like *Bacillus coagulans, Sporolactobacillus* and the thermophilic *Bacillus stearothermophilus*.

Technique

The sample or its dilutions are inoculated into the molten medium, cooled to 50°C. Then poured into Petri dishes and incubated for 72 h at 30-32°C (mesophiles) or for 48 hours at 55-60°C (thermophiles). After incubation the acid-producing colonies can be easily enumerated because they show a yellow zone that contrasts with the purple medium.

References

- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- HORWITZ, W. (2000) Official Methods of Analysis. AOAC International, Gaithersburg. MD.
- NATIONAL CANNERS ASSOCIATION (1933) Bacterial Standard for Sugar.
- NATIONAL CANNERS ASSOCIATION (1954) A Laboratory Manual for the Canning Industries. 2nd ed. Washington
- NATIONAL CANNERS ASSOCIATION (1968) Laboratory Manual for Food Canners and Processors. Vol. 1 Washington.
- VANDERZANT, C, & D. F. SPLITTSTOESSER (1992) Compendium of Methods for the Microbiological Examination of Foods. 3rd Ed. APHA. Washington D:C. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

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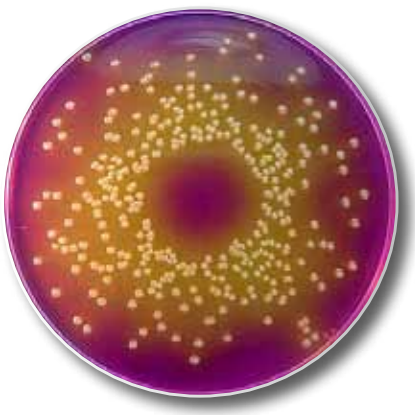
Dextrose Tryptone Purple Bromocresol Agar

Art. No. 01-556

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	Yellow medium
Bacillus cereus ATCC 10876	Productivity > 0.70	Violet medium 24 h
Geobacillus stearothermophilus ATCC	Productivity > 0.70	Yellow medium
Escherichia coli ATCC 25922	Productivity > 0.70	Yellow medium



Escherichia coli ATCC 25922

Uninoculated Plate (Control)

Bacillus cereus ATCC 10876

Dichloran Glycerin Selective Agar (DG18 Agar)

Art. No. 01-485

DANGER
H: 3.6/1A; H350
P: P281-P201-P202-P308+P313-P405-P501a

Specification

Solid differential and low water activity medium used for the determination of xerophilic fungi in low moisture food and in indoor air according to the ISO standard 16000-17:2008.

Formula* in g/L

Peptone	5,000
Dextrose	10,000
Monopotassium phosphate	1,000
Magnesium sulfate · $7H_2O$	0,500
Dichloran	0,002
Chloramphenicol	0,100
Agar	15,000

Final pH 5,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 31,7 g of powder in 1 litre of distilled water and bring to the boil. Add 220 g (~180 mL) of glycerol and homogenize. Distribute it into suitable containers and sterilize by autoclaving at 121°C for 15 minutes.

Description

Among the culture media for xerophilic fungi, those that have played a more successful role are the ones which include any agent that restrains the continuous growth of zygomycete fungal colonies. Dichloran (dichlorebenzalkonium chloride) and Rose Bengal are two of those inhibitors.

DG18 Agar formulation used is that proposed by Hocking & Pitt in 1980, and it includes Dichloran which limits the size of fungal colonies more efficiently than Rose Bengal. Chloramphenicol inhibits bacterial growth and its thermostability allows it to be included in the medium before sterilization.

The inclusion of 18% (w/w) of Glycerine gives the medium a water activity (a_w) of 0,955 without causing any of the problems that generally occur when this water activity is provided by sodium chloride or sugar.

The addition of Triton X-301® (Tapia de Daza and Beuchat, 1992) at a concentration of 0.01% (w/w) enables easier enumeration of xerophiles when *Eurotium spp*. are present (Beuchat and Hwang).

Technique

Mass inoculation is recommended by spread plating using an inoculation loop, a swab or by spreading the sample with a Drigaslky loop. Never use an inoculum volume greater than 0,1 mL.

According to the standardized technique, plates must be incubated at 22-25°C, with partial readings after 3 and 5 days, and definitive readings after 7-8 days. Results are expressed in xerophiles-CFU /g or mL of food sample or CFU/m^3 of air.

Plates of DG18 Agar in bags will keep for up to one week at (5 ± 3)°C in the dark. Due to its extreme water activity (a_w = 0.955), the plates must be rejected if any kind of dehydration is suspected.

References

- BEUCHAT, L.R. and C.A. HWANG (1995) Evaluation of modified dichloran 18% glycerol (DG18) agar for enumerating fungi in wheat flour. Int. J. Food Microbiol. 29:161-166.
- HOCKING, A.D. and J.I. PITT (1980) Dichloran-glycerol medium for enumeration of xerophilic funghi from low–moisture food. Appl. Environm. Microbiol. 39:488-492
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 16000-17 Standard. (2008) Indoor air.- Part 17: Detection and enumeration of moulds – Culture-based method.
- PITT, J.I., and A.D. HOCKING (1985) Fungi and Food Spoilage. Academic Press. Sydney.
- PITT, J.I., A.D. HOCKING and D.R: GLENN (1983) An improved medium for the detection of Apergillus flavus and A. parasiticus. J. appl. Bacteriol. 54:109-114
- SAMSON, R.A., E.S. HOEKSTRA, J.C. FRISVAD and O. FILTENBORG (2002) Introduction to the Food Borne Fungi. 6th ed. Centraalbureau voor Schimmelcultures. Utrech.
- TAPIA de DAZA, M.S. and L.R. BEUCHAT. (1992) Suitability of modified dichloran glycerol (DGH!8) agar for enumerating unstressed and stressed xerophilic molds. Food Microbiol. 9:319-333

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Dichloran Glycerin Selective Agar (DG18 Agar)

Art. No. 01-485

DANGER
H: 3.6/1A; H350
P: P281-P201-P202-P308+P313-P405-P501a

Quality control

Incubation temperature: 22 - 25°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	Black sporulation at 5 days
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-

Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)

Art. No. 01-657

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

Specification

Selective solid medium for the enumeration of moulds and yeasts in foods according to ISO 21527 - 1:2008 standard.

Formula* in g/L

Mycological peptone	5,000
Dextrose	10,000
Monopotassium phosphate	1,000
Magnesium sulfate	0,500
2-6-dichloro-4-nitro-aniline (Dichloran)	0,002
Rose bengal	0,025
Chloramphenicol	0,100
Agar	15,000

Final pH 5,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 31,6 g of the powder in 1 L of distilled water and bring to the boil, continue boiling until completely dissolved. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Dichloran Rose Bengal Chloramphenicol (DRBC) Agar is a medium based on the Dichloran Rose Bengal Chlortetracycline medium developed by King et al Cols (1979) and on the formulation of Rose Bengal Chlortetracycline medium of Jarvis (1973). The combination of Dichloran and Rose Bengal markedly restricts the size and height of mould colonies thus preventing overgrowth of luxuriant species and assisting accurate counting of colonies. The presence of Chloramphenicol and the low pH of 5.6 serve to prevent the growth of most bacteria. This medium supports good growth of yeasts and moulds and can be used to enumerate both toxigenic and non-toxigenic fungi but it is not diagnostic for detecting specific mycotoxin-producers.

In the current formulation the concentration of Rose Bengal is reduced to 25 µg/mL for optimal performance with Dichloran. Chlortetracycline is replaced by chloramphenicol as it is more stable and easier to handle. It is also preferred for use in the food and environmental sectors.

Rose Bengal is taken up by most yeasts and some moulds, which allows the easy recognition and enumeration of these colonies. Some times there can be a reduced recovery of certain yeasts due to increased activity of Rose Bengal at pH 5,6.

Technique

Using 0,1-0,2 mL of inoculum per 9 cm diameter plate, spread it over the whole surface of the plate. Incubate the plates upright at 25°C for 5 days in the dark with examination for growth after 3,4 and 5 days. Where identification is required prolong the incubation until characteristic colonies are formed. Colonies of yeast generally appear pink due the uptake of Rose Bengal.

Where separate counts of moulds and yeasts are required, identify by morphological appearance and perform microscopic examination of these two groups of microorganisms where necessary. Colonies of yeast and bacteria can be confused and microscopic examination should be carried out if unsure.

Cautions and Limitations

- Due to the selective properties of this medium and the type of specimen being cultured some strains of fungi can fail to grow or grow poorly.
- Similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
- This medium is photo-sensitive. Do not expose to light since photo-degradation of Rose Bengal produces compounds toxic to fungi.
- The prepared medium have a shelf-life of seven days at 4 ± 2°C in the dark.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Culture Media. CRC Press. Boca Raton. Fla. USA.
- BAYLIS, C.L. (2003) Manual of Microbiological Methods for the Food and Drinks Industry. CCFRA. Chipping Campden. Gloucestershire. UK.
- BEUCHAT, L.R. & M.A. COUSIN (2001) Yeasts and Molds. In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. USA.
- CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD (2003) Handbook of Culture Media for Food Microbiology. Elsevier Science. Amsterdam.
- ISO 21527-1 Standard (2008) Microbiology of food and animal feeding stuffs - Horizontal methods for the enumeration of yeast and moulds - Part1: Colony count technique in products with water activity greater than 0,95.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- JARVIS, B.(1973) Comparison of an i mproved Rose-Bengal-Chlortetracycline Agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J. Appl. Bacteriol. 36:723-727.
- KING, D.A., A.D. HOCKING & J.J.PITT (1979) Dichloran-Rose Bengal medium for enumeration and isolation of molds from foods. Appl. Environm. Microbiol. 37:959-964.

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Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)

Art. No. 01-657

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 25 - 30°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited	Selectivity
Escherichia coli ATCC 25922	Inhibited	Selectivity
Aspergillus brasiliensis ATCC 16404	Productivity > 0.50	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.50	-
Candida albicans ATCC 10231	Productivity > 0.50	-

Differential Reinforced Clostridial Medium (DRCM)

Art. No. 02-410

Specification

Liquid medium used for the enumeration of clostridia in food samples and other products using the MPN technique.

Formula* in g/L

Peptone	10,000
Meat extract	8,000
Yeast extract	1,000
Starch	1,000
Glucose	1,000
L-Cysteine	0,500
Sodium acetate	5,000
Sodium bisulfite	0,500
Ferric-ammonium citrate	0,500
Resazurin	0,002

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 27,5 g of powder in 1 L of distilled water. Bring to the boil, distribute in tubes and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is a modification by Freame and Fitzpatrick of the Gibb's classic medium, used to detect the presence of sulfite reducing clostridia. The modification is, an addition of sodium bisulfite and ferric citrate, which make colonies black and thus more visible. The current version of this medium has no agar in order to facilitate easy observation of the blackened medium. Resazurin, the redox indicator allows the verification of anaerobiosis in the medium in the same assay. L-Cysteine acts as a reducing agent in this medium.

Technique

The sample to be examined is distributed in tubes as per the MPN technique, and covered with paraffin oil to help anaerobiosis. The series of tubes is kept in a boiling water bath at 75°C for 30 minutes to remove all the dissolved oxygen and vegetative cells. Then, incubate at 30°C for up to 7 Days.

The spores of sulfate reducing clostridia usually germinate between the second and fourth day, turning the medium black.

The medium can be rendered selective by the addition of 70 IU/mL of polymyxin sulfate.

Prepared tubes without inoculation may be stored for up to 2 weeks provided the resazurin band does not show excessive oxidation (more than a 1/3 of the column).

References

- FREAME, B., FITZPATRICK, B.W.F. (1972) The use of DRCM for the isolation and enumeration of Clostridia form food. In "Isolation of Anaerobes". Ed. Shapton & Board. Academic Press. London.
- GIBBS, B.M., FREAME, B. (1965) Methods for the recovery Clostridia from foods. J. Appl. Bact. 36:23-33.
- MacFADDIN, J.M. (1985) Media for isolation -cultivation-identification-maintenance of medical bacteria. Williams & Wilkins. Baltimore.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Differential Reinforced Clostridial Medium (DRCM)

Art. No. 02-410

Quality control

Incubation temperature: 30°C
Incubation time: 48 h - 7 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity) // Anaerobic conditions

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibition	-
Clostridium perfringens ATCC 13124	Good	Black precipitate
Clostridium sporogenes ATCC 11437	Good	Black precipitate
Clostridium perfringens ATCC 10543	Good	Black precipitate

Uninoculated tube (Control)

Left: *Clostridium perfringens* ATCC 13124
Centre: *Clostridium perfringens* ATCC 10543
Right: *Escherichia coli* ATCC 25922

DNase Agar

Art. No. 01-346

Also known as

DNase Test Agar; Deoxyribonuclease Test Agar; Deoxyribonucleic Acid Agar; DNA Agar

Specification

Solid culture medium for the determination of the deoxyribonuclease activity of microorganisms, especially staphylococci and *Serratia spp.*

Formula* in g/L

Tryptose	20,00
DNA	2,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 42 g of powder in 1 L of distilled water and bring to the boil stirring constantly. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and pour into the Petri dishes.

Description

Jeffries, Holtman and Guse (1957) incorporated DNA into a general medium with agar to study bacterial and fungal DNase production. Microorganisms that produce DNase cleave DNA, reducing it to nucleotide fragments. This reaction is observed by the appearance of a clear zone surrounding the growth, the rest of the plate remains turbid. Hydrochloric acid reacts with DNA producing a white precipitates that makes the medium turbid, and it does not react with nucleotide fragments (clear zones).

DiSalvo (1958) observed that there is a correlation between coagulase production and DNase activity, thus DNase Medium may be used as a laboratory test to diagnose pathogenic staphylococci.

Mannitol fermentation may be simultaneously determined if 10 g of mannitol and 0,025 g of phenol red are added to 1 L of DNase Agar, before sterilization. Positive results in both tests will determine with more certainty that the microorganism is a pathogenic *Staphylococcus aureus.*

This medium is also useful to identify *Serratia marcescens* in clinical specimens, since it is a good DNase producer. Smith et al. (1969) modified the medium by adding toluidine blue and crystal violet, and stated that Gram negative DNase producing bacilli that grew on this medium may be described as *Serratia* species.

Technique

DNase Agar plates are inoculated with the microorganism to be studied by streaking a thick line of inoculum across the plate or by spotting onto the plate. Plates are incubated at 35-37°C for an 18-24 hour period.

To read, flood the plates with 1N hydrochloric acid and observe if there are any clear or transparent zones surrounding the streak. If the plate becomes totally turbid without any clear zone then the test is **negative**; however if any clear zones develop around the growth, the test is described as **positive.**

References

- DISALVO, J.W. (1958) Desoxyrribonuclease and Coagulase Activity of Micrococci. Med. Tech. Bull. U.S. Armed Forces. Med. J. 9:191.
- JEFFRIES, C.D., D.F. HOLTMAN y D.G. GUSE (1957) Rapid Method for Determining the Activity of Microorganisms on NucleicAcids. J. Bacteriol. 73:590-591.
- SMITH, P.B., G.A. HANCOCK & D.L. RHODEN (1969) Improved medium for detecting deoxyrribonucleaseproducing bacteria. Appl. Microbiol. 18:991-993.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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DNase Agar

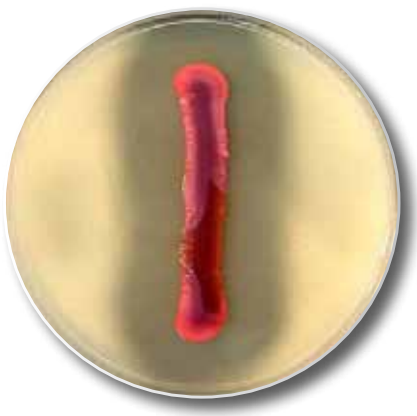
Art. No. 01-346

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Streak isolation

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Good	DNase (-)
Serratia marcescens ATCC 274	Good (red colonies)	DNase (+)
Serratia marcescens ATCC 13880	Good	DNase (+)
Staphylococcus aureus ATCC 6538	Good	DNase (+)
Staphylococcus aureus ATCC 25923	Good	DNase (+)
Escherichia coli ATCC 25922	Good	DNase (-)

Pseudomonas aeruginosa ATCC 27853



27853 *Serratia marcescens* ATCC 274

Staphylococcus aureus ATCC 25923

E. coli Broth

Art. No. 02-060

Also known as

EC Broth

Specification

Selective medium for the detection and enumeration (MPN) of enterobacteria, in water and foodstuffs according to ISO standards.

Formula* in g/L

Peptone	20,00
Bile Salts No. 3	1,50
Lactose	5,00
Dipotassium phosphate	4,00
Potassium dihydrogen phosphate	1,50
Sodium chloride	5,00

Final pH 6,9 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 37 g of powder in 1 L of distilled water. Distribute into tubes or containers with inverted Durham tubes (to detect gas production). Sterilize at 121°C for 15 minutes.

Description

EC Broth is a buffered medium containing lactose. It is part of a range of selective broths for Enterobacteriaceae. Its efficiency or selectivity is based on bile salts' inhibitory effect on other microorganisms.

This broth may be used for routine testing of water and food, either alone or by using the Most Probable Number method of enumeration.

The type of sample will determine how precise the results are. If the incubation is at 35-37°C for 48 hours, gas formation may be interpreted as presumptive evidence of coliform bacteria. Later confirmation will have to be done using any of the classical methods.

Should the incubation take place at 44,5°C, gas formation could be interpreted as a confirmation of the presence of *Escherichia coli.* Nevertheless, it must be taken into account that the validity of this test is highly limited by technical variations. A maximum incubation time of 24 hours in a water bath with very precise temperature regulation, is therefore recommended.

When using samples greater than 10 mL, the medium must be reconstituted at a concentration equivalent to that specified on the directions, taking into account the added sample volume.

References

- APHA-AWWA-WEF (1998) Standard Methods for the Examination of Water and Wastewater, 20th ed. APHA, Inc., Washington. DC. USA.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
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- ISO 9308-2 Standard (1990) Water Quality. Detection and enumeration of coliform organisms, thermotolerant coliform organisms and pressumptive *E. coli* - MPN method.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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- MARSHALL,R. (1993) Standard Methods for the Examination of Dairy Products. 16th ed. APHA. Washington. DF. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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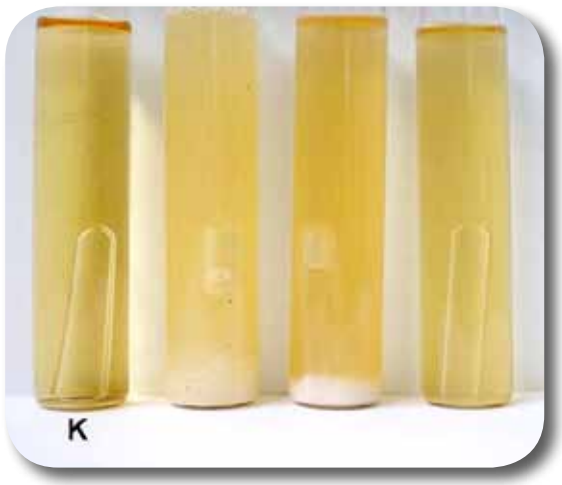
E. coli Broth

Art. No. 02-060

Quality control

Incubation temperature: 35°C ± 2,0 - 44°C ± 0,5
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Good (35°C) / Inhibited (44°C)	Gas (-)
Enterococcus faecalis ATCC 29212	Inhibited	48 h
Escherichia coli ATCC 25922	Good (35°C) / Good (44°C)	Gas (+)
Escherichia coli ATCC 8739	Good (35°C) / Good (44°C)	Gas (+)
Citrobacter freundii ATCC 43864	Good (35°C) / Inhibited (44°C)	Gas (+)



First (K): Uninoculated tube (Control)
Second: *Escherichia coli* ATCC 25922
Third: *Escherichia coli* ATCC 8739
Fourth: ***Pseudomonas aeruginosa*** ATCC 27853

Enrichment Enterobacteriaceae Broth (EE Broth) (Eur.Pharm.)

Art. No. 02-064

Also known as

EE Mossel

Specification

Liquid culture medium used for the enrichment of enterobacteria according to the ISO standard and the Pharmacopeial Harmonised Method.

Formula* in g/L

Gelatin peptone	10,000
Dextrose	5,000
Ox bile	20,000
Di-sodium phosphate ($2H_20$)	8,000
Monopotassium phosphate	2,000
Brilliant green	0,015

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 45 g of powder in 1 L of distilled water and heat at 100°C for 30 min. and cool immediately. **Do not autoclave.**

Description

As the name suggests, this medium is for the enrichment of enterobacteria, and is a modification by Mossel (1963) of the classic Brilliant Green Bile 2% Broth (Art. No. 02-041). Substitution of lactose by glucose makes it more suitable for enteric bacteria detection, (including both gas or non-gas-producers), in food and other samples.

Technique

The most common technique is as follows: the sample to be studied is added to sterile broth in a proportion of 10%. After through homogenization, the mixture is incubated for a period of 18-20 hours at 35-37°C.

After incubation, subcultures are performed on a solid media appropriate for the selective isolation of enterobacteria.

For this step, Violet Red Bile Agar (Art. No. 01-164) is recommended, although MacConkey (Art. No. 01-118), deoxycholate or brilliant green based media can also be used.

Presumptive colonies isolated on this media, can be verified following the usual methodology.

References

- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- ISO 21528-1:2004 Standard. Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 1: Detection and enumeration by MPN technique with pre-enrichment.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MOSSEL, VISSER & CORNELISSEN (1963) The examination of foods for Enterobacteriaceae using a test of the type generally adopted for the detection of *salmonellae* J. Appl. Bact. 26:444-452.
- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos. S.A. Madrid.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Enrichment Enterobacteriaceae Broth (EE Broth) (Eur.Pharm.)

Art. No. 02-064

Quality control

Incubation temperature: 30 - 35°C
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	-
Staphylococcus aureus ATCC 6538	Inhibited	-
Pseudomonas aeruginosa ATCC 9027	Good	-
Escherichia coli ATCC 25922	Good	-
Escherichia coli ATCC 8739	Good	-
Salmonella typhimurium ATCC 14028	Good	-

Left: Uninoculated tube(Control)
Centre: *Salmonella typhimurium* ATCC 14028
Right: *Escherichia coli* ATCC 25922

Endo Agar Base

Art. No. 01-589

Specification

Moderately selective solid medium used for the detection of coliforms and other enteric organisms, in milk and water, according to APHA specifications.

Formula* in g/L

Peptone	10,00
Lactose	10,00
Sodium sulfite	2,50
Di-potassium hydrogen phosphate	3,50
Agar	15,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 41 g of powder in 1 L of distilled water. Bring to the boil and distribute into suitable containers. Sterilize at 121°C for 15 minutes. Cool to 45-50°C, and add 2 vials of Basic Fuchsin 250 Supplement (Art. No. 06-607-LYO). Mix well and pour into plates.

Note: After autoclaving the medium must appear slightly pinkish. If the colour is a very intense red, it can be decolourised by adding a few drops of a sterile solution of sodium sulfite 10% before pouring it into the plates. Medium must be freshly prepared for use, and must not be used when it is red.

Description

Endo Agar is used to confirm the detection of and to count coliform bacteria following the testing of drinking water, as well as for the detection and isolation of coliforms and faecal coliforms from milk, dairy products and other food-stuffs.

The moderate selectivity is due to the formation of a fuchsine-sulfite compound. This compound reacts with the acetaldehyde formed in the lactose fermentation and frees the fuchsin dye that colours the bacterial colony. The strains that produce large amounts of the metabolite, like *E. coli*, can crystallize the fuchsin on the colony, giving rise to characteristic green metallic sheen. Inoculate the plates by the streak-plate method and incubate for 24 hours at 37°C. Colonies of coliform, which ferment lactose, are pink to pale red, with or without green metallic sheen: marked reddening of the medium may occur. Colonies of other enteric bacilli, including *Salmonella* and of non-lactose-fermenters are about the same colour as the medium, being almost colourless to faint pink.

Caution: On exposure to oxygen, the plated medium gradually becomes red due to the oxidation of sulfite and can thus no longer be used. It can only be kept for a few days even if it is stored in the dark and at refrigerator temperature.

Necessary supplements

Basic Fuchsin (250) Selective Supplement (Art. No. 06-607-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Basic fuchsin ..... 250,00 mg

Ethanol (Solvent)

References

- APHA/AWWA/WEF (1985) Standard Methods for the examination of water and wastewater. 15th ed. APHA Washington. DC. USA.
- APHA (1967) Standard methods for the examination of dairy products. 12th ed. APHA Washington. DC. USA.
- ATLAS, R.M. (1995) Handbook of Microbiological Media for the Examination of Food. CRC Press. Boca Raton. Fla. USA.
- ATLAS, R.M. y R.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- ENDO, S. (1904) Über ein verfahren zum Nachweis von typhusbazillen. Zbl. Bakt. Hyg. Abt. I Orig. 35:109.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- WINDLE TAYLOR, E. (1958) The examination of water and water supplies. 7th ed. Churchill Ltd. London.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) //1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Selectivity
Escherichia coli ATCC 8739	Productivity > 0.50	Pink-red colonies w. green metallic shine
Escherichia coli ATCC 25922	Productivity > 0.50	Pink-red colonies w. green metallic shine
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Colourless colonies w/o green metallic shine
Salmonella abony NCTC 6017	Productivity > 0.50	Colourless colonies w/o green metallic shine

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Eosin Methylene Blue Agar (EMB Agar)

Art. No. 01-068

Specification

Selective differential medium for the isolation and enumeration of coliforms according to ISO 21150 standard and USP.

Formula* in g/L

Peptone	10,000
Lactose	10,000
Dipotassium hydrogen phosphate	2,000
Eosin Y	0,400
Methylene blue	0,065
Agar	15,000

Final pH 6,90 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 37,5 g to 1 L of distilled water. Bring to the boil and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

A very versatile medium originally developed for the differentiation of *E.coli* and *Enterobacter aerogenes*. It has also proved very effective in the rapid identification of *Candida albicans* and demostrates a high correlation with the coagulase test for staphylococci.
It has been repeatedly recommended for the detection, enumeration and differentiation of members of the coliform group of bacteria.

Technique

The Weld method for the identification of *Candida albicans* uses this medium with chlortetracycline (100 mg/l) in a 10% CO_2 environment. The method's efficacy has been tested with a variety of samples, such as sputum, oral secretions, faeces, nails and vaginal secretions, all of which provide definitive results within 24-48 hours. Staphylococci are also easily identified, particularly coagulase-positive strains. These have a very characteristic appearance: small colourless colonies with a central red nucleus. The medium's prevailing application is in the differentiation of *E. coli* and *E. aerogenes*.
The medium should be sterilized once distributed into tubes containing 20 mL of product each, and then refrigerated. **Melt in a boiling water bath before use and stir until it acquires a dark purple colour**. Pour a tube into each sterile plate and allow it to solidify. It is advisable to dry the medium's surface before use, leaving the plate open but inverted.

For each doubtful lactose broth tube, inoculate one plate by streaking, and incubate for 24 to 48 hours at 37°C.

- *Escherichia coli* and *Citrobacter* form flat colonies of 2-3 mm in diameter and are dark violet in colour with a black centre which produces a distinctive green metallic sheen when light is reflected on it.
- *Enterobacter* and *Klebsiella* form convex colonies which are twice as big as the very smooth *E. coli*, have no metallic sheen and are pink in colour with a dark blue centre. Non-lactose fermenting organisms produce colourless colonies.
- *Candida albicans* colonies incubated in a CO_2 atmosphere have a very peculiar cotton-like appearance which distinguishes them from other *Candida species* that produce classical yeast like colonies.

References

- CLESCERI, L.S., A.E. GREENBERG & A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed. APHA-AWWA-WEF. Washington. DC.
- HOLT-HARRIS, J. E. y TEAGUE O.A. (1916) A New Culture Medium for the Isolation of *Bacillus typhosus* from Stools J. Infect. Dis. 18:596-600.
- ISO STANDARD 21150 (2006) Cosmetics. Microbiology - Detection of *Escherichia coli*.
- LEVINE, M (1918) Diferentation of *E. coli* and *A. aerogenes* on simplified Eosin-ethylene Blue Agar. J. Infect. Dis. 23:43-47.
- MENOLASINO, N.I., GRIEVES B. Y PAYNE P. (1960) Isolation and Identification of Coagulase Positive Staphylococci on Levine's Eosin-Methylene Blue Agar. J. Lab. Clin. Med. 56(6) 908-910.
- USP 31 - NF 26 (2008) <61> Microbial Tests. USP Con. Inc. Rockville. MD. USA.
- WELD, J. (1953) *Candida albicans:* Rapid Identification in Cultures made directly from Human materials Arch. Dermat. Syph. 67(5):473-478.
- WINDLE TAYLOR, E. (1958) The Examination of Water and Water Supplies. Churchill Ltd. 7th ed. Londres.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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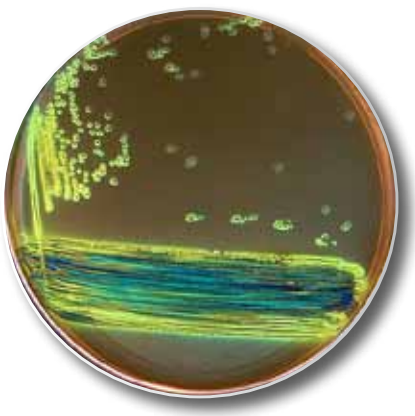
Eosin Methylene Blue Agar (EMB Agar)

Art. No. 01-068

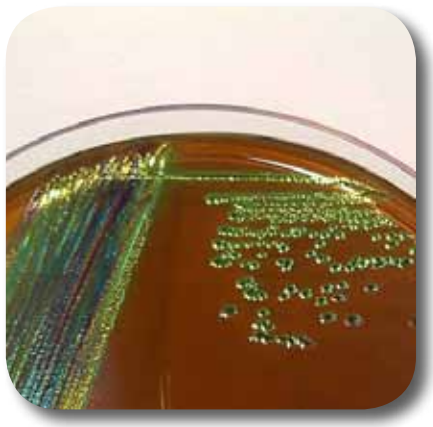
Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 h
Inoculum: Streak isolation

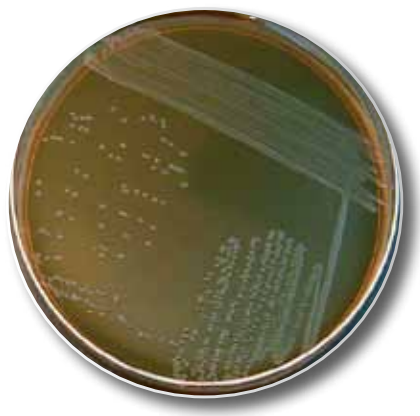
Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited to poor	48 h
Salmonella abony NCTC 6017	Good to very good	Colourless colonies w/o green metallic shine
Escherichia coli ATCC 11775	Good to very good	Dark violet colonies w. green metallic sheen
Escherichia coli ATCC 25922	Good to very good	Dark violet colonies w. green metallic sheen
Escherichia coli ATCC 8739	Good to very good	Dark violet colonies w. green metallic sheen
Salmonella typhimurium ATCC 14028	Good to very good	Colourless colonies w/o green metallic shine
Pseudomonas aeruginosa ATCC 27853	Good to very good	Colourless colonies w/o green metallic sheen



Escherichia coli ATCC 8739



Escherichia coli ATCC 8739
"Detail"



Salmonella typhimurium ATCC 14028

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Ethyl Violet Azide Broth (EVA Broth)

Art. No. 02-028

WARNING

H: 3.1.O/4; H302-4.1.C/3; H412

P: P273-P264-P270-P301+P312-P330-P501a

Also known as

Litsky Broth; Azide-Ethyl Violet Broth

Specification

Liquid medium for the confirmation of enterococci in water.

Formula* in g/L

Meat peptone	10,0000
Casein peptone	10,0000
Dextrose	5,0000
Sodium chloride	5,0000
Monopotasium phosphate	2,7000
Dipotassium phosphate	2,7000
Sodium azide	0,3000
Ethyl violet	0,0005

Final pH 7,0 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 35,6 g of powder in 1 L of distilled water, heating slightly if necessary. Distribute in tubes or flasks and sterilize in the autoclave at 121ºC for 15 minutes.

Description

EVA Broth is a highly selective medium for some enterococci, and it has been adopted by many Official Organisations, national and international. The medium's high selectivity is due to the presence of Sodium Azide and Ethyl Violet, as they inhibit other accompanying bacteria, blocking their respiratory chains, at the same time leaving enterococci unaffected. In general, this medium is used as a confirmation medium in the second stage, with an inoculum from a suitable medium such as Rothe Azide Broth (Art. No. 02-027) inoculated in this medium.

Technique

Each of the EVA Broth tubes is inoculated with one or two loops from a presumed positive Rothe Azide Broth (Art. No. 02-027), and is incubated for a 24-48 hours period at 37°C. The presence of *Enterococcus* is demostrated by turbidity in the medium.

Occasionally a slight turbidity may appear accompanied by abundant violet sediment at the bottom of the tube.

Commonly, growth in this medium is considered enough to confirm the prensence of enterococci.

However, confirmative identification must be carried out by isolation in solid media and classification in one of the four faecal enterococci species: *Enterococcus faecalis, Enterococcus faecium, Enterococcus bovis* and *Enterococcus equinum*.

References

- CLESCERI, L., A.E. GREENBERG y E.A. EATON (1998) Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF, 20th ed., Washington.
- DOWNES, F.C. y K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- GUINEA, SANCHO y PARÉS (1979) Análisis Microbiológico de Aguas: Aspectos Aplicados. Ed. Omega. Barcelona.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LITSKY,W., W.L. MALLMAN & C.W. FIFIELD (1953) A New Medium for the Detection of Enterococci in Water . Amer. J. Publ. Hlth 43(7):873.
- ROTHE (1948) Illinois State Health Department.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Ethyl Violet Azide Broth (EVA Broth)

Art. No. 02-028

WARNING

H: 3.1.O/4: H302-4.1.C/3: H412

P: P273-P264-P270-P301+P312-P330-P501a

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Enterococcus faecalis ATCC 29212	Good to very good	Violet precipitate
Enterococcus faecalis ATCC 19433	Good to very good	Violet precipitate

Left: Uninoculated tube (Control)
Centre: *Enterococcus faecalis* ATCC 29212
Right: *Escherichia coli* ATCC 25922

Enterococcus faecalis ATCC 29212 (violet precipitate)

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Eugon LT 100 Agar

Art. No. 01-654

Specification

Solid medium used for the detection and enumeration of aerobic mesophilic bacteria, in cosmetic products with and without preservatives according to ISO 21149 standard.

Formula* in g/L

Tryptone	15,00
Soy peptone	5,00
Polysorbate 80	5,00
Dextrose	5,50
Sodium chloride	4,00
Lecithin	1,00
Triton® X-100	1,00
L-Cysteine HCl	0,70
Sodium sulfite	0,20
Agar	15,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 52,4 g of powder in 1 L of distilled water. Bring it to the boil and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. The medium will become clear on cooling.

Note: The lumpy and greasy appearance of the dehydrated powder is normal due to the Polysorbate and Triton® components and does not affect its performance.

Description

Lecithin-Polysorbate-Triton® is a general culture medium that allows the growth of aerobic, and microaerophilic bacteria. Some anaerobic microorganisms, will grow due to the low redox (*Eh*) potential generated by the cysteine and sodium sulfite components.

The main use of this medium is for the total enumeration of microorganisms in cosmetic products by the Most Probable Number (MPN) method. The inclusion of Triton® X-100 in the formula enhances the release of microorganisms from the slack matrix of the cosmetic emulsion.

Lecithin and Polysorbate act as neutralizers of preservatives like quaternary ammonium compounds, phenol and aldehydes derivatives.

Technique

A 1:10 dilution of the sample is prepared using Eugon broth (Art. No. 02-654) if the sample is soluble in water. If the sample is non-water soluble, it must be emulsified with a suitable agent (e.g. Polysorbate 80) Once emulsified the sample is added to a suitable volume of Eugon broth (e.g. 1:10). The pour plate or spread plate technique can be adopted with unfilterable samples.

If the sample is filterable it is recommended filtering through a membrane filter with a nominal pore size no greater than to 0.45 µm and washing with a defined volumes of water or diluent (Art. No. 02-510 Universal Diluent). Immediately transfer the membrane to a plate of Eugon Agar.

The inoculated plates are incubated at 32,5 ± 2,5°C for 48 to 72 h and the the colonies are, then, counted.

References

- GUISNO, R., I.W. GIBBY & M.J. FOTER (1946) A neutralizing medium for evaluation of the germicidal potency of the Quaternary Ammonium Salts. Amer. J. Pharm. 118:320-323.
- ISO 21149 Standard (2006) Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- WILLIAMSON, P. & A.M. KLIGMAN (1965) A new method for the quantitative investigation of cutaneous bacteria. J. Inv. Dermatol. 45:498-503.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Eugon LT 100 Agar

Art. No. 01-654

Quality control

Incubation temperature: 32,5°C ± 2,0
Incubation time: 24 - 72 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2 :2003)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Staphylococcus epidermidis ATCC 12228	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-

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Eugon LT 100 Broth

Art. No. 02-654

Specification

Liquid medium used for the enrichment of aerobic bacteria including *E. coli*, in cosmetic products with and without preservatives according to ISO standards.

Formula* in g/L

Tryptone	15,00
Soy peptone	5,00
Polysorbate 80	5,00
Dextrose	5,50
Sodium chloride	4,00
Lecithin	1,00
Triton® X-100	1,00
L-Cysteine HCl	0,70
Sodium sulfite	0,20

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 37,4 g of powder in 1 L of distilled water. Bring to the boil gently and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. Cool rapidly. The medium will become clear on cooling.

Note: The lumpy and greasy aspect of the dehydrated powder is normal due to the polysorbate and Triton® components and does not affect its performance.

Description

Eugon LT 100 Broth is a general culture medium that allows the growth of aerobic, and microaerophilic bacteria. Some anaerobic microorganisms will grow, due to the low *Eh* potential generated by the cysteine and sodium sulfite components.

The main use of this medium is for the total enumeration of microorganisms in cosmetic products by the Most Probable Number (MPN) method. The Triton® X-100 included in the formula enhances the release of microorganisms from the slack matrix of the cosmetic emulsion.

Lecithin and polysorbate act as neutralizers of preservatives like quaternary ammonium compounds, phenol and aldehydes derivatives.

Technique

A 1:10 dilution of the sample is prepared using directly Eugon broth if the sample is soluble in water. If the sample is non-water soluble, it must emulsified with a suitable agent (e.g. Polysorbate 80). Once emulsified the sample is added to a suitable volume of Eugon broth (e.g. 1:10). If the sample is filterable it is recommended filtering it through a membrane filter with a nominal pore no greater than to 0.45 µm and washing it with defined volumes of water or diluent (Art. No. 02-510 Maximum Recovery Diluent). Immediately transfer the membrane to a suitable volume of the Eugon broth. The inoculated broth is incubated at 32,5 ± 2,5°C for 20-72 hours.

If enumeration by the MPN method is being carried out proceed as follows:

Prepare serial tenfold dilutions bank of the sample. Inoculate, incubate and enumerate as per the Most Probable Number Protocol. Carry out enumeration according to the appropriate tables in each case.

References

- GUISNO, R., I.W. GIBBY & M.J. FOTER (1946) A neutralizing medium for evaluation of the germicidal potency of the quaternary ammonium salts. Amer. J. Pharm. 118:320-323.
- ISO 21149 Standard (2006) Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria.
- ISO 21150 Standard (2006) Cosmetics - Microbiology - Detection of *Escherichia coli*.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- WILLIAMSON, P. & A.M. KLIGMAN (1965) A new method for the quantitative investigation of cutaneous bacteria. J. Inv. Dermatol. 45:498-503

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Eugon LT 100 Broth

Art. No. 02-654

Quality control

Incubation temperature: 32,5 ± 2,5°C
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) according to ISO 11133-1/2

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Staphylococcus epidermidis ATCC 12228	Good	-
Escherichia coli ATCC 25922	Good	-
Pseudomonas aeruginosa ATCC 27853	Good	-
Salmonella typhimurium ATCC 14028	Good	-

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GC Agar Base

Art. No. 01-310

Specification

Solid medium base especially recommended for the isolation and culture of fastidious microorganisms.

Formula* in g/L

Special peptone	15,00
Starch	1,00
Sodium chloride	5,00
Dipotassium phosphate	4,00
Potassium phosphate	1,00
Dextrose	1,50
Sodium bicarbonate	0,15
Yeast fractions	10,00
Agar	12,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 24,8 g of powder in 250 mL of distilled water and bring to a boil. Distribute into a 1 L flask and sterilize in the autoclave at 121°C for 15 minutes.

Dilute 5 g of haemoglobin powder in 250 mL of hot distilled water, constantly stirring, to obtain a homogeneous solution. Sterilize in the autoclave at 121°C for 15 minutes.

Cool both flasks to 50°C and aseptically add the sterile haemoglobin solution to the medium base. To facilitate *Neisseria* detection, the addition of one vial of VCAT Selective Supplement (Art. No. 06 -141-LYO) is recommended. Homogenize by rotation in order to avoid bubbles and pour into plates.

Description

The base can be used in the following applications:

Chocolate Agar

It is prepared with the Agar base and haemoglobin, without any inhibitor. Defibrinated blood can be used in the ratio of 1:10 if required i.e. 10 mL of blood per to 100 mL of prepared base, cooled to 45°C. Place the complete medium into a boiling bath for a few seconds, three consecutive times; the medium will become dark chocolate brown in colour. This medium enables the growth of very fastidious microorganisms, such as *Haemophilus influenzae*.

Thayer-Martin Agar

In 1966 Thayer and Martin described a medium that has been very effective in the isolation of Pathogens such as *Neisseria*. The medium is prepared with GC Agar base, haemoglobin and an inhibitor vial of VCNT Selective Supplement (Art. No. 06-142-LYO) that contains the antibiotics: vancomycin and colistin to inhibit the oxidase-positive contaminants; nystatin to prevent the growth of saprophytic fungi and trimethoprim to prevent *Proteus* overgrowth as demonstrated by Odegaard and Phillips in 1970. Recuperation of stressed cells is improved when a vial of GPS - Growth Promotion Supplement (Art. No. 06-144-LYO) is added.

Transgrow Agar

This medium has been demonstrated to be very effective for the storage, transport and culture of *Neisseria*.

The preparation of this medium is the same as that of Thayer-Martin Agar but it is distributed into hermetic screw-cap tubes solidified in the horizontal position.

Technique

When sampling is performed close to the laboratory, Thayer-Martin plates may be directly inoculated. If the sample has to be transported to the laboratory, Transgrow medium is preffered.

Gonococcal test:

In women, it is recommended that samples be taken from one of the following:

Where possible, from the cervix, after removing the cervical mucous.

If cervical culture is negative, rectal cultures may be carried out form samples taken form the rectum.

If a cervical sample is not suitable, e.g. in girls or after a hysterectomy, vaginal or urethral cultures may be performed with the corresponding samples.

In men, urethral culture from mucosal samples is recommended. Anal and pharynx cultures may also be appropriate.

For diagnosis of gonorrhoea in women, a growth of Gram negative cocci with a specific morphology is necessary, combined with a positive oxidase reaction. However, when diagnosing men, the demonstration of intracellular gonococci in the urethral exudations is sufficient. Culture for biochemical identification only needs to be performed when the first test is not possible. In special cases fermentations or reactions with fluorescent antibodies may be used to demonstrate the presence of *Neisseria gonorreheae*. Microscopic preparation from urethral exudates must be done very carefully in order to mantain the cellular morphology.

Plate inoculation is performed by drawing a Z over the surface with the swab; ising a rolling motion. After this, the sample is dispersed with a loop. Incubate at 35°C, in a very moist, 10% CO_2 enriched atmosphere.

N. gonorrheae and *N. meningitidis* produce colourless and translucent colonies.

Antibiotic incorporated in the medium with inhibitory supplement avoids the growth of almost all the non pathogenic microorganisms in the sample, including the saprophytic species of *Neisseria*. Thayer-Martin Medium inhibits also *Mima polymorpha* var *oxidans*, a microorganism that sometimes may be confused with *Neisseria gonorrheae.*

Transgrow tubes are inoculated by introducing the swab very carefully, squeezing it out against the walls and reaching the bottom of the tube. Extract the swab carefully to reduce the CO_2 lost.

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GC Agar Base

Art. No. 01-310

Transport:

If possible, the sample should be incubated at 35°C for 12-16 hours before being transported to the laboratory. Transgrow Medium keeps *neisseria* alive for up to 48 hours, even at room temperature. In the laboratory, incubate the tubes or, if they have been already incubated, examine the growth.

Transgrow Medium, with 10% CO_2 allows the growth of pathogenic *neisseria* and inhibits all the other contaminating microorganisms in the same way as Thayer-Martin medium.

Transgrow Medium tubes, if well closed, with a CO_2 atmosphere and refrigerated, are usable for at least 3 months after preparation.

Necessary supplements

VCAT Selective Supplement (Art. No. 06-141-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Vancomycin	1,00 mg
Colistin sulfate	3,75 mg
Amphotericin B	0,50 mg
Trimethoprim	1,50 mg

Distilled water (Solvent)

References

- ATLAS, R.M. & L.C. PARKS (1997) Handbook of microbiological media. CRC Press. BocaRaton .Fla. USA.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs. -Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MACFADDIN, J. (1985) Media for isolation-cultivation-Identification-maintenance of medical bacteria. Vol. I. William & Wilkins. Baltimore.
- ODEGAARD, K. (1971) Trimethoprim for the prevention of overgrowth by swarming *Proteus* in the cultivation of gonococci. Acta. Path. Microbiol. Scand. Sect. (B) 79:545-548.
- THAYER, J. D. & J. E. MARTIN (1966). Improved medium selective for cultivation of *Neisseria gonorrheae* and *N. meningitidis* Pub. Health Rep. 81:559-562.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 48 h

Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Candida albicans ATCC 10231	Poor to inhibited	-
Neisseria gonorreae ATCC 19424	Good	-
Neisseria meningitidis ATCC 13090	Good	-

Giolitti-Cantoni Broth

Art. No. 02-230

Specification

Liquid medium used for the recovery and enumeration of low numbers of coagulase-positive staphylococci in foods according to ISO, FIL-IDF and EN standards.

Formula* in g/L

Tryptone	10,00
Meat extract	5,00
Yeast extract	5,00
Lithium chloride	5,00
D-Mannitol	20,00
Sodium chloride	5,00
Glycine	1,20
Sodium pyruvate	3,00
Polysorbate 80	1,00

Final pH 6,9 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 55,2 g of powder in 1 L of distilled water. The medium can be prepared at single strength or double strength by using double the quantity of powder. Distribute into tubes dispensing 10 mL/tube (single strength) or 20 mL/tube (double strength). Sterilize in the autoclave at 121°C for 15 minutes. Cool and add 1% Potassium Tellurite Sterile Solution (Art. No. 06-089) using 0,1 mL/tube for single strength and 0,2 mL/tube for double strength.

Description

This medium for the selective enrichment of staphylococci was formulated in 1966 by Giolitti and Cantoni.

The growth of staphylococci is promoted by pyruvate, glycine and above all by a high concentration of mannitol. Addition of Polysorbate 80 is necessary for the successful recovery of *Staphylococcus aureus* (Chopin et al., 1985). Accompanying flora are inhibited by lithium chloride and potassium tellurite.

Anaerobic growth conditions increase the selectivity of the medium. Generally, growth of staphylococci can be recognized by a blackening or black precipitates in the culture medium due to reduction of tellurite to metallic tellurium.

The prepared basal culture medium (without sodium tellurite) can be stored for about 1-2 weeks in the refrigerator. The ready-to-use medium (with the sodium tellurite added) must be used on the same day of preparation. It is advisable that the stored medium base be degasified at the moment of use by heating for 15 minutes at 100°C, cooled rapidly and sterile potassium tellurite solution added.

Technique

Refer to the standard protocol for specific products (Food and animal feeding stuffs EN-ISO 6888-3:2003; Milk and milk based products ISO 5944:2001 and FIL-IDF 60:2001). As a general technique the following is suggested:

Use food macerates or a 10-fold serial dilution and inoculate 1 mL in single strength medium. To lower the detection limit, 10 mL of the test sample (liquid products) or the first dilution (other products) may be inoculated in double strength medium. MPN procedures need at least three tubes for at least three dilution steps. If no anaerobic jar is available, overlay with a layer of sterilized vaseline (Art. No. 06-077) or vaspar. Incubate anaerobically for 24-48 hours at 37°C.

After 24 hours, subculture any tubes showing blackening or black precipitate by streaking onto Baird-Parker Agar (Art. No. 01-030). Incubate the remainder of the tubes for a further 24 hours and subculture all tubes showing growth (irrespective of blackening) to Baird-Parker Agar.

When determining the bacterial count by the MPN method, all tubes showing growth are considered as presumptive positive for staphylococci and they are confirmed only if they produce a positive result in the coagulase test.

References

- CHOPIN, A. et al. (1985) ICMSF Methods Studies XV. Comparison of four media and methods for enumerating *Staphylococcus aureus* in powdered milk. J. Food Protect. 48:21-27.
- EN-ISO 6888-3 Standard (2003) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase positive staphylococci (*Staphylococcus aureus* and other species). Part 3: Detection and MPN technique for low numbers.
- FIL-IDF (2001) Milk and milk based Products. Detection of coagulase-positive staphylococci. MPN technique. Standard 60:2001. Brussels.
- GIOLITTI, G. A. CANTONI, C. (1966) A medium for the isolation of staphylococci from foodtuffs. J.Appl. Bact. 29, 395-398.
- HARRIGAN, WF. &. McCANCE, M.E. (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Press. London.
- ISO 5944 Standard (2001) Milk and milk based Products. Detection of coagulase-positive staphylococci. MPN technique.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Giolitti-Cantoni Broth

Art. No. 02-230

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	Black precipitate to 48 h
Staphylococcus aureus ATCC 6538	Good	Black precipitate to 48 h
Staphylococcus epidermidis ATCC 12228	Poor	-
Escherichia coli ATCC 8739	Inhibited	-
Bacillus subtilis ATCC 6633	Inhibited	-

First: *Escherichia coli* ATCC 8739
Second: *Staphylococcus aureus* ATCC 25923 (24 h)
Third: *Staphylococcus aureus* ATCC 6538 (48 h)
Fourth: *Bacillus subtilis* ATCC 6633

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Glucose Bromocresol Purple Agar

Art. No. 01-502

Specification

Solid medium for the confirmation of enterobacteria in diverse samples according to ISO standards.

Formula* in g/L

Tryptone	10,000
Yeast extract	1,500
Dextrose	10,000
Sodium chloride	5,000
Bromocresol purple	0,015
Agar	15,000

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 41,5 g of powder in 1 L of distilled water and bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is used to confirm enterobacteria by their ability to ferment glucose. Glucose fermentation can be detected by the production of acid that change pH and hence the colour of the medium to yellow.

References

- ATLAS, R.M. y L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla.
- ISO 8523 Standard (1991) Microbiology: Guidance for the detection of Enterobacteriaceae with pre-enrichment. Ginebra.
- ISO 4702 Standard (1993) Microbiology: Guidance for the enumeration of Enterobacteriaceae without resuscitation. MNP technique and colony count technique. Ginebra.
- ISO 21528-1 Standard (2004) Microbiology of food and animal feeding stuffs. - Horizontal methods for the detection and enumeration of Enterobacteriaceae. - Part 1: Detection and enumeration by MPN technique with pre-enrichment.
- ISO 21528-2 Standard (2004) Microbiology of food and animal feeding stuffs. - Horizontal methods for the detection and enumeration of Enterobacteriaceae. - Part 2: Colony-count method.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Glucose Bromocresol Purple Agar

Art. No. 01-502

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Productivity > 0.70	Yellow medium D (+)
Escherichia coli ATCC 25922	Productivity > 0.70	Yellow medium D (+)
Salmonella typhimurium ATCC 14028	Productivity > 0.70	Yellow medium D (+)
Salmonella abony NCTC 6017	Productivity > 0.70	Yellow medium D (+)

Salmonella typhimurium ATCC 14028

Glucose Peptone Chloramphenicol Agar (GP Agar + Antibiotic)

Art. No. 01-692

DANGER
H: 3.6/1A; H350
P: P281-P201-P202-P308+P313-P405-P501a

Specification

Solid culture medium used for enumeration of yeast and mould in cosmetics according to the ISO standard 16212:2008.

Formula* in g/L

Dextrose	20,00
Peptone	5,00
Yeast extract	2,00
Potassium phosphate	1,00
Magnesium sulfate	0,50
Chloramphenicol	0,05
Agar	15,00

Final pH 5,7 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 43,5 g of the powder in 1 L of distilled water and bring to the boil to dissolve completely. Distribute in suitable containers and sterilize into autoclave at 121°C for 15 minutes.

Description

This medium is proposed in the ISO Standard 16212:2008 as an alternative medium for the enumeration of yeast and mould in cosmetics. Glucose provides carbon and energy to the fungi. Peptone is the source of nitrogen and nitrogen compounds. Yeast extract provides growth factors and vitamins. Mineral salts help provide the correct osmotic pressure and maintain the optimal pH for growth. Agar is the only solidifying agent.

Technique

Refer to the ISO Standard for the procedure.
The inoculated plates are incubated at 25 ± 2,5°C for 3 to 5 days. After incubation the plates should, if possible, be examined immediately. Alternatively, they may be stored, unless otherwise specified, for up to a maximum of 24 hours in the refrigerator at 5 ± 3°C.

References

- ISO 16212 Standard (2008) Cosmetics - Microbiology - Enumeration of yeast and mould.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 25 ± 2,5°C
Incubation time: 3 - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Bacillus cereus var. mycoides ATCC 11778	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-

Gram Negative Broth (GN Broth)

Art. No. 02-093

Also known as

Hajna Broth; GN Enrichment Broth

Specification

Liquid culture medium for enteric bacteria according to Hajna's formulation.

Formula* in g/L

Peptone	20,00
Dextrose	1,00
D-Mannitol	2,00
Sodium citrate	5,00
Sodium deoxycholate	0,50
Di-potassium phosphate	4,00
Monopotassium phosphate	1,50
Sodium chloride	5,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 39 g of powder in 1 L of distilled water. Dispense in tubes or flasks and sterilize in the autoclave at 121°C for 15 minutes.

Description

GN Broth (Gram Negative Broth) is an enrichment and selective medium for enterobacteria, with a strong inhibitory action against Gram positive bacteria because of its high content of citrate and deoxycholate. Mannitol restrains the growth of *Proteus* and facilitates the proliferation of *Salmonella* and *Shigella*.

The medium is strongly recommended for primary enrichment, (14-16 hours), before proceeding to selective media such as EMB (Art. No. 01-068) or MacConkey (Art. No. 01-118). Its author, Hajna, declares an extraordinary selectivity of the medium, whatever the origin of the sample, if it is kept in a transport medium prior to inoculation.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media CRC Press. BocaRaton. Fla. USA.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington DC. USA.
- EDWARS & EWING (1973) Identification of *Enterobactericeae*. Burgess Pub. Co. Minneapolis.
- HAJNA, A.A. (1955) A new enrichment medium for Gram negative organisms of the intestinal group. Pub. Hlth. Lab 13:83.
- ISENBERG, H.D. (1998) Essential Procedures for Clinical Microbiology. ASM Press. Washington. DC. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MaCFADDIN, J.F. (1985) Media for Isolation-cultivation-identification-maintenance of Medical Bacteria. Vol. I. Williams & Wilkins. Baltimore. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Gram Negative Broth (GN Broth)

Art. No. 02-093

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	-
Staphylococcus aureus ATCC 6538	Inhibited	-
Escherichia coli ATCC 25922	Good	-
Escherichia coli ATCC 8739	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Salmonella abony NCTC 6017	Good	-
Shigella flexneri ATCC 12022	Good	-

m-Green Agar

Art. No. 01-633

Also known as

m-Green yeast & Mold Agar

Specification

Solid selective culture medium for enumeration of fungi according to ISO standard 10718:2002.

Formula* in g/L

Dextrose	50,000
Peptone	10,000
Yeast extract	9,000
Magnesium sulfate	2,100
Potassium phosphate	2,000
Diastase	0,050
Thiamine	0,050
Bromocresol green	0,026
Agar	15,000

Final pH 4,6 ± 0,2

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 87,3 g of powder in 1 L of distilled water and bring to the boil to dissolve. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Pour plates immediately. Do not overheat or remelt: The low pH of the media softens the jellification of the agar, giving a consistency not suitable to streak plating, but able to support the membrane filter.

Description

This classical formulation used by the food industry for the detection and enumeration of yeast and moulds by the membrane filter method was adopted by ISO for application in cork stoppers for alcoholic or non-alcoholic beverages in the 10718:2002 Standard.

The composition of the culture broth includes Bromocresol Green indicator that facilitates the visualization and counting of fungal colonies. The fungal colonies are green due to the diffusion of the dye into the colonies (alkaline reaction). The end products of the microbial growth diffuse into the medium, reducing the pH and turn the indicator to yellow (acid reaction). Bacterial growth is inhibited by the acid pH.

Technique

Roll the membrane filter used to filter the test sample onto the surface of the medium, avoiding the formation of air bubbles. Incubate the plates at 30 ± 2°C for 3 days. Observe and count the colonies on each plate at least every 24 hours.

After incubation colonies appearing on the filter surface can be counted. Mould colonies generally appear green and filamentous, whereas yeast colonies are green and opaque.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London. ISO Standard 10718:2002 Cork stoppers. Enumeration of colony-forming units of yeasts, moulds and bacteria capable of growth in an alcoholic medium.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60 % RH).

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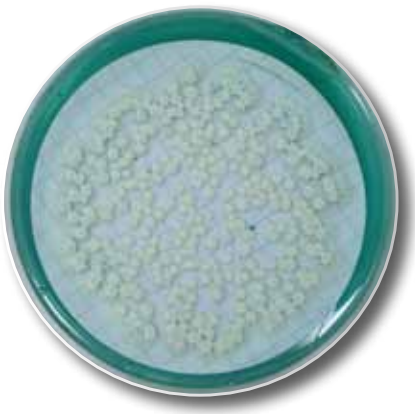
m-Green Agar

Art. No. 01-633

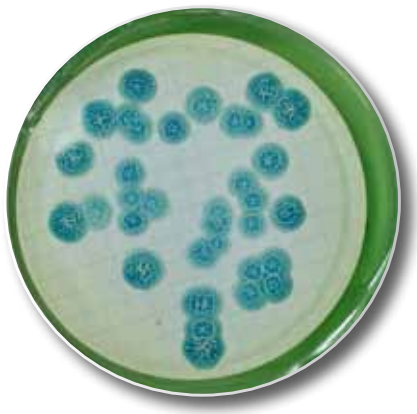
Quality control

Incubation temperature: 30 - 35°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003). Membrane Filter Methods.

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited to poor	-
Aspergillus brasiliensis ATCC 16404	Good	Black sporulation at 5 days
Saccharomyces cerevisiae ATCC 9763	Good	-
Candida tropicalis ATCC 1369	Good	-
Candida albicans ATCC 10231	Good	-



Saccharomyces cerevisiae ATCC 9763



Candida albicans ATCC 10231

Aspergillus brasiliensis ATCC 16404

m-Green Broth

Art. No. 02-633

Also known as

m-Green Yeast & Mould Broth

Specification

Liquid selective culture medium used for the enumeration of fungi according to ISO standard 10718:2002.

Formula* in g/L

Dextrose	50,000
Peptone	10,000
Yeast extract	9,000
Magnesium sulfate	2,100
Potassium phosphate	2,000
Diastase	0,050
Thiamine	0,050
Bromocresol green	0,026

Final pH 4,6 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 73,22 g of powder in 1 L of distilled water, heating if necessary. Distribute it into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

m-Green Broth is a classical formulation used in the food industry for the detection and enumeration of yeast and moulds by the membrane filtration method and was adopted by ISO in its 10718:2002 Standard for application in cork stoppers for alcoholic beverages.

The composition of the culture broth includes Bromocresol Green indicator which facilitates the visualization and counting of fungal colonies. The fungal colonies are green due to the diffusion of the dye into the colonies (alkaline reaction). The end products of the microbial growth diffuse into the medium, reducing the pH and turning the indicator to yellow (acid reaction). Bacterial growth is inhibited by the acidic pH.

Technique

Saturate the blotting pad for the membrane filter in a sterile Petri dish with 2.0 to 2.5 mL of m-Green Broth. Roll the membrane filter used for filtering the test sample onto the surface of the moistened pad. Avoid formation of air bubbles. Incubate the plates at 30 ± 2°C for 3 days. Observe and count the colonies on each filter at least every 24 hours.

After incubation, the colonies that appear on the surface of the filter can be counted. Mould colonies generally appear green and filamentous, whereas yeast colonies are green and opaque.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- ISO Standard 10718:2002 Cork stoppers. Enumeration of colony-forming units of yeasts, moulds and bacteria capable of growth in an alcoholic medium.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60 % RH).

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m-Green Broth

Art. No. 02-633

Quality control

Incubation temperature: 30 - 35°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited to poor	-
Aspergillus brasiliensis ATCC 16404	Good	Black sporulation at 5 days
Saccharomyces cerevisiae ATCC 9763	Good	Gas (+)
Candida tropicalis ATCC 1369	Good	-
Candida albicans ATCC 10231	Good	-

Hektoen Enteric Agar

Art. No. 01-216

Also known as

HE Agar; HEA

Specification

Solid, selective and differential culture medium for isolation of pathogenic enterobacteria from contaminated samples according to ISO 21567 standard.

Formula* in g/L

Meat peptone	12,00
Yeast extract	3,00
Bile salts	9,00
Lactose	12,00
Sucrose	12,00
Salicin	2,00
Sodium chloride	5,00
Sodium thiosulfate	5,00
Ammonium ferric citrate	1,50
Acid fuchsin	0,10
Bromothymol blue	0,06
Agar	15,00

Final pH 7,5 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 77 g of powder in 1 litre of distilled water and let it soak. Heat stirring constantly, until boiling. Cool to 55-60°C and pour into sterile plates. **Do not autoclave.** This medium is very thermolabile and overheating should be avoided.

Description

This culture medium, originally developed by King and Metzger, has a high nutrient content, peptones, fermentable sugars and combination of indicators. All these characteristics and the bile salts make it a very selective and effective medium.

Technique

In order to avoid the spreading of *Proteus*, it is necessary that the agar surface be perfectly dry at the moment of inoculation. Inoculation must be carried out by surface streaking, directly from rectal swabs or faeces. If colonies are well separated after 18 hours of incubation, the first characteristic appearances or colony morphology may be observed:

- *Shigella spp.*, *Proteus inconstans*: Raised colonies, green colour.
- *Salmonella spp.*: Green-blue colonies, with or without black centre.
- Pseudomonas *spp.*: Irregular colonies, plain, green or brown.
- Companion and non pathogenic bacteria: Salmon coloured colonies.

References

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- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods.4th ed. APHA. Washington DC. USA.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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- US FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual 8th ed. AOAC International. Gaithersburg, Md. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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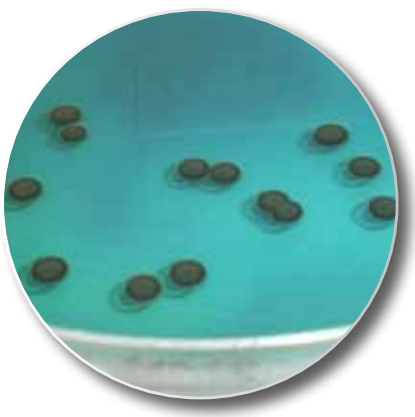
Hektoen Enteric Agar

Art. No. 01-216

Quality control

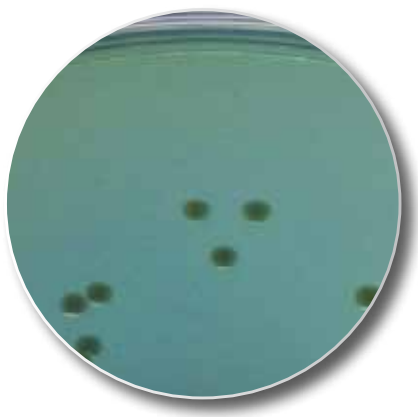
Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Light Pink small colonies
Escherichia coli ATCC 25922	Inhibited	-
Proteus mirabilis ATCC 43071	Productivity > 0.50	Black colonies, Greenish-Blue medium
Salmonella enteritidis ATCC 13076	Productivity > 0.50	Black colonies, Greenish-Blue medium
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Black colonies, Greenish-Blue medium
Shigella sonnei ATCC 25931	Productivity > 0.50	Green to blue colonies
Shigella flexneri ATCC 12022	Productivity > 0.50	Green to blue colonies



Salmonella typhimurium ATCC 14028

Uninoculated Plate



Shigella flexneri ATCC 12022

Iron Sulfite Modified Agar

Art. No. 01-634

Specification

Solid differential medium used for the enumeration of sulfite-reducing bacteria from foods and animal feeding stuffs according to ISO 15213:2003 standard.

Formula* in g/L

Tryptone	15,00
Soya peptone	5,00
Yeast extract	5,00
Disodium disulfite ($Na_2S_2O_5$)	1,00
Iron ammonium citrate	1,00
Agar	15,00

Final pH 7,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 42 g of powder in 1 L of distilled water. Bring to the boil and distribute into suitable containers.
Sterilize in the autoclave at 121°C for 15 minutes. If the medium is not used on the same day of preparation, the medium must be reduced before use.

Description

This modification of the Iron Sulfite Agar is formulated according to ISO 15213:2003 Standard that specifies a horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.
The method can be used with foods and animal feeding stuffs and environmental samples in the food production and handling area. In the *Nordisk Metodikkommitté för Livsmedel* Standard (NMKL No. 95:1997 Sulfite-reducing Clostridia: Determination in food) this medium is used in the clostridia presumptive test, before the confirmatory (respiratory tests, spore-forming test) step. In the ISO Standard also it is also stated that this method is applicable only for clostridia and after the isolation on this medium a confirmatory study of black colonies must be performed.

Technique

Transfer aliquots from the dilution bank of the sample into sterile Petri dishes in duplicate. Into each inoculated Petri dish, add 15 mL of melted, reduced medium cooled to 44-47°C. Carefully mix the inoculum with the medium and allow it to solidify. After the medium has solidified, overlay with another 10 mL of the same medium. The time elapsing between inoculation of Petri dishes and the addition of the melted medium should not exceed 15 min.
The inoculated Petri dishes are incubated in anaerobic conditions at 37 ± 1°C for 24-48 h. If thermophilic bacteria are suspected a second set of petri dishes must be incubated at 50 ± 1°C for 24-48 h.
The black colonies, surrounded or not by a black zone are considered as sulfite-reducing bacteria, presumptive clostridia. Their identity must be confirmed with suitable biochemical and serological tests.

References

- ISO 15213:2003 Standard. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.
- NMKL Standard 95 (1997) Sulfite-reducing Clostridia: Determination in food.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Iron Sulfite Modified Agar

Art. No. 01-634

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method. Anaerobic condition

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited to poor	at 50°C
Escherichia coli ATCC 25922	Inhibited to poor	at 50°C
Clostridium sporogenes ATCC 11437	Productivity > 0.70	Black colony
Clostridium perfringens ATCC 10543	Productivity > 0.70	Black colony
Clostridium perfringens ATCC 13124	Productivity > 0.70	Black colony

Left: Uninoculated tube (Control)
Centre: *Clostridium perfringens* ATCC 13124
Right: *Clostridium perfringens* ATCC 10543

K Agar

Art. No. 01-674

Specification

Selective solid medium used for the isolation of *Alicyclobacillus acidoterrestris*, in fruit juices according to IFU Standard Method No. 12.

Formula* in g/L

Yeast extract	2,50
Peptone	5,00
Dextrose	1,00
Polysorbate 80	1,00
Agar	20,00

Final pH 3,7 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 29,5 g in 1 L of distilled water and bring to the boil, to dissolve. Distribute in suitable containers and sterilize in the autoclave at 121ºC for 15 minutes. Cool to 45-50ºC and adjust the pH to 3,7 ± 0,2 with sterile 25% L-Malic acid solution. Mix well to homogenize and pour into sterile Petri dishes. **Avoid heating or remelting the medium after the pH adjustment.**

Description

Since the early 1980s, when spoilage of fruit juices by acid dependent thermotolerant spore-forming bacteria was recognised (Cerny et al., 1984) members of the genus *Alicyclobacillus* have emerged as food spoilage organisms of major significance to the fruit juice industry (Baumgart & Menje, 2000). Spoilage is generally manifested as the formation of off-flavours and odours from compounds such as guaiacol and the halogenated phenols. To date, no human risk are known to be associated with the consumption of juices and other food products containing *Alicyclobacillus* bacteria, but the economic impact can be very high.

The low pH-value of the media, in combination with the high incubation temperature inhibits the growth of contamining flora. K Agar (Art. No. 01-674) when incubated at 45ºC supports the growth of predominantly *A. acidoterrestris* and limited growth of other species of the genus (*A. acidocaldarius, A. cycloheptanicus* and *A. hesperidium*). Therefore, K Agar (Art. No. 01-674) can be used to detect predominantly *A. acidoterrestris* strains. This medium complies the Standard IFU Method on the detection of taint producing *Alicyclobacillus* in fruit juices.

Technique

The IFU Standard provides three methods of detection depending on the sample composition and the time since processing:

1. Raw materials (including process water): A heat shock treatment is prescribed followed by direct plating (optional), filtration or enrichment in liquid medium of the heated material.
2. End products sampled directly after (heat) processing where an additional heat shock is unnecessary: Pre-incubation of the sample in liquid medium is recommended.
3. End products taken from the market: Pre-incubation of the sample, and heat shock treatment is carried out. If spoilage is suspected and no Alicyclobacilli are detected after direct plating, a heath shock and enrichment treatment follow up is recommended.

In all the methodologies, incubation for 3-5 days at 45 ± 1ºC is recommended. Count all colonies growing on the K Agar as presumptive *Alicyclobacillus acidoterrestris*. Confirm these colonies by further testing.

References

- BAUMGART, J. (2003) Media for detection and enumeration of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* in foods. In Handbook of Culture Media for Food Microbiology. J.E.L. Corry *et al.* (Eds.) Elsevier Sci B.V. Amsterdam.
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- CERNY, G., W. HENNLICH & K. PORALLA (1984) Fruchsaftverderb durch Bazillen: Isolierung und Charakterisierung des Verderberregers. Z. Lebens. Unter Forsch. 179:224-227.
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- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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K Agar
Art. No. 01-674

Quality control

Incubation temperature: 45°C ± 2,0
Incubation time: 72 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Alicyclobacillus acidoterrestris ATCC 49025	Good	-
Alicyclobacillus acidocalcarius ATCC 27009	Good	-
Escherichia coli ATCC 25922	Inhibited	-

Kanamycin Esculin Azide Agar (KAA Agar)

Art. No. 01-263

H: 4.1.C/3; H412
P: P273-P501a

Specification

Solid medium for confirmative detection and isolation of Lancefield's group D streptococci in food samples, according to Mossel *et al.*

Formula* in g/L

Tryptone	20,00
Yeast extract	5,00
Sodium chloride	5,00
Disodium citrate	1,00
Esculin	1,00
Ferric-ammonium citrate	0,50
Sodium azide	0,15
Kanamycin sulfate	0,02
Agar	15,00

Final pH 7,0 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 48 g of powder in 1 L of distilled water and let it soak. Heat to boiling point and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

KAA confirmative Agar is a medium that several organisations and institutes recommend for detecting, enumerate and isolate Lancefield's group D streptococci in samples of food and beverages e.g.: bottled water, fresh/refrigerated/frozen/minced meat, fish, molluscs, soft drinks, pastries and spices. Kanamycin and sodium azide are the selective inhibitory compounds.

Technique

From samples considered positive, aliquots of 0,1 mL are inoculated onto the surface of the plates of KAA, spreading with a Drigalsky loop. Incubate the plates, in an inverted position, at 37°C for 24 hours. Colonies that appear surrounded by a black halo are considered as group D streptoccoci, and are isolated to confirm them biochemically and morphologically with the following tests: microscopical examination; catalase assay (that should be negative) in an azide-less medium; growth at 45°C and resistance to a high saline concentration [6,5% of NaCl in BHI Broth (Art. No. 02-599)].

Finally, they have to grow in Bile Esculin Agar (Art. No. 01-265) with an appearance similar to the colonies on the KAA Confirmative Agar. Nonetheless, there are some exceptions to this rule, i.e. *Streptococcus equinus* and *S. bovis* do not grow in the hypersaline broth, and therefore, definitive identification has to be performed by serological methods.
This methodology does not allow the enumeration of bacteria from the original sample, and as this is a necessary, the Most Probable Number (MPN) technique is recommended with KAA Presumptive Broth (Art. No. 02-263), using double strength broth if necessary.

References

- CeNAN. (1982) Técnicas para el Examen Microbiológico de Alimentos y Bebidas. Madrid.
- DOWNES, F.P. y K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- GUINEA, J., SANCHO, J. y PARES, R. (1979) Análisis Microbiológico de Aguas. Ed. Omega. Barcelona.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MOSSEL, D.A.A., P.G.M. BUKER, J. ELDERING (1978) Streptokokken der Lancefield Gruppe D in Lebensmitteln und Trinkwasser. Arch. F. Lebensmittelhyg. 29:121-127.
- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Kanamycin Esculin Azide Agar (KAA Agar)

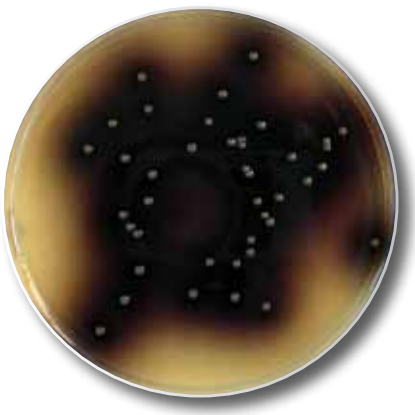
Art. No. 01-263

H: 4.1.C/3; H412
P: P273-P501a

Quality control

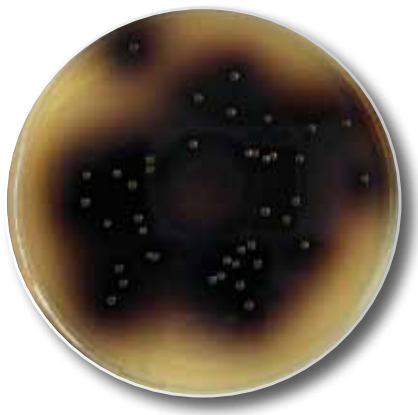
Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibited	-
Staphylococcus aureus ATCC 25923	Inhibited	-
Enterococcus faecalis ATCC 29212	Productivity > 0.70	Brown to black colonies (Esculin +)
Enterococcus faecalis ATCC 19433	Productivity > 0.70	Brown to black colonies (Esculin +)



Enterococcus faecalis ATCC 29212

Uninoculated plate (Control)



Enterococcus faecalis ATCC 19433

Kanamycin Esculin Azide Broth (KAA Broth)

Art. No. 02-263

WARNING
H: 3.1.O/4; H302-4.1.C/3; H412
P: P273-P264-P270-P301+P312-P330-P501a

Specification

Liquid medium for the presumptive detection of Lancefield's group D streptococci in food samples, according to Mossel *et al.*

Formula* in g/L

Tryptone	20,00
Yeast extract	5,00
Sodium chloride	5,00
Disodium citrate	1,00
Esculin	1,00
Ferric-ammonium citrate	0,50
Sodium azide	0,15
Kanamycin sulfate	0,02

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 33 g of powder in 1 L of distilled water. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

KAA Presumptive Broth is a medium that several International Organizations recommend to detect, enumerate and isolate Lancefield's group D streptococci in samples of food and beverages like: bottled water, fresh, refrigerated, or frozen minced meat, fish, molluscs, soft drinks, pastries, spices and other products. Kanamycin and sodium azide are the selective inhibitory compounds.

Technique

Prepare tubes with 9 mL of broth, and Petri plates with the agar. Make a 10-fold serial dilution bank from the sample in duplicate, and inoculate 1 mL amounts in the tubes. Incubate at 37°C for 24 hours.

The presumptive presence of streptococci is indicated by the development of a blackish-brown colour and the loss of fluorescence using a Wood's lamp. These tubes are considered as positive, and then, form these, inoculate 0,1 mL aliquots over the surface of the KAA plates spreading with a Drigalsky loop. Incubate the plates, in an inverted position, at 37°C for 24 hours. Colonies surrounded by a black halo are considered as group D streptoccoci, and are isolated to confirm their identity biochemically and morphologically with the following tests: microscopic examination, catalase assay (that should be negative) in an azide free medium, growth at 45°C and resistance to a high saline concentration (6,5% of NaCl in BHI Broth (Art. No. 02-599). Finally, they have to grow in Bile Esculin Agar (Art. No. 01-265) with a similar appearance to that of the colonies on the KAA. Nonetheless, there are some exceptions to this rule, i.e. *Streptococcus equinus* and *S. bovis* do not grow in the hypersaline broth, and therefore, definitive identification has to be performed by serological methods.

This methodology does not allow the enumeration of bacteria from the original sample, and as this is a necessary, the Most Probable Number (MPN) technique using the presumptive broth, is recommended, using double strength broth if necessary.

For bottled water, soft drinks and molluscs, CeNAN (*Centro Nacional de Alimentación y Nutrición,* in Spain) suggest the following technique:

Prepare broth tubes at normal concentration and at double strength. Using a sterile pipette, inoculate five broth tubes of double strength with 10 mL of sample. Inoculate five tubes of normal concentration with 1 mL of sample and five tubes of normal concentration with 0,1 mL of sample. Homogenize them well and incubate at 37°C for 48 hours. Tubes that show a blackish-brown colour after the incubation period, are considered positive. Carry out the counting using the MPN tables.

References

- CeNAN (1982) Técnicas para el Examen Microbiológico de Alimentos y Bebidas. Madrid.
- GUINEA, J., J. SANCHO & R. PARES (1979) Análisis Microbiológico de Aguas. Ed. Omega, Barcelona.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MOSSEL, D.A.A., P.G.M. BUKER, J. ELDERING (1978) Streptokokken der Lancefield Gruppe D in Lebensmitteln und Trinkwasser. Arch F. Lebensmittelhyg. 29:121-127.
- PASCUAL ANDERSON, M^{a}R^{a} (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- VANDERZANT & SPLITTSTOESSER (1992) Compendium of Methods for the Microbiological Examination of Food. 3rd ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Kanamycin Esculin Azide Broth (KAA Broth)

Art. No. 02-263

WARNING

H: 3.1.O/4; H302-4.1.C/3; H412

P: P273-P264-P270-P301+P312-P330-P501a

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity) (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibited	-
Staphylococcus aureus ATCC 25923	Inhibited	-
Enterococcus faecalis ATCC 29212	Good	Black medium
Enterococcus faecalis ATCC 19433	Good	Black medium

Left: Uninoculated Tube
Right: *Enterococus faecalis* ATCC 29212

Kenner Fecal Agar (KF Agar)

Art. No. 01-294

WARNING

H: 3.1.O/4; H302-4.1.C/3; H412

P: P273-P264-P270-P301+P312-P330-P501a

Specification

Solid and selective medium for enterococci enumeration and detection.

Formula* in g/L

Proteose peptone	10,000
Yeast extract	10,000
Sodium chloride	5,000
Sodium glycerophosphate	10,000
Maltose	20,000
Lactose	1,000
Sodium azide	0,400
Bromocresol purple	0,015
Agar	20,000

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 76,4 g of powder in 1 L of distilled water and bring to the boil stirring constantly. If it is to be used immediately, sterilisation is not necessary. If sterilization is necessary, sterilize in the autoclave in small volumes, at 121°C for 10 minutes maximum. Let agar cool to 50°C and add 10 mL/L of TTC Sterile Solution 1% (Art. No. 06-023). Homogenize well and distribute into Petri dishes.

Note: A non-homogeneous appearance is normal, and does not affect the medium´s quality and efficacy.

Description

Kenner, Clark and Kabler (1960, 1961) discovered that KF medium was excellent for detecting enterococci in polluted water. Carbohydrates in this medium (lactose and maltose) are utilised by most of enterococci, producing a big amount of acid and causing the indicator to turn from violet to yellow. Streptococci that do not belong the D group may also grow in the medium, but they do not produce enough acid to change the colour of the indicator. Other microorganisms are strongly inhibited by sodium azide. Enterococci reduce TTC to formazan and so their colonies are red in colour.

Technique

If the sample is suspected of being highly contaminated, prepare ten-fold serial dilutions bank and inoculate the surface with 0,1 mL of sample using a Drigalsky loop (spread plate method) or, if desired, inoculate the medium with 1 mL of sample using the pour plate method. Incubation should be carried out at 37°C for a 48 hours period.

After incubation, readings are performed by observing whether the indicator has turned from violet to yellow, and whether colonies are pink or red in colour.

It is very important to maintain the pH of the medium over 7,0, or otherwise, false results may occur. Sterilization for longer than the specified period could result in darkening of the sugar and thereby resulting in a decrease in the pH.

References

- CLESCERI, L.S., A.E. GREENBERG y A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater 20th ed. APHA, Washington.
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- KENNER, B.A., CLARK, H.F. y KABLER, P.W. (1961) Fecal Streptococci II. Quantification of Streptococci in faeces. Am. Inst. Publ. Health, 50:1553.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Kenner Fecal Agar (KF Agar)

Art. No. 01-294

WARNING

H: 3.1.O/4; H302-4.1.C/3; H412

P: P273-P264-P270-P301+P312-P330-P501a

Quality control

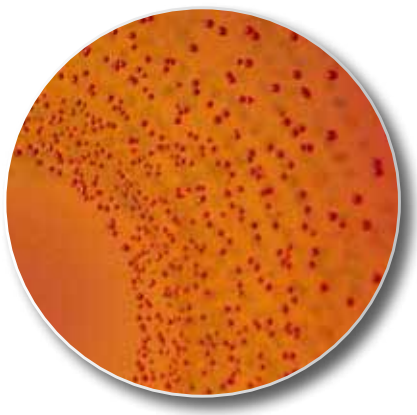
Incubation temperature: 35°C ± 2.0

Incubation time: 48 h

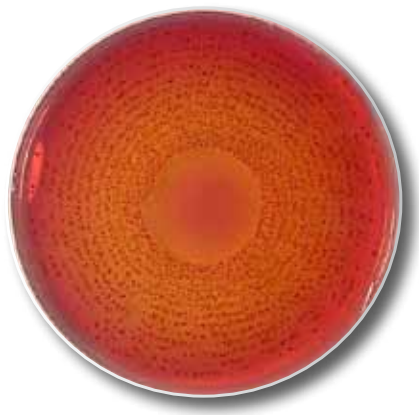
Inoculum: 10-100 CFU (Productivity)/1.000-10.000 CFU (Selectivity). Spiral Plate Method (or Membrane Filter Method)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Total inhibition	Selectivity
Escherichia coli ATCC 25922	Total inhibition	Selectivity
Enterococcus faecalis ATCC 29212	Productivity > 0.50	Dark red colonies.Yellow medium
Enterococcus faecalis ATCC 19433	Productivity > 0.50	Dark red colonies.Yellow medium

Enterococcus faecalis ATCC 29212



Enterococcus faecalis ATCC 29212
"Detail"



Enterococcus faecalis ATCC 19433

King A Agar (P Agar)

Art. No. 01-001

Also known as

Pigment Production Agar A; Ps Medium A; PsP; Tech Agar; King A Medium; Pseudomonas Agar Medium for detection of Pyocyanin.

Specification

Solid medium to enhance the pyocyanin production by *Pseudomonas aeruginosa* according to ISO 16266 and 22717 standards.

Formula* in g/L

Peptone	20,00
Magnesium chloride	1,40
Potassium sulfate	10,00
Agar	15,00

Final pH 7,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 46,4 g of powder in 1 L of distilled water with glycerol 10 mL and let soak. Heat, stirring constantly, until boiling. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. If tubes are used, let them solidify with short slant and good sized butt.

Description

This A medium was formulated by King, Ward and Raney in 1954 to enhance the pyocyanin production by *Pseudomonas aeruginosa.* The blue pigment, Pyocyanin, diffuses into the culture medium. Pyocyanin production varies depending on the strains of *Pseudomonas aeruginosa* present and on the growth conditions. Although this medium enhances blue pigment production in particular, it is possible that green (pyoverdine) or brown (pyomelanin) pigments also appear and mask the pyocyanin. Production of fluorescein and other pseudomonas pigments can be observed on other more suitable media, like King B Agar (Art. No. 01-029).

Technique

Slanted tubes or Petri dishes are inoculated by streaking and are then incubated at 30-32°C for 4-5 days. The disadvantage of using Petri plates is that the medium is subject to dehydration during incubation. Therefore, it is better to use slanted tubes being careful to aerate by loosening the screw caps or replacing them with cotton or aluminium caps.

In freshly isolated pathogenic strains from the pathological material, pigment production is often shown early i.e. after 24-48 hours of incubation, however if the material is non pathogenic or if it comes from water, food or soil, then the pigmentation can be delayed.

When the pigment is not the usual blue colour, it may be due to the production of two or more coloured substances. If it is not confirmed on other culture medium, it is recommended to confirm by extraction:

Using the culture slant, 0,5-1 mL chloroform is added, and is shaken for a few minutes until the pyocyanin is diffused, which turns the solvent blue. After that, the chloroform is acidified with a few drops of HCl, obtaining a rapid change in colour from blue to red, this colour changes confirms the presence of pyocyanin.

References

- ISO 16266:2006 Standard. Water Quality - Detection and enumeration of *Pseudomonas aeruginosa.* Method by membrane filtration.
- ISO 22717:2006 Standard. Cosmetics - Detection of *Pseudomonas aeruginosa.*
- KING E.O., M. WARD y D.E. RANEY (1954) Two simple media for the demonstration of pyocyanin and fluorescein. J.Lab.Clin.Med. 44:301-307.
- LENNETTE, E.H., E.W. SPAULDING y J.P. TROUANT (1974) Manual of Clinical Microbiology. 2nd. Ed. ASM. Washington.
- USP (2008) 31th ed. <61> Microbial Limit Tests. US Pharmacopeial Convention Inc. Rockville MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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King A Agar (P Agar)

Art. No. 01-001

Quality control

Incubation temperature: 30 - 32°C
Incubation time: 48 - 72 h
Inoculum: Streak Inoculation

Microorganism	Growth	Remarks
Pseudomonas fluorescens ATCC 49838	Good to very good	Without pigments. F (-)
Escherichia coli ATCC 8739	Good to very good	-
Pseudomonas aeruginosa ATCC 10145	Good to very good	Dark green
Pseudomonas aeruginosa ATCC 27853	Good to very good	Green to blue
Pseudomonas aeruginosa ATCC 25668	Good to very good	Green to blue
Pseudomonas aeruginosa ATCC 9027	Good to very good	Green to blue

Pseudomonas aeruginosa ATCC 9027

King B Agar (F Agar)

Art. No. 01-029

Also known as

Pigment Production Agar B; Ps Medium B; Fluorescein Agar; Flo Agar; Pseudomonas Agar Medium for the Detection of Fluorescein.

Specification

Culture media for enhancing the fluorescein production by Pseudomonas *spp*. according to EN 12780:2002 and ISO 16266, 22717 standards.

Formula* in g/L

Meat peptone	10,00
Casein peptone	10,00
Dipotassium phosphate	1,50
Magnesium sulfate	1,50
Agar	15,00

Final pH 7,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 38 g of powder in 1 L of distilled water with 10 mL of glycerol and let it soak. Heat to boiling and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. Cool by solidifying in slanted position with a long slant.

Description

F Medium was formulated by King, Ward and Raney in 1954 to enhance green fluorescent pigment (pyoverdine) production by *Pseudomonas fluorescens* and *P. aeruginosa*, in which pyocyanin production is restricted.

Green-yellowish pigments, soluble and fluorescent, define Pseudomonas group I according to the 9th edition of Bergey's Manual of Systematic Bacteriology, and therefore, detection of their production is critical.

Technique

Slanted tubes are inoculated with *Pseudomona* strains and incubated at 30-32°C for a 2-4 days period. If after this time a green-yellowish colour does not appear on the medium, the tubes should be kept under observation at room temperature for an additional period of 6-20 days before the culture can be regarded as negative. It should be noted that *Pseudomonas aeruginosa* and Pseudomonas *putida* strains obtained from water, soil or food, produce pigments slowly.

Pyoverdine is not soluble in chloroform, so the confirmation of its presence is usually done by a characteristic fluorescence verification under Wood's light (365 µm), comparing the suspected positive tube to another un-inoculated F Medium tube, which is considered as the control.

References

- DIN 38411 Standard (1991) Parte 6: Mikrobiologischen Verfahren (Gruppe K) Nachweis von *Escherichia coli* und coliformen keimen (K6).
- EN 12780 Standard (2002) Water Quality. Detection and enumeration of *Ps aeruginosa* by membrane filtration. CEN. Bruselas.
- ISO 16266 Standard (2006) Water Quality. Detection and enumeration of *Ps aeruginosa*. Method by membrane filtration.
- ISO 22717 Standard (2006) Cosmetics. Detection of *Pseudomonas aeruginosa*.
- KING, E.O., M.WARD y D.E. RANEY (1954) Two simple media for the demonstration of pyocyanin and fluorescein J.Lab.Clin.Med. 44:30-307.
- LENNETTE, E.H., E.W. SPAULDING y J.P. TROUANT (1974) Manual of Clinical Microbiology. 2nd ed. ASM. Washington.
- PALLERONI, N. (1984) The genus Pseudomonas, in Bergey's Manual of Systematic Bacteriology.
- USP (2008) 31th ed. <61> Microbial Limit Tests. US Pharmacopeial Convention Inc. Rockville. MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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King B Agar (F Agar)

Art. No. 01-029

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 48 h
Inoculum: 10-100 CFU (Productivity)/1.000-10.000 CFU (Selectivity). Spiral Plate Method (or Membrane Filter Method)

Microorganism	Growth	Remarks
Pseudomonas fluorescens ATCC 49838	Good to very good	F (+)
Esterobacter aerogenes ATCC 13048	Good to very good	Without pigment
Pseudomonas aeruginosa ATCC 27853	Good to very good	Yellow-green
Pseudomonas aeruginosa ATCC 25668	Good to very good	Yellow-green
Pseudomonas aeruginosa ATCC 9027	Good to very good	Yellow-green
Pseudomonas aeruginosa ATCC 10145	Good to very good	Yellow-green

Pseudomonas aeruginosa ATCC 9027

Pseudomonas fluorescens ATCC 49838

Pseudomonas aeruginosa ATCC 10145

Kligler Iron Agar (KIA)

Art. No. 01-103

Also known as

Iron Agar; Kligler's Iron Agar

Specification

Solid differential medium for primary identification of enterobacteria based on the fermentation of two sugars and the hydrogen sulfide production according to ISO standard 6340.

Formula* in g/L

Meat extract	3,00
Yeast extract	3,00
Peptone	20,00
Lactose	10,00
Sodium chloride	5,00
Dextrose	1,00
Ammonium ferrous citrate	0,50
Sodium tiosulfate	0,50
Phenol red	0,03
Agar	15,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 58 g of powder to 1 L of distilled water and bring to the boil. Distribute in tubes and sterilize in the autoclave at 121°C for 15 minutes. Let it solidify with a short slant and large butt.

Description

Kligler Agar is a differential medium that has all the characteristics of the 2-Sugar Russell Agar and Lead Acetate Medium for H_2S detection. In this medium, lactose fermentation and hydrogen sulfide production can be detected, allowing a presumptive identification of most enterobacteria. Sugar fermentation is shown by acid production, which turns the indicator from red to yellow. Since there is only a small amount of sugar (dextrose) in the medium, acid production due to its fermentation is very limited and re-oxidation of the indicator occurs on the surface of the medium, causing the indicator to remain red. When lactose is fermented, a large amount of acid is produced re-oxidation does not occur and the entire medium turns yellow.

Hydrogen sulfide production is indicated by the medium turning black, due to the reaction of H_2S (liberated from thiosulfate) with the iron ions presents in the ammonium iron citrate.

Technique

Kligler Iron Agar is used in slanted tubes with short slant and a generous butt, which are inoculated on the surface and also stab inoculated. The inoculum must be copious; it has to come from a solid medium, otherwise, readings may be delayed (up to additional 2-3 days). Normal incubation is 18 hours at 37°C.

Tubes with caps that allow ventilation, are recommended, such as cotton caps, cellulose caps or cap-o-test.

Should screw caps be used, do not tighten them otherwise they can hinder the re-oxidation of the indicator.

Kligler's medium provides excellent results if used freshly prepared, however if it has been prepared a few days beforehand, it is advisable to re-melt it and solidify it again to obtain more accurate readings.

A large production of H_2S may make the readings difficult, and hence early readings are strongly recommended.More precise readings are obtained if Three Sugar Iron Agar (Art. No. 01 -192) is used, since this contains sucrose allowing a greater differentiation between members of *Proteus*, *Salmonella* and *Shigella* spp.

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Typical Reaction of Enterobacteria on KIA

Genus and species	Slant	Butt	SH_2
Enterobacter aerogenes	Ac.	Ac. and G	-
Enterobacter cloacae	Ac.	Ac. and G	-
Escherichia coli	Ac.	Ac. and G	-
Proteus vulgaris	N	Ac. and G	+
Morganella morganii	N	Ac. or Ac. and G	-
Salmonella ssp.	N or Alk	Ac.	+
Salmonella cholerasuis	N or Alk	Ac. and G	+
Salmonella typhi	N or Alk	Ac.	+

Ac. = Acidification (Yellow); **N** = Neutral (No change); **Alk** = Alkalinization;
G = Gas production (bubbles); **+** = SH_2 production (Blackness)

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Kligler Iron Agar (KIA)

Art. No. 01-103

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Ratón. Fla. USA. DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods.4th ed. APHA. Washington. DC. USA.
- ISO 6340:1995 Standard. Water Quality - Detection of *Salmonella* species. Geneva.
- KLIGLER (1918) Modification of culture media used in the isolation and differentiation of thyphoid, dyesentery and allied bacilli. J.Exper Med. 28:319-332.
- KLIGLER (1917) A simple medium for the differentiation of members of thyphoid-parathyphoid groups. Am. J. Pub. Hlth 7:1042-1044.
- MaCFADDIN, J.F. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. William & Wilkins. Baltimore. MD. USA.
- RUSELL, F.F. (1911) The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. 25:217-220.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 18 - 24 h
Inoculum: Stab the butt and streak the slant

Microorganism	Growth	Remarks
Proteus vulgaris ATCC 6380	Good	Slant: N blackness; Butt: Ac H_2S (+)
Shigella flexneri ATCC 12022	Good	Slant: N; Butt: Ac; H_2S (-). Yellow medium
Escherichia coli ATCC 8739	Good	Slant: Ac; Butt: Ac / Gas; H_2S (-)
Escherichia coli ATCC 25922	Good	Slant: Ac; Butt: Ac / Gas; H_2S (-)
Salmonella enteritidis NCTC 6017	Good	Slant: N blackness; Butt: Ac H_2S (+)
Salmonella typhimurium ATCC 14028	Good	Slant: N blackness; Butt: Ac H_2S (+)

First: Uninoculated Tube (Control)
Second: *Shigella flexneri* ATCC 12022
Third: *Escherichia coli* ATCC 25922
Fourth: *Salmonella typhimurium* ATCC 14028

Lactose Broth

Art. No. 02-105

Specification

Medium for pre-enrichment and detection of enterobacteria and coliforms in milk and water according to ISO standards.

Formula* in g/L

Peptone	5,00
Meat extract	3,00
Lactose	5,00

Final pH 6,9 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 13 g of powder to 1 L of distilled water, or in the quantity required for the desired concentration.
Dissolve and distribute into containers fitted with Durham tubes. Sterilize in the autoclave at 121°C for 15 minutes. **Avoid further reheating.**

Description

Lactose Broth is a classical medium for use in the presumptive testing for coliforms and for the enrichment of *Salmonella*. This formulation is per the standards recommended by APHA, AWWA, USP-NF and ISO.
It is commonly used with Durham fermentation tubes for the detection of gas formation. If a specific volume of sample is to be inoculated this must be taken into consideration when making up the medium as the concentration must not be alteredon addition of the inoculum.
Although it is not Eijkman's original formulation, this broth provides excellent results in assays of gas production at 45°C, which is a characteristic of *Escherichia coli.*
While preparing this medium it is important to avoid overheating and to distribute it into tubes before sterilization.

References

- APHA-AWWA-WPCF (1998) Standard methods for the examination of water and wastewater. 20th ed. APHA Washington
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual 8th ed. Rev A. AOAC International. Gaithersburg. VA. USA.
- ISO 9308-2 Standard. (1990) Water Quality - Detection and enumeration of coliform organisms, thermotolerant coliform and presumptive *E. coli* - MPN technique.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 21150:2006 Standard. Cosmetics - Detection of *Escherichia coli*.
- US PHARMACOPOEIA (2005) <61> Microbial limit test. US Pharmacopeial Conv. Inc. Rockville. MD. USA.
- VANDERZANT & SPLITTSTOESSER (1992) Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Lactose Broth

Art. No. 02-105

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) (ISO/TS 11133-1/2).

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Good	Gas (-)
Enterococcus faecalis ATCC 29212	Good	Gas (-)
Escherichia coli ATCC 8739	Good	Gas (+)
Escherichia coli ATCC 25922	Good	Gas (+)
Salmonella typhimurium ATCC 14028	Good	Gas (-)
Citrobacter freundii ATCC 43864	Good	Gas (+)

Left: Uninoculate tube (Control)
Centre: *Escherichia coli* ATCC 25922
Right: *Salmonella typhimurium* ATCC 14028

"Detail"

Lactose Gelatin Medium

Art. No. 03-632

Specification

Solid medium used for the biochemical confirmation of *Clostridium perfringens*, according to ISO 7937 standard.

Formula* in g/L

Tryptone	15,00
Yeast Extract	10,00
Lactose	10,00
Gelatin	120,00
Phenol red	0,05

Final pH 7,50 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 155 g of powder in 1 L distilled water, heating if necessary. Dispense in tubes in suitable volumes and sterilize at 121°C for 15 minutes. If not used the same day, store in the refrigerator at 5 ± 3°C. Just prior to use heat in a boiling water bath or flowing steam for 15 minutes, then cool rapidly to the incubation temperature. Discard unused medium 3 weeks after preparation.

Description

This medium with the Nitrate Motility Medium (Art. No. 03-612) are used in the confirmation technique for *Clostridium perfringens* according to the 7937:2004 ISO Standard.

Technique

Inoculate each selected colony from the Tryptose-Sulfite-Cicloserine Agar (Art. No. 01-278) into the Lactose Gelatine Medium and incubate under anaerobic conditions for 24 hours at 37°C. Examine the tubes of Lactose Gelatine Medium for the presence of gas and a yellow colour due to acid formation indicating fermentation of lactose. Chill the tubes for 1 hour at 5 ± 3°C and check for gelatine liquefaction. If the medium has solidified, re-incubate for an additional 24 hours and again check for gelatine liquefaction.

Interpretation

Non-motile bacteria that produce black colonies in Tryptose Sulfite Cicloserine Agar (Art. No. 01-278) liquefy gelatine in 48 hours are considered to be *Clostridium perfringens.*

Cultures that show a faint reaction for nitrite should be eliminated, since *C. perfringens* consistently gives an intense and immediate reaction.

References

- ISO 7937 Standard (2004) Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of *Clostridium perfringens* -Colony count technique.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Pure cultures using and inoculating needle

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Poor	-
Escherichia coli ATCC 25922	Good	-
Clostridium perfringens ATCC 10543	Good	*L (+) Gas (+) Gelatinase (+)
Clostridium perfringens ATCC 13124	Good	*L (+) Gas (+) Gelatinase (+)
Clostridium sporogenes ATCC 11437	Good	*L (+) Gas (**D) Gelatinase (+)

*L = Lactose

**D = Production Doubtful

Left: Uninoculated Tube (Control)
Centre: *Clostridium sporogenes* ATCC 11437
Right: *Clostridium perfringens* ATCC 10543

Lactose Neutralizing Broth

Art. No. 02-666

Specification

Liquid medium used for the enrichment of *E. coli* from cosmetics according to ISO 21150:2006.

Formula* in g/L

Tryptone	5,00
Beef extract	3,00
Lactose	5,00
Lecithin	1,00
Polysorbate 80	5,00
Triton® X-100	1,00

Final pH 6,9 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20 g of the powder in 1 L of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. The medium is opalescent when hot, but clears as cooling.

Description

Lactose Neutralizing Broth is a liquid medium for *E. coli* recommended in the ISO: 21150:2006 Standard as an alternative enrichment medium. It is the classic Lactose Broth in which the dispersing agent Tritón® X-100 is added. Two other components, lecithin and polysorbate, are included. These substances neutralize almost all the preservatives in the matrix of the cosmetic product.

Technique

Disperse the sample in a suitable volume (1:10) of Lactose Neutralizing Broth and incubate at 32,5 ± 2,5°C for at least 20 hours to a maximum of 72 hours. Alter incubation subculture for isolation or for counting on suitable media as per an elected method.

References

- ISO 21150:2006 Standard. Cosmetics - Microbiology - Detection of *Escherichia coli.*
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 72 h
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Bacillus subtilis ATCC 6633	Good	-
Escherichia coli ATCC 25922	Good	-
Escherichia coli ATCC 8739	Good	-
Pseudomonas aeruginosa ATCC 27853	Good	-

Lactose Sulfite Broth Base

Art. No. 02-519

Also known as

CLS

Specification

Liquid medium for the determination of H_2S production by *Clostridium perfringens* according to ISO 7937 standard.

Formula* in g/L

Peptone	5,000
Yeast extract	2,500
Sodium chloride	2,500
Lactose	10,000
L-Cysteine	0,300

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve completely 10,1 g of powder in 500 mL of distilled water and sterilize in the autoclave at 121°C for 15 minutes. Cool and aseptically add a flask sterile sodium meta-bisulfite solution (Art. No. 06-114CASE or 06 -114-LYO) and a flask of sterile ammonium ferric citrate solution (Art. No. 06-113CASE or 06-113-LYO). Mix well and distribute into sterile containers containing Durham tubes.

Description

This is a simple medium that selects *C. perfringens* over other sulfite reducing clostridia by their ability to produce gas from lactose, at 46°C. *C. paraperfringens* also has this ability, however this microorganism is very rare in food samples.

Technique

All of the freshly prepared or reconstituted media tubes are inoculated in duplicate with 1 mL of the sample dilution. The sample dilution must have previously been kept in a boiling water bath, for 10 minutes. Tubes are incubated in anaerobic conditions at 46°C for a period of 18-24 hours. *C. perfringens* presence is observed by an iron sulfide precipitate appearing in the tubes. It indicates sulfite reducing activity. Accumulation of gas in the Durham's tubes is a sign of lactose fermentation.

Necessary supplements

Sodium Disulfite (Sodium Meta-Bisulfite) for Bacteriology (Art. No. 06-114CASE / 06-114-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Di-sodium sulfite ..... 375,00 mg

Distilled water (Solvent)

Ferric Ammonium Citrate Supplement (Art. No. 06-113CASE / 06-113-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Ferric ammonium citrate ..... 312,00 mg

Distilled water (Solvent)

References

- ISO Standard 7937 (2004) Microbiology of food and animals feeding stuffs. Horizontal method for enumeration of *Clostridium perfringens.* Colony count technique.
- PASCUAL ANDERSON, Mª R. (1992) Microbiología Alimentaria. Díaz de Santos. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 46°C ±1,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Inhibited	-
Staphylococcus aureus ATCC 25923	Inhibited	-
Escherichia coli ATCC 8739	Inhibited	-
Clostridium perfringens ATCC 13124	Good	H_2S (+) Gas (+)
Clostridium sporogenes ATCC 19404	Good	H_2S (+) Gas (-)
Clostridium perfringens ATCC 10543	Good	H_2S (+) Gas (+)

Left: *Clostridium perfringens* ATCC 10543
Centre: *Clostridium perfringens* ATCC 13124
Right: Uninoculated tube

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m-Lauryl sulfate Agar

Art. No. 01-524

Specification

Solid medium for the isolation and enumeration of coliform organisms and *E. coli* from water by membrane filtration.

Formula* in g/L

Peptone	39,00
Yeast extract	6,00
Lactose	30,00
Sodium Lauryl sulfate	1,00
Phenol red	0,20
Agar	15,00

Final pH 7,40 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 91,2 g of powder in 1 L of distilled water and bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. **Please note: Overheating can cause the lactose to darken**.

Description

MF-Laurylsulphate Agar is the solid version of the broth of the same name, and is successor to the Enrichment Teepol Broth formulated in 1976, after Teepol 610 disappeared off the market.

In liquid form this medium is recommended by the Dept. of Environment, Health & Social Security and the Public Health and Medical Service of the United Kingdom. It is recommended for the detection and enumeration of coliforms and *Escherichia coli* by the membrane-filtration technique without pre-enrichment. The solid version can be used in the same way as the absorbent pad with the broth.

The Lauryl sulfate acts as selective inhibitor of sporulating contaminants. At the formulated concentration the tension-active agent (Lauryl sulfate) has no effect over coliforms, and they grow quickly and abundantly from minute inocula.

The acid production from lactose is shown by the phenol red indicator turning from red to yellow. This change results in yellow colonies over a yellow zone in the medium.

Technique

Coliform enumeration and *E. coli* enumeration must be done in separate volumes of sample. The volume to be filtered must be careful selected to obtain 10-100 colonies on the membrane.

Water samples once filtered through a sterile membrane are placed on the surface of the MF Lauryl sulfate agar and incubated. Burman (1976), recommended the following times and temperatures of incubation for non-chlorinated waters:

Coliforms: 4 h at 30°C followed by 14 h at 35°C

Escherichia coli: 4 h at 30°C followed by 14 h at 44°C

In chlorinated waters it is better to change the first incubation step to 6 h at 25°C. The 44°C incubation is more reliable if is carried out in a hermetically sealed container in a water bath with rigorous control of the temperature. The presumptive colonies growing at 44°C must be confirmed with the production of gas from lactose and production of indol at 44°C.

References

- ATLAS R.M & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- BURMAN, N.P. (1976) Recent advances in Bacteriological Examination of Water, in Progress in Microbiological Techniques, edited by C.H. Collins. Butterworth. London.
- CORRY, J.E.L, G.D.W. CURTIS & R.M. BAIRD (2003) Handbook of Culture Media for Food Microbiology. Elsevier. Amsterdam.
- HOLDEN, W.S. (1970) Water Treatment and Examination. J & A Churchill. London.
- PHLS and DEPT. of ENVIRONMENT, HEALTH & SOCIAL SECURITY (1982) The Bacteriological Examination of Drinking Water Supplies. Report on Public Health and Medical Subjects No. 17. HMSO. London.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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m-Lauryl sulfate Agar

Art. No. 01-524

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10 - 100 CFU. Membrane filter Methods

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 19433	Inhibited	-
Escherichia coli ATCC 8739	Productivity > 0.50	Orange-Yellow media. Yellowish colonies L (+)
Escherichia coli ATCC 25922	Productivity > 0.50	Orange-Yellow media. Yellowish colonies L (+)
Pseudomonas aeruginosa ATCC 27853	Good	Red media. Colourles colonies L (-)
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Red media. Colourless colonies L (-)
Salmonella abony NCTC 6017	Productivity > 0.50	Red media. Colourless colonies L (-)

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LB Broth

Art. No. 02-384

Specification

Liquid medium used for general purposes, especially recommended for molecular genetics studies of *Escherichia coli*.

Formula* in g/L

Casein peptone.....10,00
Yeast extract.....5,00
Final pH 7,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 15 g of powder in 1 L of distilled water. Distribute into suitable containers and sterilize in the autoclave at 121ºC for 15 minutes.

Description

Formulation of this broth is according to the Luria and Bartani medium base, in which sodium chloride has been omitted to allow for the variation in saline concentration due to other additives.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- AUSUBEL, F.M., R. BRENT, R.E. KINGSTON, D.D. MORE, J.G. SEIDMAN, J.A. SMITH, & K. STRUHL (1994) Current Protocols in molecular Biology. Greene Pub.Assoc. Inc. Brooklyn. NY.
- GHERNA, R., P. PIENTA & R. COTE (Eds.) (1992) ATCC Catalogue of Bacteria and Bacteriophages. Medios #1065, #1082, #1226, #1235, #1236, #1315 y #1364. American Type Culture Collection. Rockville. MD. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LENNOX, E.S. (1955) Transduction of linked genetic carácter of the host bacteriophage P1. Virology 1:190-206.
- LURIA, S.E. & J.W. BURROUS (1955) Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. 74:461-476.
- MILLER, J.H. (1972) Experiments in molecular genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour. NY.
- SAMBROOK, J., E.F. FITSCH & T. MANIATIS (1989) Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbour Laboratory. Cold Spring Harbour. NY.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	-
Escherichia coli ATCC 8739	Good	-
Escherichia coli ATCC 35218	Good	-
Escherichia coli ATCC 11775	Good	-

LB Broth (Lennox)

Art. No. 02-406

Specification

Liquid medium used for general purposes, especially recommended for molecular genetics studies with *Escherichia coli.*

Formula* in g/L

Casein peptone.....10,00
Yeast extract.....5,00
Sodium chloride.....5,00
Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20 g of powder in 1 L of distilled water. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Formulation of this liquid medium is according to the Luria and Bertani Broth Base modified by Lennox.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- AUSUBEL, F.M., R. BRENT, R.E. KINGSTON, D.D. MORE, J.G. SEIDMAN, J.A. SMITH, & K. STRUHL (1994) Current Protocols in Molecular Biology. Greene Pub. Assoc. Inc. Brooklyn. NY.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LENNOX, E.S. (1955) Transduction of linked genetic carácter of the host bacteriophage P1. Virology 1:190-206.
- SAMBROOK, J., E.F. FITSCH & T. MANIATIS (1989) Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbour Laboratory. Cold Spring Harbour. NY.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	-
Escherichia coli ATCC 8739	Good	-
Escherichia coli ATCC 35218	Good	-
Escherichia coli ATCC 11775	Good	-

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LB Agar (Miller)

Art. No. 01-385

Specification

Solid medium for general purposes and recommended for use in molecular genetics studies of *Escherichia coli*.

Formula* in g/L

Casein peptone	10,00
Yeast extract	5,00
Sodium chloride	10,00
Agar	15,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 40 g of powder in 1 L of distilled water and bring to the boil stirring constantly. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

LB Media was originally formulated by Luria and Burrous, but Lennox added sodium chloride to improve the osmolarity of the medium. Formulation of this solid medium is according to the Lennox formulation, modified by Miller, who increased the sodium chloride concentration.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- AUSUBEL, F.M., R. BRENT, R.E. KINGSTON, D.D. MOORE, J.G. SEIDMAN, J.A. SMITH & K. STRUHL (1994) Current protocols in molecular biology. Greene Pub. Assoc. Inc. Brooklyn, N.Y.
- GHERNA, R., P. PIENTA, R. COTE (Eds.) 1992. ATCC Catalogue of Bacteria and Bacteriophages. Media #1065, #1226, #1226, #1235, #1236, #1315, #1364. American Type Culture Collection. Rockville MD. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LENNOX, E.S. (1955) Transduction of linked genetic character of the host bacteriophage P1. Virology 1:190-206.
- LURIA, S.E. & J.W. BURROUS (1955) Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. 74:461-476.
- MILLER, J.H. (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
- SAMBROOK, J., E.F. FITSCH & T. MANIATIS (1989) Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 11775	Productivity > 0.70	-
Escherichia coli ATCC 8739	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Escherichia coli ATCC 35218	Productivity > 0.70	-

LB Broth (Miller)

Art. No. 02-385

Specification

Liquid medium for general purposes, recommended for molecular genetics studies of *Escherichia coli.*

Formula* in g/L

Casein peptone.....10,00
Yeast extract.....5,00
Sodium chloride.....10,00
Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 25 g of powder in 1 L of distilled water. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

LB Media was originally formulated by Luria and Burrous, but Lennox added sodium chloride to improve the osmolarity of the media. The formulation of this broth is according to the Lennox recipe, modified by Miller, who has increased the sodium chloride concentration.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- AUSUBEL, F.M., R. BRENT, R.E. KINGSTON, D.D. MOORE, J.G. SEIDMAN, J.A. SMITH & K. STRUHL (1994) Current protocols in molecular biology. Greene Pub. Assoc. Inc. Brooklyn. NY.
- GHERNA, R., P. PIENTA & R. COTE (Eds.) 1992. ATCC Catalogue of Bacteria and Bacteriophages. Media #1065, #1226, #1226, #1235, #1236, #1315, #1364. American Type Culture Collection. Rockville. MD. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LURIA, S.E. & J.W. BURROUS (1955) Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. 74:461-476.
- LENNOX, E.S. (1955) Transduction of linked genetic character of the host bacteriophage P1. Virology 1:190-206.
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- SAMBROOK, J., E.F. FITSCH & T. MANIATIS (1989) Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory. Cold Spring Harbor. NY.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35 °C
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	-
Escherichia coli ATCC 35218	Good	-
Escherichia coli ATCC 25922	Good	-
Escherichia coli ATCC 11775	Good	-

Right: uninoculated tube (Control)
Centre: *Escherichia coli* ATCC 11775
Left: *Escherichia coli* ATCC 8739

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Legionella BCYE Agar Base

Art. No. 01-687

Also known as

CYE

Specification

Solid medium base used for the detection, isolation and enumeration of *Legionella* from water according to the ISO standards 11731:1998 and 11731-2:2004.

Formula* in g/L

Activated Charcoal	2,00
Yeast Extract	10,00
Agar	15,00

Final pH 6,9 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 13,5 g of powder in 500 mL of distilled water and bring to the boil dissolving completely. Sterilize by autoclaving at 121°C for 15 minutes. Allow to cool to 47-50°C and add aseptically a reconstituted vial (Art. No. 06-137-LYO) of *Legionella* BCYE Growth Supplement. Mix gently and pour into Petri dishes. If a selective medium for *Legionella* is desired, it can be obtained by the addition of a vial (Art. No. 06-138-LYO) of *Legionella* GVPC Selective Supplement to 500 mL of BCYE Medium melted and cooled to 47-50°C.

If the control medium BCYE – Cys is desired it can be obtained by the addition of a reconstituted vial of (Art. No. 06-134-LYO) *Legionella* BCYE w/o Cysteine Growth Supplement to the sterile, melted and cooled *Legionella* BCYE Agar Base.

Description

The actual formulation of this medium is according to the ISO Standards 11731 and 11731-2, but BCYE Agar is based in a modification of a previously described media. In 1979 Feeley and collaborators described Charcoal Yeast Extract (CYE) Agar as a modification of the F-G Agar. They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in a better recovery of *Legionella pneumophila*. Pasculle, in 1980, reported that CYE Agar could be improved by buffering the medium with ACES buffer and a year later Edelstein increased the sensitivity of the medium by adding α-ketoglutarate which is the present formulation (BCYE Agar).

The medium consist of a Medium base supplemented with growth factors (BCYE Agar) and the Selective Medium supplemented with inhibitors of undesirable accompanying flora. The yeast Extract supplies the basic nutrients as the medium contains no fermentable carbohydrates. L-Cysteine, Ferric pyrophosphate and α-ketoglutarate are incorporate to satisfy the specific nutritional requirements of *Legionella* species.

The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO_2 and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth. The selectivity is increased by the addition of Vancomycin and polymyxin B which inhibit Gram-positive bacteria and cycloheximide or natamycin which are antifungal agents and inhibits the yeast growth.

Technique

Refer to the ISO Standards 11731 and 11731-2 or other standard procedures to obtain isolated colonies from specimens and samples.

Allow the inoculated plates to stand until the inocula has been absorbed. Invert the plates and incubate at 36 ± 1°C for up to 10 days. To ensure the atmosphere in the incubator is humid, place a tray of water in the bottom of the incubator. Top up this tray with fresh water (if necessary) each time the plates are examined. Incubation in an atmosphere of air with 2,5% (volume fraction) CO_2 may be beneficial for the growth of some *Legionella*, but it is not essential.

Examine the plates with a plate microscope on at least three occasions at intervals of 2 to 4 days during the 10-day incubation period, as *Legionella* grow slowly an can be masked by the growth of other organisms. Record the number of each type of colony present.

Colonies of *Legionella* are often white-grey-blue-purple in colour, but may be brown, pink, lime-green or deep-red. They are smooth with a smooth edges and exhibit a characteristic ground-glass appearance. Under ultraviolet light colonies of several species autofluoresce brilliant white, but others are red and *L. pneumophila* appear dull green often tinged with yellow. All presumptive colonies must be confirmed by cultural, biochemical, serological or genetic methods.

Necessary supplements

Legionella BCYE Growth Suplement (Art. No. 06-137-LYO)

ACES Buffer	3,600 g
Potassium hydroxide	1,400 g
Ferric pyrophosphate	0,125 g
L-Cysteine HCl	0,200 g
Potassium α–ketoglutarate	0,500 g

Distilled water (Solvent)

Legionella GVPC Selective Suplement (Art. No. 06-138-LYO) and

Vancomycin	0,50 mg
Polymyxin B sulfate	40000,00 IU
Cycloheximide	40,00 mg
Glycine (ammonia free)	1,50 g

Distilled water (Solvent)

(continues on the next page)

Legionella BCYE Agar Base

Art. No. 01-687

***Legionella* BCYE w/o Cysteine NO Growth Suplement (Art. No. 06-134-LYO)**

Vial Contents:

Necessary amount for 500 mL of complete medium.

ACES Buffer	3,600 g
Potassium hydroxide	1,400 g
Ferric pyrophosphate	0,125 g
Potassium α–ketoglutarate	0,500 g

Distilled water (Solvent)

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. BocaRaton. Fla. USA
- CLESCERI, L.S., A.E. GREENBERG & A.D. EATON (1998) Standard methods for the examination of water and wastewater. 9-106. 20th edition. APHA-AWWA-WEF. Washington DF, USA.
- EDELSTEIN, P.H., (1981) Improved semiselective medium for the isolation of *Legionella pneumoniae* from contaminated clinical and environmental specimens. J. Clin Microbiol. 14(3):298
- FEELEY, J.C., R.J. GIBSON, G.W. GORMAN, N.C. LANGFORD, J.K. RASHEED, C.D. MACKEL, & W.B. BAINE (1979) Charcoal-Yeast Extract Agar: Primary isolation medium for *Legionella pneumophila*. J. Clin. Microbiol. 10(4) 437
- ISO 11731 Standard (1998) Water Quality – Detection and Enumeration of *Legionella*.
- ISO 11731-2 Standard (2004) Water Quality – Detection and Enumeration of *Legionella* – Part 2: Direct membrane filtration method for waters with low bacterial counts. Williams & Wilkins, Baltimore, Md, USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MacFADDIN, J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria.
- PASCULLE, A.W., J.C. FEELEY, R.J. GIBSON, L.G. CORDES, R.L. MYEROWITZ, C.M. PATTON, G.W. GORMAN, C.L. CARMACK, J.W. EZZELL & J.N. DOWLING (1980) Pittsburgh pneumonia agent: Direct isolation from human lung tissue. J. Infect. Dis., 141:727.
- WARD, K.W. (1995) Processing and interpretation of specimens for *Legionella spp*. In "Clinical Microbiology Procedures Handbook" Chap. 12.1 edited b H.D. Isenberg. ASM Press. Washington DC, USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 3 - 10 days

Inoculum: 150-300 CFU (Productivity) // 1.000-10.000 CFU (Selectivitu). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Legionella pneumophila ATCC 33152	Good	Grey - white colonies
Staphilococcus epidermidis ATCC 12228	Inhibited	-

Letheen Agar

Art. No. 01-236

Also known as

AOAC Letheen Agar; TGE w: Lecithin w. Polysorbate 80

Specification

Solid medium for assays of antimicrobial action of Quaternary Ammonium Compounds (QAC's).

Formula* in g/L

Tryptone	5,00
Meat extract	3,00
Dextrose	1,00
Lecithin	1,00
Agar	15,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 25 g of powder in 1 L of distilled water with 7 mL of Polysorbate 80 (Art. No. TW0080). Let it soak and bring to the boil stirring constantly. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Letheen Agar is formulated according to AOAC guidelines, that were taken from the research work of Weber and Black for the assay of bactericidal action of Quaternary Ammonium Compounds (QAC's). In fact, the medium is the classical formulation for standard counting methods with the addition of lecithin and polysorbate, which act as the neutralizers of the QAC's.

References

- ASTM Standard E 640-78 (1991) Test method for preservatives in water-containing cosmetics. Philadelphia. PA.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- FDA (Food and Drug Adminstrations) (1998) Bacteriogical Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburg. MD.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- LUCAS, J.P. (1977) Microbiological Examination of Cosmetics. Newburguers' Manual of Cosmetic Analysis. AOAC.
- WEBER, G.R. & L.A. BLACK (1948) Relative efficiency of quaternary inhibitors. Soap and Sanit Chem. 24:134-139.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Staphylococcus epidermidis ATCC 12228	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-

Letheen Broth

Art. No. 02-236

Specification

Liquid culture medium used for the determination of germicidal activity coefficients of cationic detergents.

Formula* in g/L

Peptone	10,00
Meat extract	5,00
Lecithin	0,70
Sodium chloride	5,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20,7 g of powder in 1 L of distilled water with 5 mL of Polysorbate 80 (Art. No. TW0080). Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This is the liquid version of Letheen Agar (Art. No. 01-236), recommended by AOAC to verify the germicidal activity coefficients in cationic soaps. The formulation is not the same as the agar.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- HORWITZ, W. (2000) Official Methods of Analysis. AOAC International. Gaithersburg. MD. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- LUCAS, I.P. (1977) Microbiological Examination of Cosmetics. Newburger's Manual of Cosmetic Analysis AOAC. Washington.
- WEBER, G.R. & L.A. BLACK (1948) Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. 24:134-139.

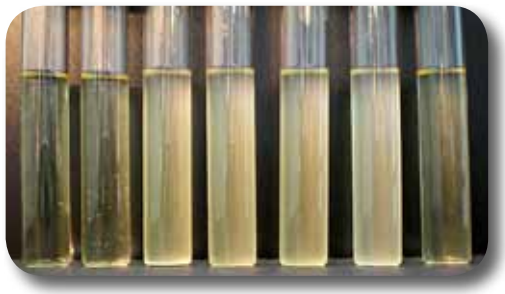
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	-
Staphylococcus aureus ATCC 6538	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Escherichia coli ATCC 8739	Good	-
Enterococcus faecalis ATCC 19433	Good	-
Yersinia enterocolitica ATCC 9610	Good	-



Letheen Modified Agar

Art. No. 01-237

Specification

Solid medium for the primary screening of microorganisms in cosmetics according to the FDA.

Formula* in g/L

Casein peptone	10,00
Meat peptone	10,00
Meat extract	3,00
Yeast extract	2,00
Dextrose	1,00
Lecithin	1,00
Sodium chloride	5,00
Sodium bisulfite	0,10
Agar	15,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 47 g of powder in 1 L of distilled water and add 7 mL of Polysorbate 80 (Art. No. TW0080). Allow it to soak and bring to the boil stirring constantly. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

In the early 40's, Weber and Black recommended the use of lecithin and polysorbates to neutralize the antimicrobial action of the Quaternary Ammonium Compounds (QAC's).

In 1965 the methodology was accepted by the AOAC for the antimicrobial assays and extends their use to all the cationic surfactants (detergents).

The TAT (Tryptone-Azolectin-Polysorbate) medium, in the Newburger Cosmetic Analysis Manual, (2nd ed., 1977) is similar in composition and uses the AOAC formulation. In 1978 the FDA (Bacteriological Analytical Manual, 5th ed., 1978) incorporated it as primary presumptive and enrichment medium for all microbial examinations of cosmetics.

The present formulation appears in the 8th ed. (1998) of the BAM and the notable modifications are the inclusion of sodium chloride providing suitable osmotic pressure and a increased amount of peptones and tissue extracts to promote good growth, these transforms this medium into a very rich all-purpose medium suitable for neutralizing almost all preservatives present in samples for examination.

References

- ASTM Standard E 640-78 (1991) Test Method for the preservatives in water-containing cosmetics. Philadelphia. PA. USA.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburg. MD. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- WEBER, G.R. & L.A. BLACK (1948). Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. 24:134-139.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

(continues on the next page)

Letheen Modified Agar

Art. No. 01-237

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Staphylococcus epidermidis ATCC 12228	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-

Enterococcus faecalis ATCC 19433

Escherichia coli ATCC 25922

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Letheen Modified Broth

Art. No. 02-237

Specification

FDA recommended liquid medium for the primary recovery of stressed microorganisms in the microbial examination of cosmetics.

Formula * in g/L

Casein peptone	15,00
Meat peptone	10,00
Meat extract	5,00
Yeast extract	2,00
Sodium chloride	5,00
Lecithin	0,70
Sodium bisulfite	0,10

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 37,8 g of the powder in 1 L of distilled water with 5 mL of Polysorbate 80 (Art. No. TW0080). Distribute in suitable containers and autoclave at 121°C for 15 minutes.

Description

In the early 40's, Weber and Black recommended the use of lecithin and polysorbates to neutralize the antimicrobial action of the Quaternary Ammonium Compounds (QAC's).

In 1965 the methodology was accepted by the AOAC for antimicrobial assays and extends their use to all the cationic surfactants (detergents).

The TAT (Tryptone-Azolectin-Polysorbate) medium, in the Newburger Cosmetic Analysis Manual, (2nd ed., 1977) is similar in composition and uses the AOAC formulation. In 1978 the FDA (Bacteriological Analytical Manual, 5th edition, 1978) incorporated it as a primary presumptive and enrichment medium for all microbial examination of cosmetics.

The present formulation appears in the 8th edition (1998) of the BAM and the notable modification are the inclusion of sodium chloride providing suitable osmotic pressure and an increased amount of peptones and tissue extracts to promote good growth, these transform this medium into a very rich all-purpose medium suitable for neutralizing almost all preservatives presents in samples under examination.

The ISO Technical Committee on Cosmetics (ISO/TC 217) (2006) has also adopted the present formulation as an alternative enrichment medium prior to microbiological examination but ideally Eugon LT100 Broth should be employed for this. (Art. No. 02-654).

References

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- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
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- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 21149 Standard (2006) Cosmetics - Enumeration and detection of aerobic mesophilic bacteria.
- ISO 22717 Standard (2006) Cosmetics - Microbiology - Detection of *Pseudomonas aeruginosa*.
- ISO 22718 Standard (2006) Cosmetics - Detection of *Staphylococcus aureus*.
- LUCAS, I.P. (1977) Microbiological Examination of Cosmetics. Newburger's Manual of Cosmetic Analysis AOAC. Washington.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Letheen Modified Broth

Art. No. 02-237

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	-
Staphylococcus aureus ATCC 6538	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Escherichia coli ATCC 8739	Good	-
Enterococcus faecalis ATCC 19433	Good	-
Yersinia enterocolitica ATCC 9610	Good	-

Liebermeister & Braveny Agar

Art. No. 01-446

Specification

Solid medium for the selective isolation of β-haemolytic streptococci from throat samples.

Formula* in g/L

Meat peptone	1,00
Meat extract	0,60
Yeast extract	0,50
L(+)Lysine	0,02
Sodium chloride	6,00
Disodium phosphate	2,00
Agar	15,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 25 g of powder in 930 mL of distilled water and bring to the boil. Distribute into containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45°C, then add 70 mL/L of defibrinated Sheep blood. Homogenize well and pour into plates.

Description

Despite its simplicity, this medium has a better yield in the recovery of β-haemolytic streptococci than commonly used Blood Agar.

Okamoto *et al.* and, later Bernheimer & Rodbart demonstrated the strong stimulatory effect of nucleic acids on the haemolytic properties of streptococci. Liebermeister & Braveny formulated a medium with insufficient nutrients for the normal development of microorganisms but with an increased amount of nucleic acids in the yeast extract. Moreover, they also included lysine, which has a stimulatory effect on haemolysis similar to that the nucleic acids.

The result is that β-haemolysis streptococci form only small colonies that have zones of haemolysis of average or greater than average size. Viridans streptococci (α-haemolytic) show virtually no growth, and if haemolysis zones form at all, they are minimal.

Technique

Plates are surface inoculated and incubated at 37°C for 24-48 hours. After incubation small colonies form which are surrounded by large, well defined haemolytic zone. Staphylococci and enterococci are almost completely inhibited and also the majority of viridans streptococci.

References

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- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Liebermeister & Braveny Agar

Art. No. 01-446

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	β -haemolysis
Escherichia coli ATCC 8739	Productivity > 0.70	γ -haemolysis
Enterococcus faecalis ATCC 29212	Productivity > 0.70	γ -haemolysis
Enterococcus faecalis ATCC 19433	Productivity > 0.70	γ -haemolysis
Staphylococcus aureus ATCC 6538	Productivity > 0.70	β -haemolysis
Streptococcus pneumoniae ATCC 49619	Productivity > 0.70	β -haemolysis
Streptococcus pyogenes ATCC 19615	Productivity > 0.70	α -haemolysis

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Listeria Enrichment Broth Base (UVM)

Art. No. 02-472

Specification

Liquid culture medium for the enrichment of *Listeria spp.*

Formula* in g/L

Proteose peptone	5,00
Tryptone	5,00
Meat extract	5,00
Yeast extract	5,00
Sodium chloride	20,00
Esculin	1,00
Disodium phosphate	12,00
Dipotassium phosphate	1,35

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 54,35 g of powder into 1 L of distilled water. Distribute 500 mL in each container and autoclave at 121°C for 15 minutes. Cool to 50°C and then aseptically add the selective supplement to each 500 mL: UVM I for primary enrichment (Art. No. 06-106CASE or 06-106-LYO) and UVM II/ Fraser for secondary enrichment (Art. No. 06-111CASE or 06-111-LYO).

Note: Prepared medium (broth + supplement) must be kept away from light, since it promote the production of acriflavine-oxidised photo-complexes that repress *Listeria* growth.

Description

This broth base for the enrichment of *Listeria* is made according to the AOAC modifications of the Vermont University Medium (UVM). Having an increase in acriflavine concentration in the secondary enrichment, and a strong reduction in the amount of nalidixic acid in all stages allows more positive isolations of *Listeria*.

Technique

Primary enrichment

Add 25 g or 25 mL of sample to 225 mL of primary enrichment broth (Broth Base, Art. No. 02-472, and UVM I, Art. No. 06-106CASE or 06-106-LYO). Homogenize in a Stomacher® for 2 minutes and incubate the mixture at 30°C for 24 hours. After the first 4 hours inoculate aliquots of 0,2 mL on Oxford Selective Agar (Art. No. 01-471) plates for isolation purposes.

Secondary enrichment

After 24 hours of primary enrichment, inoculate the secondary enrichment broth (Broth Base, Art. No. 02-472, and UVM II/Fraser, Art. No. 06-111CASE or 06-111-LYO) in a ratio of 1:100. Incubate at 30°C. After 4 and 24 hours inoculate aliquots of 0,2 mL on Oxford Selective Agar (Art. No. 01-471) plates for isolation purposes.

Isolation

Isolation is carried out on the Oxford Selective Agar (Art. No. 01-471 + Selective Supplement, Art. No. 06-127-LYO) plates. Incubate for 24-48 hours at 30-37°C. It can be advantageousto alkalinize the inoculum before inoculation, by mixing 1 mL of enrichment broth with 5 mL of 0,5% sterile KOH solution.

Necessary supplements

Listeria Selective Supplement for Primary Enrichment (UVM I) (Art. No. 06-106CASE or 06-106-LYO)

Vial contents:

Necessary amount for 500 mL of complete medium.

Nalidixic acid, sodium salt	10,00 mg
Acriflavine	6,00 mg

Distilled water (Solvent).

Listeria Selective Supplement for Secondary Enrichment (UVM II / FRASER) (Art. No. 06-111CASE or 06-111-LYO)

Vial contents:

Necessary amount for 500 mL of complete medium.

Nalidixic acid, sodium salt	10,00 mg
Acriflavine	12,50 mg

Distilled water (Solvent).

References

- ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. FL.
- McCLAIN, D. & W.H. LEE (1988) Development of USDA-FSIS Method for isolation of *Listeria monocytogenes* from raw meat and poultry. JAOAC 71:3:660-664.
- VANDERZANT, C & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Listeria Enrichment Broth Base (UVM)

Art. No. 02-472

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibited	-
Staphylococcus aureus ATCC 25923	Inhibited	-
Listeria monocytogenes ATCC 19112	Good	-
Listeria monocytogenes ATCC 19115	Good	-
Listeria monocytogenes ATCC 7644	Good	-

Left: Uninoculated tube (Control)
Centre: *Listeria monocytogenes* ATCC 19115
Right: *Listeria monocytogenes* ATCC 7644

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Listeria Enrichment Broth Base (Lovett)

Art. No. 02-498

Specification

Liquid culture medium for the enrichment of *Listeria*, according to Lovett *et al.*

Formula* in g/L

Tryptone	17,00
Yeast extract	6,00
Soy peptone	3,00
Sodium chloride	5,00
Dextrose	2,50
Dipotassium phosphate	2,50

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 36 g of powder in 1 L of distilled water and distribute 500 mL per flask. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and aseptically add to each flask the contents of one vial of *Listeria* Supplement for Selective Enrichment according to FDA/IDF (Art. No. 06-107CASE or 06-107-LYO). Homogenize and distribute into suitable containers.

Note: Prepared medium (broth + supplement) must be kept away from light, since it promotes the production of acriflavine oxidised photo complexes that repress *Listeria* growth.

Description

This media formulation according to Lovett *et al.* has been adopted by the FDA for the analysis of food, and it is recommended by the IDF/FIL for the selective enrichment of *Listeria* in milk samples, due to its good results in the recovery of stressed bacteria.

Technique

Mix the sample (25 mL or 25 g) with 225 mL of complete enrichment broth and incubate at 30°C for 7 days. Make subcultures after 24 hours, 48 hours and 7 days in the following way:

- Inoculate 0,5 mL of enrichment culture onto solid medium for the *Listeria* isolation (Oxford Agar Base, Art. No. 01-471, or Palcam Agar Base, Art. No. 01-470, with their respective selective supplements).
- Alkalinize 0,5 mL of enrichment culture by mixing with 4,5 mL of 0,5% sterile KOH solution and inoculate onto solid medium for *Listeria* isolation.

Necessary supplements

Listeria Selective Supplement for Enrichment according to FDA/IDF (Art. No. 06-107CASE or 06-107-LYO)

Vial contents:

Necessary amount for 500 mL of complete medium.

Nalidixic acid, sodium salt	20,00 mg
Cycloheximide	25,00 mg
Acriflavine	7,50 mg

Distilled water (Solvent)

References

- ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.
- LOVETT, J., D.W. FRANCIS & J.M. HUNT (1988) *Listeria monocytogenes* in raw milk: Detection, incidence and pathogenicity. J. Food Protect. 50:188-192.
- LOVETT, J. & A.D. HITCHINS (1989) *Listeria* isolation. FDA (Food and Drug Adminstrations) Bacteriological Analytical Manual. 6th ed. Supplement Sept. 1987 (2nd Print):29.01.
- VANDERZANT, C & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Listeria Enrichment Broth Base (Lovett)

Art. No. 02-498

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Listeria monocytogenes ATCC 19112	Good	-
Listeria monocytogenes ATCC 19114	Good	-
Listeria monocytogenes ATCC 7644	Good	-

Left: Uninoculated tube(Control)
Centre: *Listeria monocytogenes* ATCC 19114
Right: *Listeria monocytogenes* ATCC 19112

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Listeria Enrichment Broth Base (Fraser)

Art. No. 02-496

Specification

Liquid culture medium used for the enrichment and detection of *Listeria spp.*, according to ISO standards.

Formula* in g/L

Proteose peptone	5,00
Tryptone	5,00
Meat extract	5,00
Yeast extract	5,00
Sodium chloride	20,00
Esculin	1,00
Disodium phosphate	12,00
Monopotassium phosphate	1,35
Lithium chloride	3,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 57,4 g of powder in 1 L of distilled water. Distribute 500 mL per flask and sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C. Aseptically add one vial of Ferric Ammonium Citrate for Bacteriology (Art. No. 06-112-LYO) and one vial of *Listeria* Supplement for Secondary Enrichment UVM II/Fraser (Art. No. 06-111CASE or 06-111-LYO) to each flask and homogenize well.

To obtain the Half Fraser Broth, two vials of (Art. No. 06-112-LYO) and one vial of 06-145-LYO to 1000 mL of Broth Base. Only acriflavine and nalidixic acid are reduced to half concentration.

Note: Prepared medium (broth + supplement) must be kept away from light, since it promotes the production of acriflavine oxidised photocomplexes that repress *Listeria* growth.

Description

This broth base for *Listeria* enrichment is according to the modifications made to the University of Vermont Medium (UVM) by Fraser and Sparber. This formulation has been adopted by the USDA-FSIS. The inclusion of lithium chloride inhibits the development of enterococci which can also hydrolyze esculin in the same way as *Listeria*. Any blackening of the medium produced by the reaction of esculetin due to esculin hydrolysis, with iron present in the medium, can be taken as presumptive *Listeria*. The ferric citrate also helps with the development of *L. monocytogenes*.

Technique

Although some authors use Fraser Broth as the only enrichment medium, it has been verified than better results are obtained if it is employed as a secondary enrichment step, according to the following methodology:

- Inoculate the sample in a primary enrichment broth (UVM I, Art. No. 02-472 or Lovett Broth, Art. No. 02-498) and incubate for 18-24 hours.
- Take aliquots of 0,1 mL, and inoculate them in tubes with 10 mL of Fraser Broth and incubate for 24-28 hours.
- Tubes that blacken are considered presumptively positive and must be sub-cultured on isolation and confirmation solid media, such as Oxford Agar Base (Art. No. 01-471) or Palcam Agar Base (Art. No. 01-470) or ALOA Medium. Tubes that remain clear are considered negative and can be discarded or incubated for a further 24 hours if in doubt.

Necessary supplements

Listeria Selective Supplement for Secondary Enrichment (UVM II / Fraser) (Art. No. 06-111CASE / 06-111-LYO)

Vial contents:

Necessary amount for 500 mL of complete medium.

Nalidixic acid, sodium salt	10,00 mg
Acriflavine	12,50 mg

Distilled water (Solvent)

Ferric Ammonium Citrate Supplement (Art. No. 06-112-LYO)

Vial contents:

Necessary amount for 500 mL of complete medium.

Ferric ammonium citrate	250,00 mg

Distilled water (Solvent)

Listeria Selective Supplement for Primary Enrichment (Half Fraser) (Art. No. 06-145-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Nalidixic acid, sodium salt	5,00 mg
Acriflavine	6,20 mg
Ammonium ferric citrate	250,00 mg

Distilled water (Solvent)

Listeria Selective Supplement for Primary Enrichment (Half Fraser) (Art. No. 06-136-LYO)

Vial Contents:

Necessary amount for 225 mL of complete medium.

Nalidixic acid, sodium salt	2,25 mg
Acriflavine	2,80 mg
Ammonium ferric citrate	112,50 mg

Distilled water (Solvent)

References

- ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.
- FRASER, J.A. & W.H. SPERBER (1988) Rapid detection of *Listeria spp.* In food and environmental samples by esculin hydrolysis. J. Food Prot. 51:762-765.
- ISO 11290 Standard (1996) Microbiology of food ad animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria*

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Listeria Enrichment Broth Base (Fraser)

Art. No. 02-496

monocytogenes-Part 1: Detection Method.

- ISO 11290 Standard (1996) / Amd 1 (2004) Microbiology of food ad animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes*-Part 1: Detection Method-Amendment 1: Modification of the isolation media and the haemolysis test and inclusion of precision data.
- ISO 11290 Standard (1998) Microbiology of food ad animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes*.-Part 2: Enumeration method.
- ISO 11290 Standard (1998) / Amd 1 (2004) Microbiology of food ad animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes*-Part 2: Enumeration method-Amendment 1: Modification of the enumeration media.
- MacCLAIN, D. & W.H. LEE (1988) Development of a USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J.AOAC 71:660-664.
- VANDERZANT, C & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity).

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 19433	Partial inhibition	-
Escherichia coli ATCC 25922	Partial inhibition	-
Listeria monocytogenes ATCC 19112	Good	Black medium. Esculin (+)
Listeria monocytogenes ATCC 7644	Good	Black medium. Esculin (+)
Listeria monocytogenes ATCC 19115	Good	Black medium. Esculin (+)
Staphylococcus aureus ATCC 25923	Inhibited	-

Left: Uninoculated Tube (Control)
Centre: *Listeria monocytogenes* ATCC 19112
Right: *Listeria monocytogenes* ATCC 19112

Lysine Iron Agar (LIA)

Art. No. 01-094

Specification

Differential medium for Enterobacteria, recommended by Edwars and Ewing for *Salmonella* and *Arizona arizonae* (now know as *Salmonella choleraesuis subsp. arizonae*) identification.

Formula* in g/L

Gelatin peptone	5,00
Yeast extract	3,00
Dextrose	1,00
Lysine	10,00
Ammonium ferric citrate	0,50
Sodium thiosulfate	0,04
Bromocresol purple	0,02
Agar	15,00

Final pH 6,7 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 34,5 g of powder in 1 L of distilled water and bring to the boil. Dispense in tubes and sterilize in the autoclave at 121°C for 15 minutes. Allow to solidify in a slanted position, with a long butt and short slant.

Description

Lysine and Iron medium has been widely used for the differentiation of different biotypes of *Salmonella*, especially *S. arizonae*, which, on standard selective isolation media, such as MacConkey or deoxycholate, may give rise to coloured or colourless colonies due to the fact that their lactose fermentative capacity is quite variable.

By using LIA in combination with Kligler Iron Agar (Art. No. 01-103) or Triple Sugar Iron (Art. No. 01-192), when identifying isolates false negative results due to lactose negative *Salmonella* can be avoided.

Salmonella is the only genus of enterobacteria that normally decarboxylates lysine and produces substantial amounts of hydrogen sulfide.

LIA works perfectly verifying these two characteristics.

Technique

Presumptive colonies form the primary isolation media are. Inoculated into a Kligler's tube, and without reloading the inoculation loop, surface streak the slant and stab inoculate the butt of an LIA tube. Incubate them with loose lids, at 35-37°C for 24 hours.

Microorganisms that decarboxylate the Lysine, rapidly, produce a strong alkalinization in the entire medium turning the indicator purple. Those that have no Lysine decarboxylase activity, acidify the medium at the bottom producing a yellow colouration, whilst the surface of the medium remains the original colour or shows an alkaline reaction.

Proteus are distinguished easily, since, above the yellow butt, they produce a typical red or orange colour on the surface, due to the oxidative deamination of Lysine. The microorganisms which produce of hydrogen sulfide blacken the medium due to iron sulphur precipitates.

Although the gas production may be observed, generally this medium does not offer optimal conditions for this, and gives very irregular results, with total inhibition in some cases.

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- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington.
- EDWARS, P.R., y FIFE, MARY A.(1961) Lysine-Iron Agar in the detection of *Arizona* cultures. Appl. Microbiol 99, 478-480.
- EWING, J. (1982) Edwars and Ewing's identification of Enterobacteriaceae. 4th ed. Elsevier Sci. Pub. Co. Inc. N.Y.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburg. MD. USA.
- MARSHALL, R.T. (1992) Methods for the examination of dairy products. 16th ed. APHA. Washington.
- McFADDIN, J.F. (1985) Media for the isolation, cultivation, identification and maintenance of medical bacteria. William & Wilkins. Baltimore.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Lysine Iron Agar (LIA)

Art. No. 01-094

TYPICAL REACTIONS OF DIFFERENT TYPES OF ENTEROBACTERIA ON LIA

Genus and species	Slant	Depth	Gas	SO_2
Escherichia	Alk. (Blue)	Alk or N	- or +	-
Shigella	Alk.	Ac.	-	-
Salmonella spp.	Alk.	Alk. Or N.	-	+ (-)
Salmonella typhi	Alk.	Alk.	-	+ or -
Salmonella arizona	Alk.	Alk. Or N	-	+ (-)
Citrobacter	Alk.	Ac.	- or +	+ or -
Edwarsiella	Alk.	Alk.	- or +	+
Klebsiella	Alk or N	Alk or N	+ or -	-
Enterobacter cloacae	Alk.	Alk. or N	+ or -	-
Enterobacter aerogenes	Alk.	Alk or N	+ (-)	-
Hafnia	Alk.	Alk or N	- or +	-
Serratia	Alk or N	Alk or N	-	-
Proteus vulgaris	R	Ac.	-	- (+)
Proteus mirabilis	R	Ac.	-	- (+)
Morganella morganii	Alk or R	Ac.	-	-

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 16 - 24 h
Inoculum: Stab the butt and streak the slant

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Good	Slant: Yellow, Depth: Yellow, Gas: -, H_2S: -
Escherichia coli ATCC 25922	Good	Slant: Alk (violet), Depth: Alk, Gas: -, H_2S: -
Proteus mirabilis ATCC 43071	Good	Slant: Red, Depth: Ac, Gas: -, H_2S: +
Salmonella abony NCTC 6017	Good	Slant: Alk (violet), Depth: Alk, Gas: -, H_2S: +
Salmonella typhimurium ATCC 14028	Good	Slant: Alk (violet), Depth: Alk, Gas: -, H_2S: +

MacConkey Agar (Eur. Pharm.)

Art. No. 01-118

CE IVD

Specification

Selective and differential medium used in the detection, isolation and enumeration of *Salmonella* and coliforms in clinical specimens according to the Pharmacopoeial Harmonized Methodology and in foodstuffs specimens according to ISO standard 21150:2006.

Formula* in g/L

Peptones	20,000
Lactose	10,000
Bile salts	1,500
Sodium chloride	5,000
Neutral red	0,030
Crystal violet	0,001
Agar	15,000

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 51,5 g of powder in 1 L of distilled water. Bring to the boil and sterilize in the autoclave at 121°C for 15 minutes.

Description

At the beginning of the last century, MacConkey made the original formulation and included ox bile as inhibitor of Gram positive bacteria and litmus as an indicator of acid production from lactose sugar. More recently litmus has been substituted by a phenol red indicator making interpretations easier and more precise. Advancements in the understanding of bacterial physiology has meant that the medium has now been adapted to facilitate the detection of coliforms. The two most significant modifications to the original formulation are as follows:

- The substitution of ox bile by purified bile salts that improves the selectivity and avoids the inherent turbidity, which is due to the fat composition of bile. The efficiency of the inhibition due to bile salts is variable and depends on the relative concentration of cholate and taurocholate.
- The inclusion of supplementary inhibitors such as crystal violet and/or brilliant green. A popular formulation in America, but not in Europe where lower selectivity is preferred.
- Lactose positive bacteria grown on this medium form red colonies due to acid production resulting form lactose fermentation and thus *Escherichia coli* colonies can be easily distinguished as they also form a small precipitation zone of bile salts around them.

Some enterococci may also grow, but they are easy to distinguish from coliforms, as they form smaller colonies and have no precipitation zone.

Technique

From a ten-fold serial dilution bank, 1 mL samples are inoculated into empty sterile Petri dishes in duplicate. Then, 15 mL of molten medium at 45°C is poured into every plate and mixed carefully. After solidification, a second layer of another 5 mL of sterile medium is poured into every plate to seal the surface and facilitate enumeration of colonies.

For enumeration, after an incubation of 24 hours at 35°C, select plates with 30-150 colonies. The characteristic colonies must be confirmed as coliforms by gas production from lactose in a broth culture.

References

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M

MacConkey Agar (Eur. Pharm.)

Art. No. 01-118

CE IVD

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Inhibited	Selectivity
Enterococcus faecalis ATCC 29212	Inhibited	Selectivity
Escherichia coli ATCC 8739	Productivity > 0.50	Dark violet colonies w. precipitate zone
Escherichia coli ATCC 25922	Productivity > 0.50	Dark violet colonies w. precipitate zone
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Colourless colonies w/o precipitate zone
Salmonella abony NCTC 6017	Productivity > 0.50	Colourless colonies w/o precipitate zone
Pseudomonas aeruginosa ATCC 9027	Good	Colourless colonies w/o precipitate zone

Escherichia coli ATCC 8739

Uninoculated Plate (Control)

Salmonella typhimurium ATCC 14028

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MacConkey No. 2 Agar

Art. No. 01-682

Specification

A modification of MacConkey Agar containing bile salts No. 2 for the identification of enterococci.

Formula* in g/L

Peptones	20,000
Lactose	10,000
Bile salts No. 2	1,500
Sodium chloride	5,000
Neutral red	0,050
Crystal violet	0,001
Agar	15,000

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 51,5 g of powder in 1 L of distilled water. Bring to the boil and sterilize in the autoclave at 121°C for 15 minutes.

Description

MacConkey No. 2 Agar is a modification of the MacConkey Agar (Art. No. 01-118) Due to the use of a different mixture of bile salts the agar is less inhibitory. This medium is specially used for the identification of enterococci in the presence of coliforms and non-lactose fermenters, in food, water, sewage or any other sample.

Enterococci appear as small deep red colonies with a pale peripheral zone about 1 mm in diameter. Enterococci can be considered as an index of faecal pollution.

Non-lactose fermenters produce colourless colonies.

Non-faecal streptococci, staphylococci and other bile-tolerant Gram positive cocci are completely inhibited.

References

- ATLAS, R.M. y L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Londres.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Total inhibition	-
Enterococcus faecalis ATCC 29212	Poor to good	48 h. Pink coloured colonies red centres
Enterococcus faecalis ATCC 19433	Poor to good	48 h. Pink coloured colonies red centres
Escherichia coli ATCC 25922	Good	Pink- red colonies w. slow precipitation
Escherichia coli ATCC 8739	Good	Pink- red colonies w. slow precipitation
Salmonella typhimurium ATCC 14028	Good	Colourless colonies w/o precipitate

MacConkey Broth

Art. No. 02-118

Specification

Liquid medium used for the detection and enumeration of coliforms by MPN technique.

Formula* in g/L

Peptone	20,000
Lactose	10,000
Bile salts	5,000
Sodium chloride	5,000
Neutral red	0,075

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 40 g of powder in 1 L of distilled water. Bring to the boil and distribute into suitable containers fitted with Durham tubes. Sterilize in the autoclave at 121°C for 15 minutes.

Description

MacConkey media is a well known popular enrichment media for coliform bacteria.

At the beginning of the last century, MacConkey made the original formulation and included ox bile as an inhibitor of Gram positive bacteria and litmus as the indicator of acid production from lactose sugar. More recently litmus has been substituted by a phenol red indicator making interpretations easier and more precise.

Advancements is knowledge of bacterial physiology has allowed adaptation of this medium to facilitate coliform detection. The most significant modification to the original formulation has been the substitution of ox bile by purified bile salts that improve the selectivity and avoid the inherent turbidity which is due to the fat composition of bile. The efficiency of the inhibition due to bile salts is variable and depends on the relative concentration of cholate and taurocholate.

Technique

MacConkey Broth can be used for the enumeration of coliforms by the MPN technique, selecting positive tubes that show turbidity, a colour change to red purple and gas production.

Broth is prepared at single (40 g/L) and double strength (80 g/L) and distributed in a series of five tubes fitted with Durham tube. It is recommended that the single strength Broth is distributed in volumes of 10 mL in tubes sized 16 x 160 mm and the double strength 10 mL volumes in tubes on 20 x 200 mm and 50 mL in flask of 100 mL also fitted with Durham tubes.

The inoculation must be done in the following way: Each flask with 50 mL double strength is inoculated with 50 mL of sample. Each tube with 10 mL of double strength Broth is inoculated with 10 mL of sample. Each tube with 10 mL of single strength broth is inoculated with 1 mL of sample.

All the inoculated tubes and flasks are incubated at 30°C for 48 hours. The tubes that show turbidity change to red purple in colour and show gas production (bubble in the Durham tube) are considered positive.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MacCONKEY, A.T. (1905) Lactose-Fermenting Bacteria in Faeces. J. Hyg 5:333.
- WHO (1963) International Standards for Drinking Waters. 7th ed. Churchill Ltd. London.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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MacConkey Broth

Art. No. 02-118

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Total to partial inhibition	48 h
Citrobacter freundii ATCC 43864	Good	Medium (Red) Gas (+)
Escherichia coli ATCC 8739	Good	Medium (Red) Gas (+). 18 h
Escherichia coli ATCC 25922	Good	Medium (Red) Gas (+). 18 h
Salmonella typhimurium ATCC 14028	Good	Medium (Yellowish) Gas (-)
Pseudomonas aeruginosa ATCC 27853	Good	Gas (-)

Left: Uninoculate tube (Control)
Centre*: Escherichia coli* ATCC 25922
Right: *Salmonella typhimurium* ATCC 14028

"Detail"
Left: *Escherichia coli* ATCC 25922
Right: *Salmonella typhimurium* ATCC 14028

MacConkey Broth (Eur. Pharm.)

Art. No. 02-611

Also known as

Medium 6

Specification

Liquid medium, for the detection and enumeration of coliforms, according to the Pharmacopeial Harmonised Method.

Formula* in g/L

Peptone	20,00
Lactose	10,00
Bile	5,00
Bromocresol purple	0,01

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 35 g of powder in 1 L of distilled water. Heat only if necessary. Distribute into suitable containers fitted with Durham tubes. Sterilize in the autoclave at 121°C for 15 minutes.

Description

MacConkey media is a well known popular enrichment media for coliform bacteria. This MacConkey Broth is a modification of the classic medium, where neutral red is replaced by a less aggressive indicator, according to the European Pharmacopoeia.

At the beginning of the last century, MacConkey made the original formulation and included ox bile as an inhibitor of Gram positive bacteria and litmus as the indicator of acid production from lactose sugar. More recently the litmus has been substituted by a phenol red indicator making interpretations easier and more precise.

Advancements is the knowledge of bacterial physiology has allowed adaptation of this media faciliting coliform detection. The most significant modification to the original formulation has been:

- The substitution of the ox bile by purified bile salts that improve selectivity and avoid the inherent turbidity due to the fat composition of bile. The efficiency of the inhibition due to bile salts is variable and depends on the relative concentration of cholate and taurocholate.
- In the 60's, the toxicity of neutral red on the stressed cells of coliforms was demonstrated, especially on some strains of *Escherichia coli*, and so the pH indicator was changed to the bromocresol purple, being less harmful than the neutral red.

Technique

The MacConkey Broth is used for the enumeration of coliforms by the MPN technique, selecting the positive tubes that show turbidity, a colour change to yellow and gas production.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MacCONKEY, A.T. (1905) Lactose-fermenting bacteria in faeces. J. Hyg 5:333.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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MacConkey Broth (Eur. Pharm.)

Art. No. 02-611

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Inhibited	-
Enterococcus faecalis ATCC 29212	Inhibited to poor	-
Escherichia coli ATCC 25922	Good	Medium (yellow) Gas (+)
Escherichia coli ATCC 8739	Good	Medium (yellow) Gas (+)
Salmonella typhimurium ATCC 14028	Good	Medium (violet) Gas (-)
Citrobacter freundii ATCC 43864	Good	Medium (yellow) Gas (+)
Pseudomonas aeruginosa ATCC 9027	Good	Medium (violet) Gas (-)

Left: Uninoculate tube (Control)
Centre: *Escherichia coli* ATCC 25922
Right: *Salmonella typhimurium* ATCC 14028

"Detail"

MacConkey Sorbitol Agar

Art. No. 01-541

Also known as

SMAC Agar.

Specification

Selective and differential solid medium for the detection of Enterohaemorrhagic *Escherichia coli* (EHEC O157:H7).

Formula* in g/L

Peptone	20,000
Sorbitol	10,000
Bile salts No. 3	1,500
Sodium chloride	5,000
Neutral red	0,030
Crystal violet	0,001
Agar	15,000

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 51,5 g of powder into 1 L of distilled water and bring to the boil. Sterilize in the autoclave at 121°C for 15 minutes. If the medium is used on the same day of preparation autoclaving is not necessary but boiling must be carried out for at least 3 minutes.

To obtain the CT-SMAC Agar (according to ISO 16654:2001 Standard) add aseptically, the contents of a vial of the CT SMAC Selective Supplement (Art. No. 06-146-LYO) to 500 mL of the medium sterilized, melted and cooled to 45-50°C. Homogenize and pour plates.

Description

The substitution of lactose by sorbitol for the isolation of enteropathogenic *Escherichia coli* serotypes O111 and O55 was proposed in 1952 by Rappaport and Hening. The usefulness of the medium was shown by March and Ratman (1986) and Adams (1991) for the detection, differentiation and isolation of enterohaemorrhagic (EHEC) and the verotoxin-producing (VTEC) strains of serotype O157:H7 *E. coli.*

The only modification of the typical MacConkey Medium formulation is the replacement of lactose with sorbitol. The enterohaemorrhagic strains do not use this substrate and produce colourless colonies. The other serotypes can ferment sorbitol and hence produce red colonies.

In all others aspects, MacConkey Agar with sorbitol works similarly to other media in the MacConkey group. Peptone supplies the nitrogen and sodium chloride provides an osmotic environment. Crystal violet and bile salts inhibit the growth of Gram positive bacteria and neutral red acts as the pH indicator.

The sensitivity of SMAC is limited by the difficulty in identifying non-sorbitol-fermenting colonies amongst the accompanying microflora and the possible presence of other non-sorbitol-fermenters such *Proteus spp.,* Aeromonas spp. and some other *E. coli* which make it necessary to test colonies for confirmation.

Zadik *et al.* (1993) reported a further improvement in EHEC O157 isolation rates by using SMAC supplemented with cefixime and potassium tellurite (CT-SMAC). Cefixime inhibits *Proteus* at a concentration not inhibitory to *Escherichia coli*. EHEC O157 strains are generally less susceptible to tellurite than many other non-sorbitol-fermenters such as Aeromonas spp., *Plesiomonas spp., Morganella spp., Providencia spp.* and most other *E. coli* strains. The use of cefixime and tellurite in Sorbitol MacConkey Agar (CT-SMAC) for isolation of *E. coli* O157:H7 is described in the FDA Bacteriological Analytical Manual and ISO adopted it as the preferred selective medium in its 16654:2001 Standard.

Technique

Spread the inoculum onto the dry surface of the medium and incubate at 35 ± 2°C for 24 hours. Usually, the O157:H7 serotype forms colourless colonies and the other strains of *E. coli* produce red colonies. The results must be recorded at 24 hours because an extended incubation produces a decreasing colouration in the sorbitol-fermenting colonies and sometimes a colony of the O157:H7 serotype may begin to ferment sorbitol.

Some Gram negative bacteria such as Pseudomonas, *Proteus* and *Klebsiella* can growth on MacConkey Agar with Sorbitol but their colonies are diverse and easy to differentiate from *E. coli.*

Because of the failure to ferment sorbitol by some strains of no-enterotoxigenic *E. coli* and atypical colony production by some enterohaemorrhagic strains, the use of other media concurrently with MacConkey Agar with Sorbitol or CT-SMAC is recommended. Confirmation of suspect colonies by serological, biochemical or molecular techniques is also necessary.

Necessary supplements

CT SMAC Selective Suplement (Art. No. 06-146-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Cefixime	0,025 mg
Potassium tellurite	1,250 mg

Distilled water (Solvent)

References

- ADAMS, S. (1991) Screening for verotoxin-producing *E. coli.* Clin Lab. Science 4:1:19-20.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc., London.
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MacConkey Sorbitol Agar

Art. No. 01-541

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- MACCONKEY, A.T. (1905) Lactose-fermenting Bacteria in faeces. J. Hyg 5:333.
- MURRAY, P.R., E.J. BARON, M.A. PFALLER, F.C. TENOVER, & R.H. YOLKEN (Eds) (1995) Manual of Clinical Microbiology 6th ed. A.S.M. Washington. DC. USA.
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- ZADIK, P.M., P.A. CHAPMAN, & C.A. SIDDONS (1993) Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J. Med. Microbiol. 39:155-158.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	-
Escherichia coli ATCC 25922	Productivity > 0.50	Pink-Red colonies, with precipitate
Escherichia coli ATCC 35150 ser 0157 H7	Productivity > 0.50	Colourless colonies w/o precipitate

E. coli ATCC 35150 ser 0157:H7
Sorbitol (-)

Uninoculated plate (Control)

Escherichia coli ATCC 25922
Sorbitol (+)

Malt Extract Agar No. 1

Art. No. 01-111

Specification

Culture medium for moulds and yeast.

Formula* in g/L

Malt extract	13,00
Dextrine	2,50
Gelatin peptone	5,00
Agar	15,00

Final pH 5,5 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 35,5 g of powder in 1 L of distilled water and heat gently, constantly stirring until boiling. Dispense in suitable containers and sterilize in the autoclave at 115°C for 15 minutes. **Avoid overheating** since the low pH of the medium may hydrolyze the agar.

Description

Malt Extract Agar is a classic culture medium for moulds and yeast. Malt extract has enough sugar (maltose, glucose, sucrose) to allow excellent growth, and additional necessary growth factors are provided by the gelatine peptone.

Malt Extract Agar has been widely used for the maintenance, isolation and identification of fungi, and it is also proposed in several pharmacopoeias as a medium for the control of sterility in pharmaceutical products. It is most often used for comparative morphological studies.

Should more selectivity be desired, a few millilitres of 10% lactic acid, or 5% tartaric acid can be added, but this makes the solidification of the agar more difficult. When acidification is below pH 5,0 do not re-melt the agar since the solidifying agent will be hydrolyzed.

Technique

See appropriate references for specific procedures and techniques.

References

- BOOTH, C. (1972) Fungal Culture Media. In Methods in Microbiology Vol. 7B, edited by J.R. Norris and D.W Ribbons. Acadenic Press. Londres.
- HARRIGAN, W.F. & M. McCANCE (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Press. London.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- SAMSON, R.A., E.S. HOEKSTRA, J.C. FRISVAD and O. FILTENBORG (2002) Introduction to Food and Airborne Fungi. 6th ed. CBS. Utrecht. Holland.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Malt Extract Agar No. 1

Art. No. 01-111

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 48 h - 7 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Poor to good	24-48 h
Escherichia coli ATCC 8739	Poor to good	24-48 h
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	5-7 days (green to black - spores)
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-
Penicillium aurantiogriseum ATCC 16025	Good	-

Penicillium aurantiogriseum ATCC 16025

Saccharomyces cerevisiae ATCC 9763

Aspergillus brasiliensis ATCC 16404

Malt Extract Agar No. 2

Art. No. 01-573

Specification

Solid medium for the isolation and enumeration of fungi.

Formula* in g/L

Malt extract	30,00
Soy peptone	3,00
Agar	15,00

Final pH 5,6 ± 0,2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 48 g of powder in 1 L of distilled water. Bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Malt Extract Agar No. 2 promotes the growth of almost all fungi because of its balanced composition, and its ability to inhibit most bacteria due its low pH.

Should greater inhibition of bacterial growth be desired, readjust the pH to 3,5 by adding a sterile solution of 10% lactic acid or 5% tartaric acid to the molten medium. **Do not reheat the medium after these additions.**

Technique

See appropriate references for specific procedures and techniques.

References

- ATLAS, R.M. y L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- REIS, J. (1972) Ein selektives kulturmedium für der Nachweiss von *Aspergillus flavus*. Zbl. Bakt. Hyg. I. Abt. Orig. 220:564-566.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Malt Extract Agar No. 2

Art. No. 01-573

Quality control

Incubation temperature: 25°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	5 days (black)
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	2 days (white)
Candida albicans ATCC 10231	Productivity > 0.70	2 days (white)

Saccharomyces cerevisiae ATCC 9763

Aspergillus brasiliensis ATCC 16404

Malt Extract Agar No. 3

Art. No. 01-574

Specification

Solid medium for the isolation and enumeration of fungi.

Formula* in g/L

Malt extract	30,00
Mycological peptone	5,00
Agar	15,00

Final pH 5,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 50 g of powder in 1 L of distilled water. Bring to the boil and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

The balanced and rich nutrient composition of the medium makes it suitable for morphogenetic and general morphological studies of fungi. Due to its low pH bacterial growth is largely restricted. Total suppression can be achieved by adding 20 mL of a sterile solution of 10% Lactic acid or 5% tartaric acid, to the melted medium at 55°C, reducing the pH to 3,5. **In these conditions, do not reheat the medium to avoid hydrolysis of the agar.**

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BALLOWS, HAUSLER, HERMAN, ISENBERG & SHADOMY (eds.) (1991) Manual of Clinical Microbiology. ASM. Washington.
- DOWNES, F.P. y K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1978) Bacteriological Analytical Manual A.O.A.C. Washington.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- RAPP, M (1974) Indikator-Zusätze zur Keimdifferenzierung auf würze und Malzextrakt Agar. Milchwiss. 29:341-34.
- REIS, J. (1972) Ein selektives kulturmedium für der Nachweiss von *Aspergillus flavus.* Zbl. Bokt. Hyg. I. Abt. Orig. 220:564-566.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Candida albicans ATCC 10231	Productivity > 0.70	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	5 days (black spores)

Candida albicans ATCC 10231

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Malt Extract Agar (Blakeslee)

Art. No. 01-672

Specification

Solid medium to achieve typical growth and sporulation of fungi according to the methodology of CBS and IFU.

Formula* in g/L

Malt extract	20,00
Peptone	1,00
Dextrose	20,00
Agar	18,00

Final pH 5,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 59 g of powder in 1 L of distilled water and let it soak. Heat gently bringing it to the boil and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. **Avoid overheating or remelting** since the low pH of the medium may hydrolyse the agar.

Description

Blackeslee's Malt Extract Agar is a medium recommended for the morphological studies of the fungal mycelium and for sporulation and colour development needed in the specific identification of fungi. The International Federation of Fruit Juice Producers (IFU) uses this medium to count preservative-resistant yeasts, mainly *Zygosaccharomyces bailii* in fruit juices.

Technique

Prepare three sets of three Petri dishes and distribute 1 mL of the sample or dilution in each dish. Each set is poured with molten sterile medium, cooled to 45°C, to which an amount of 0 ppm, 400 ppm and 800 ppm benzoic acid (calculated as Na benzoate) has been added respectively before pouring into Petri dishes.

The inoculated plates are incubated at 27 ± 2°C for 5 days with the first count on the third day and the final count on the fifth day. The results are expressed as number of yeast per g of product and the concentration of preservative is reported with its respective count.

The generic and specific identification must be verified by microscopic examination of the hyphae, asci and cell shape. Some species require addition biochemical tests.

References

- ATLAS, R.M. & R.C. PARKS (1993) Handbook of microbiological media. CRC Press. London.
- DOWNMES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. USA.
- IFU Method No. 3 (1996) Yeast Count Procedure. III. Preservative-resistant Yeasts Count. Schweizerischer Obstverbant. Zug.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SAMSON, R.A., E.S. HOEKSTRA, J.C. FRISVAD & O. FILTENBORG (2002) Introduction to food- and airborne fungi. 6th ed. Centraal Bureau voor Schimmelcultures (CBS) Utrech. Netherlands.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 25°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2 :2003)

Microorganism	Growth	Remarks
Zygosaccharomyces bailii	Productivity > 0.70	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-

Malt Extract Broth No. 1

Art. No. 02-111

Specification

Liquid culture medium for moulds and yeast.

Formula* in g/L

Malt extract.....13,00
Dextrine..... 2,50
Gelatin peptone.....5,00
Final pH 5,5 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20,5 g of powder in 1 L of distilled water, heating if necessary. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat.**

Description

Malt Extract Broth is a classic culture medium for moulds and yeasts. Malt Extract has sufficient sugar (maltose, glucose, sucrose) to allow copious growth, and growth factors are provided by the gelatine peptone. Malt Extract Broth has been widely used in maintenance, isolation and identification of fungi, and it is also proposed in several pharmacopoeias as a medium for the control of sterility in pharmaceutical products. It's use mostly use is mostly for comparative morphological studies.

Technique

The Galloway & Burgess Technique for morphogenetic studies is as follows: A short shaped cone made of filter paper is put in a Petri dish with 7-8 mL of liquid medium. The sample to test is inoculated on the wet surface of the filter paper cone and incubation is carried out at room temperature with illumination.
Should more selectivity be desired, a few millilitres of 10% lactic acid, or 5% tartaric acid can be added.

References

- FDA (Food and Drug Adminstrations) (1995) Bacteriological Analytical Manual. 8th ed. Rev A AOAC International Inc. Gaithersburg.
- GALLOWAY, L.D. & R. BURGESS (1952) Applied Mycology and Bacteriology. 3rd ed. Leonard Hill. London.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- VANDERZANT & SPLITTSTOESSER (1992) Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Poor to good	24-48 h
Escherichia coli ATCC 8739	Poor to good	24-48 h
Aspergillus brasiliensis ATCC 16404	Good - very good	-
Saccharomyces cerevisiae ATCC 9763	Good - very good	-
Candida albicans ATCC 10231	Good - very good	-

Malt Extract Broth No. 2

Art. No. 02-491

Specification

Liquid medium for cultivation of yeasts and moulds.

Formula* in g/L

Malt extract.....17,00
Peptone.....3,00
Final pH 5,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20 g of powder in 1 L of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat**, since - Maillard reaction browing may occur.

Description

This formulation of the classic Malt Extract Broth is according to Reiss's modification in order to achieve better results for the cultivation of *Aspergillus flavus*.
Malt Extract Broth has been widely used in maintenance, isolation and identification of fungi, and it is also proposed in several pharmacopoeias as a medium for the control of sterility in pharmaceutical products. It is frequently used in comparative morphological studies.

Technique

Should more selectivity be desired, a few millilitres of 10% lactic acid, or 5% tartaric acid, but the pH should not be below 3,5 as this makes growth slow and difficult.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- RAPP, M. (1974) Indikator-Zusätze zur Keimdifferenzierung auf Würze und Malzextrakt Agar. Milchwissenschaft 29:341-344.
- REIS, J. (1972) Ein selektives kulturmedium für der Nachweiss von *Aspergillus flavus*. Zbl. Bakt. Hyg. I. Abt. Orig. 220:564-566.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 25°C ± 2,0
Incubation time: 24 h - 5 days
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	-
Bacillus subtilis ATCC 6633	Good	-
Aspergillus brasiliensis ATCC 16404	Good	Black sporulation at 5 days
Saccharomyces cerevisiae ATCC 9763	Good	-
Candida albicans ATCC 10231	Good	-

M

Mannitol Salt Agar (Eur. Pharm)

Art. No. 01-116

CE IVD

Also known as

Chapman Agar

Specification

Selective medium for the isolation of pathogenic staphylococci according to the Pharmacopoeial Harmonized Methodology and the ISO standard 22718:2006.

Formula* in g/L

Meat extract	1,000
Casein peptone	5,000
Meat peptone	5,000
Sodium chloride	75,000
D-Mannitol	10,000
Phenol red	0,025
Agar	15,000

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 111 g of powder in 1 L of distilled water and bring to the boil. Dispense in tubes or flasks and sterilize in the autoclave at 121°C for 15 minutes.

Description

Mannitol Salt Agar is a classical medium for the detection and enumeration of staphylococci. It was described by Chapman and has been adopted by many official organisations. Several modifications of it have been developed, all formulations resulting in media with similar efficiency.

This medium takes advantage of the high saline tolerance of staphylococci, and uses sodium chloride as a selective agent. Only staphylococci and halophilic enterobacteria are able to grow freely at the concentration of salt employed in this medium, while other bacteria are inhibited. It also exploits the correlation between the pathogenicity of staphylococci and their ability ferment mannitol.

Mannitol fermentation results in an accumulation of acid products, indicated by the phenol red indicator turning yellow. A yellow halo surrounds the presumptive pathogenic colonies, while the rest of the medium remains red/orange in colour.

Technique

Inoculate the plates and incubate at 37°C for 36 hours or at 32°C for 3 days.

The typical appearance of the colonies after the correct incubation is as follows:

- Presumptive pathogenic staphylococci (coagulase +) are mannitol positive and produces large colonies with a yellow halo.
- Non-pathogenic Staphylococci (coagulase -) are usually mannitol negative and produce small colonies without a halo or change in colour.

Coagulase presence must be tested by the classical technique in order to establish its true pathogenic potential.

References

- ATLAS, R.M. & L.C.PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
- CHAPMAN (1945) The significance of sodium chloride in studies of staphylococci. J. Bact 50:201.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods.4th ed. APHA. Washington. DC. USA.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- FDA (Food and Drug Adminstrations) (1995) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC Internacional Inc. Gaithersburg. MD. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 22718:2006 Standard. Cosmetics - Detection of *Staphylococcus aureus.*
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30°C and <60% RH).

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Mannitol Salt Agar (Eur. Pharm)

Art. No. 01-116

CE IVD

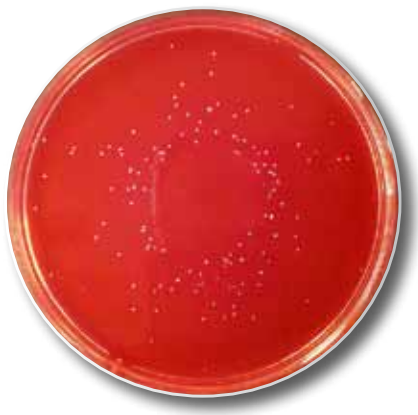
Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Inhibited	Selectivity
Staphylococcus epidermidis ATCC 12228	Productivity > 0.50	White-pink colonies; Red medium Man (-) 72 h
Staphylococcus aureus ATCC 25923	Productivity > 0.50	White colonies; Yellow medium Man (+)
Staphylococcus aureus ATCC 6538	Productivity > 0.50	White colonies; Yellow medium Man (+)

Staphylococcus aureus ATCC 25923
48 h

Staphylococcus aureus ATCC 6538
48 h



Staphylococcus epidermidis ATCC 12228
72 h

Mannitol Selenite Broth Base

Art. No. 02-652

Specification

Liquid enrichment medium specially recommended for *Salmonella typhi* and *Salmonella paratyphi B*.

Formula* in g/L

Peptone	5,00
Mannitol	4,00
Sodium phosphate	10,00

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 19 g of the powder in 1 L of distilled water and add 4 g of sodium bi-selenite (Art. No. SO0160). Homogenize and boil for a few seconds. Distribute it into suitable containers. A it is a thermolabile medium use immediately. **Do not autoclave nor overheat**. Discard the prepared medium if a large amount of reduced selenite (red precipitate) is seen at the bottom of the container.

Description

This medium is a modification of the *Salmonella* Enrichment Broth described by Leifson (1936) in which the lactose is substituted by mannitol. Anderson & Kennedy (1965) showed that Mannitol Selenite Broth is better than other enrichment broths for the isolation of *Salmonella typhi*. But, for all other species of *Salmonella* the selective enrichment medium of choice is Rappaport Vassiliadis Broth (Art. No. 02-379).

Technique

An incubation at 37°C for a period not exceeding 18 hours is initially recommended, since within this period there is growth of coliforms but enhanced growth of pathogens. After 24 hours this effect seems to diminish and the growth of accompanying organisms may mask the growth of *Salmonella*.

Appearance of a red precipitate before inoculation is indicative of overheating of the medium, this indicates that the selective properties are significantly reduced. The presence of abundant sample residues may also inactivate the selective property of the medium, if the sample is e.g. faeces or egg powder. In such cases, it is better to make a 1:10 dilution and let the larger particles settle out inoculating the Selenite Cystine Broth.

It has been demonstrated that when *Salmonella* isolation from faeces is required, the results are improved if the enrichment medium is incubated at 43°C. However this procedure does not work for the isolation of *Salmonella typhi*.

In urine samples use Selenite Cystine Broth (Art. No. 02-602) in double concentration, and inoculate it with an equal volume of urine. In all cases, sub-culturing must be performed between 6 hours and 24 hours.

Most authors recommend the simultaneous use of another enrichment broth, such as Muller-Kauffmann Tetrathionate Medium Base (Art. No. 02-335).

References

- ANDERSON, K. & H. KENNEDY (1965) Comparison of selective media for the isolation of *Salmonellae*. J. clin. Path. 18:747-749.
- ATLAS, R.M., LC. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BÄNFFER, J.R. (1971) Comparison of the isolation of *Salmonellae* from human faeces by enrichment at 37°C and 43°C. Zbl. Bakt. I Orig. 217:(35-40).
- HEALTH PROTECTION AGENCY (2004) Mannitol selenite broth. National Standard Method MSOP 51 Issue 1. http://www.hpa-standardmethods.org.uk/pdf.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LEIFSON, E. (1936) A new Selenite Selective Enrichment media for the Isolation of Typhoid and Paratyphoid (*Salmonella*) *Bacilli*. Am.J.Hyg. 24:423-432.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Mannitol Selenite Broth Base

Art. No. 02-652

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Total inhibition	-
Escherichia coli ATCC 25922	Total inhibition	-
Salmonella typhimurium ATCC 14028 + (1) + (2)	Good	Recovery in XLD (Mixed cultures)
Salmonella enteritidis ATCC 13076 + (1) + (2)	Good	Recovery in XLD (Mixed cultures)
Escherichia coli ATCC 8739 + (1)	Inhibition	Recovery in XLD (Mixed cultures)
Pseudomonas aeruginosa ATCC 27853 + (2)	Inhibition	Recovery in XLD (Mixed cultures)

Marine Agar

Art. No. 01-291

Specification

Solid culture medium for heterotrophic marine bacteria.

Formula* in g/L

Meat peptone	5,0000
Yeast extract	1,0000
Iron citrate	0,1000
Sodium chloride	19,4500
Sodium sulfate	3,2400
Sodium bicarbonate	0,1600
Sodium silicate	0,0040
Sodium fluoride	0,0024
Disodium phosphate	0,0080
Calcium chloride	1,8000
Magnesium chloride	8,8000
Potassium chloride	0,5500
Potassium bromide	0,0800
Strontium chloride	0,0340
Ammonium nitrate	0,0016
Boric acid	0,0220
Agar	15,0000

Final pH 7,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 55,1 g of the powder in 1 L of distilled water and bring to the boil. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Marine Agar was formulated according to the original description of ZoBell that tries to duplicate the major mineral concentration found in sea water. Included in its composition are mineral salts, peptone and yeast extract, and growth factors necessary to sustain the growth of heterotrophic marine bacteria.

The gelling agent is agar and it is often found to be liquefied by marine bacteria.

Marine bacteria are thermo-sensitive and streak-plates are recommended, if pour-plates are preferred, the molten medium must be cooled to 45°C before pouring it over the sample.

Marine Agar is a very hygroscopic medium: Keep the bottle tightly caped in a dry place.

References

- BUCK, J. D. & R.C. CLEVERDON (1960) The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr. 5:78-80.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SIZEMORE, R.K. & L.H. STEVENSON (1970) Method for the isolation of proteolytic marine bacteria. Appl. Microbiol. 20:991-992.
- ZOBELL, C.E. (1941) Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Mar. Res. 4:42-75.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Marine Agar

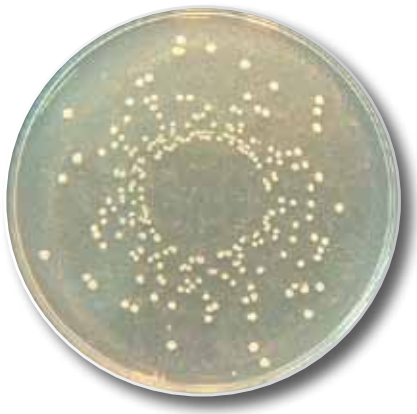
Art. No. 01-291

Quality control

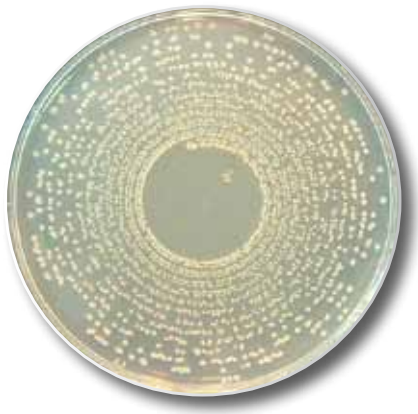
Incubation temperature: 20 - 25°C ± 2,0
Incubation time: 48 - 72 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	-
Vibrio parahaemolyticus ATCC 17802	Productivity > 0.70	-
Vibrio alginolyticus ATCC 17749	Productivity > 0.70	-

Vibrio alginolyticus ATCC 17749



Vibrio parahaemolyticus ATCC 17802



Escherichia coli ATCC 25922

Maximum Recovery Diluent (MRD)

Art. No. 02-510

Also known as

Universal diluent

Specification

Isotonic diluent for the maximal recovery of stressed microorganisms according to ISO standards.

Formula* in g/L

Peptone	1,00
Sodium chloride	8,50

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 9,5 g of powder in 1 L of distilled water and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This formulation combines the osmotic pressure of the physiological saline solution with the protective action of the peptone to obtain good recovery of stressed microorganisms.
The sodium chloride ensures isotonic conditions and the low concentration of peptone does not allow cellular growth in the short period (2-4 hours) of time required for the preparation of the dilution bank of the sample.

Technique

According to the ISO method, the sample is diluted in a ratio 1:10 with the Maximum Recovery Diluent and homogenized by a vortex mixer or Stomacher®. After a short period (10-15 minutes) of rest, a 1/10 dilution bank with the same diluent is prepared following standard procedures. Plates are inoculated using the range of different concentrations.

References

- ISO 6887-1:1999 Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions - Part 2 (2003): Specific rules for the preparation of meat and meat products.
- ISO 8261:2001 Standard. Milk and milk products - General guidance for the preparation of test samples, initial suspension and decimal dilution for microbiological examination.
- ISO 21149:2006 Standard. Cosmetics - Enumeration and detection of aerobic mesophilic bacteria.
- ISO 21150:2006 Standard. Cosmetics - Detection of *Escherichia coli.*
- ISO 22717:2006 Standard. Cosmetics - Detection of *Pseudomonas aeruginosa*.
- ISO 22718:2006 Standard. Cosmetics - Detection of *Staphylococcus aureus*.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. (Productivity) at 0, 45 minutes and 3 h. (20 - 25°C)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Satisfactory
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory
Escherichia coli ATCC 8739	Good	Satisfactory
Candida albicans ATCC 10231	Good	No significant reduction
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Enterococcus faecalis ATCC 29212	Good	Satisfactory

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Methyl Red Voges Proskauer Broth (MRVP)

Art. No. 02-207

Also known as

Clarks Lubs Medium

Specification

Classic liquid medium used for differential tests (Voges-Proskauer and Methyl Red) in Enterobacteriaceae according to ISO and FIL-IDF standards.

Formula* in g/L

Peptone	7,00
Dextrose	5,00
Potassium phosphate	5,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 17 g of powder into 1 L of distilled water, heating if necessary. Dispense in tubes and sterilize in the autoclave at 121°C for 15 minutes.

Description

This classical Lubs and Clark medium is used to perform Methyl Red (MR) and Voges-Proskauer (VP) tests, and together with Indol and Citrate tests allows the differentiation of coliforms. These reactions are as follows:

Methyl Red test

Amongst the Enterobacteriaceae, *E. coli* ferments glucose by the mixed acid pathway, accumulating acidic substances, which produces a large decrease in the initial pH. This change is detected by the methyl red indicator, that is yellow above pH 5,1 and red below pH 4,4.

Voges-Proskauer test

Enterobacteria of *Klebsiella-Enterobacter* biotype ferment glucose by the 2,3-butanediol pathway. Although acidic substances are produced in this way, at the end products are mostly neutral or alkaline. Due to this, incubation must be extended to 3 days. After this period, the methyl red reaction is negative. Nonetheless, Voges-Proskauer test is complementary to Methyl Red test. It shows the 2-3-butanediol and acetone production, (substances hard to identify in the mixed acid pathway). It takes advantage of the fact that these two products, in an alkaline environment, oxidize to diacetyl, which reacts with guanidine and produces coloured compounds.

Technique

There are several techniques used to carry out these tests. An example of one is as follows:

The medium is inoculated with a pure culture of the microorganism to study and incubated at 30°C for at least 3 days or for a maximum of 5 days. Just before reading, the culture is separated in two aliquots, one for each test.

Methyl Red test

Add 4-5 drops of Methyl Red Reagent (Art. No. RE0057) to the culture, and shake in order to homogenize. The test is considered positive if it turns red and negative if it remains yellow.

- Positive (turning red): *E.coli, Edwarsiella, Shigella, Salmonella, Citrobacter, Proteus, Klebsiella ozoenae, Klebsiella rhinoscleromatis, Yersinia.*
- Negative (turning yellow): *Enterobacter, Hafnia, Serratia, Klebsiella pneumoniae.*
- With *Erwinia*, this reaction is very variable.

Voges-Proskauer test

Add to the medium Barrit's Reagent (Art. No. RE0100) until it becomes a milky in appearance. Then, add O'Meara's Reagent (Art. No. RE0060) until this milky appearance disappears. Shake vigourously. The test is positive if medium turns a pink-violet colour, beginning at the top of the tube. If the test is negative, it remains the same colour. Relative amounts of each reagent depend on initial volumes of medium. Never incubate above 30°C.

- Positive (pink-red): *Enterobacter, Hafnia, Klebsiella pneumoniae, Serratia.*
- Negative (no colour change): *Escherichia, Edwarsiella, Citrobacter, Salmonella, Shigella, Yersinia, Klebsiella ozonae, Klebsiella rhinoscleromatis.*
- With *Proteus* and *Erwinia* spp, this reaction is meaningless as it can be very variable.

Voges-Proskauer test may be performed more rapidly, using very small volumes of medium and large inocula. This allows short incubations (18-20 hours), and also, reading may be accelerated by heating the culture almost to boiling after adding the reagents. However, false results are more probable using this method.

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Methyl Red Voges Proskauer Broth (MRVP)

Art. No. 02-207

der Voges-Proskauer Reaktion duch fumarat. Zbt. Bakt. Hyg. I Abt. Orig. A 252:316-323.
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- VOGES & PROSKAUER (1898) Beitrag zur Ernährungsphysiologic und zur Differentialdiagnose der hämorrhagischen Septicäemia. Z. Hyg 28:20-32.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 - 72 h
Inoculum: 1.000 - 10.000 CFU

Microorganism	Growth	Remarks
Citrobacter freundii ATCC 8090	Good	VP (-) RM (+)
Enterobacter aerogenes ATCC 13048	Good	VP (+) RM (-)
Escherichia coli ATCC 25922	Good	VP (-) RM (+)
Escherichia coli ATCC 8739	Good	VP (-) RM (+)
Salmonella typhimurium ATCC 14028	Good	VP (-) RM (+)
Klebsiella pneumoniae ATCC 13883	Good	VP (+) RM (-)
Serratia marcenses ATCC 13880	Good	VP (+) RM (-)

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Methyl Red Voges Proskauer Saline Broth

Art. No. 02-456

Also known as

MRVP saline broth

Specification

Liquid culture medium for Methyl Red and Voges-Proskauer (VP) tests in marine bacteria.

Formula* in g/L

Peptone	7,00
Dextrose	5,00
Sodium chloride	30,00
Dipotassium phosphate	5,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 47 g of powder in 1 L of distilled water, heating only if necessary. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is used to perform Methyl Red and Voges-Proskauer tests on marine enteric bacteria. The fundamentals of these reactions are described in Methyl-Red Voges-Proskauer Broth (Art. No. 02-207).

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h - 3 days
Inoculum: 10.000 - 100.000 CFU

Microorganism	Growth	Remarks
Vibrio parahaemolyticus ATCC 17802	Good	VP (-) RM (+)
Vibrio alginolyticus ATCC 17749	Good	VP (-) RM (+)

Vogues Proskauer test

Methyl Red test

Left: Uninoculated tube (Control)
Centre: *Vibrio alginolyticus* ATCC 17749
Right: *Vibrio parahaemolyticus* ATCC 17802

Microbial Content Test Agar

Art. No. 01-613

Also known as

TSA with Lecithin and Polysorbate, TSA Lecithin & Polysorbate

Specification

Solid medium for the sampling of surfaces of sanitary importance using the contact plate technique.

Formula* in g/L

Tryptone	15,00
Soy peptone	5,00
Sodium chloride	5,00
Lecithin	0,70
Polysorbate 80	5,00
Agar	15,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 45,7 g of powder in 1 L of distilled water and bring to the boil, distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is a modification of the classical TSA for surface sampling by the contact plate technique. Lecithin and Polysorbate 80 are incorporated to neutralize quaternary ammonium compounds, phenolic disinfectants, hexachlorophene, formalin and ethanol.
The dehydrated medium has a characteristic “brown sugar” appearance and may seem moist due to the inclusion of these agents.
Collection of samples from identical areas (replicate) “before and after” treatment with disinfectant yields data useful for evaluating cleaning procedures in environmental sanitation.

References

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- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Microbial Content Test Agar

Art. No. 01-613

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Enterococcus faecalis ATCC 29212	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-

Enterococcus faecalis ATCC 29212

Bacillus subtilis ATCC 6633

Escherichia coli ATCC 25922

Mineral Modified Glutamate Agar Base

Art. No. 01-659

Specification

Solid medium for resuscitation and recovery of *E. coli* cells damaged by heat, freezing or chemical processes according to FIL-IDF and ISO standards.

Formula* in g/L

Lactose	10,000
Sodium formate	0,250
L-Cysteine HCl	0,020
L-Aspartic acid	0,024
L-Arginine	0,020
Thiamine	0,001
Nicotinic acid	0,001
Pantothenic acid	0,001
Magnesium sulfate	0,100
Ammonium-iron citrate	0,010
Calcium chloride	0,010
Dipotassium phosphate	0,900
Agar	15,000

Final pH 6,7 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

The sodium glutamate (6,35 g/L) and ammonium chloride (2,5 g/L) are not included in the formulation of the dehydrated powder to improve its stability. Moreover a longer shelf-life and a better performance of the medium is obtained.

Directions

Suspend 26,33 g of the powder in 1 L of distilled water in which 2,5 g of ammonium chloride (Art. No. AM0273) is dissolved and add 6,35 g of Sodium Glutamate (Art. No. SO0400). Bring the mixture to the boil to dissolve.

Distribute it into suitable containers and sterilize in the autoclave at 115°C for 10 minutes.

Note: The pH value is critical for performance of the medium. The heating process can affect the pH and care must be taken to adjust the medium to give a final pH of 6,7.

Description

This medium is produced according to the formulation established by standards ISO 11866-2:2005, ISO 16649 -1:2001 and FIL-IDF 170A:1999 for the enumeration of presumptive testing of *Escherichia coli* in milk and other foods.Also recommended for the resuscitation step in the colony-count technique at 44°C using membrane filtration. This method is preferred by FIL-IDF for the examination of milk samples and milk products in which comparatively large numbers of *Escherichia coli* are suspected (more than 100 per g or 10 per mL).

Technique

Using sterile forceps place a cellulose acetate membrane in the dried surface of each of two plates of Glutamate Agar aseptically. Take care to avoid trapping air bubbles beneath the membranes and gently flatten the membranes with a sterile spreader (Drigalsky loop).

Put 1 mL of the test sample to the centre of each membrane. Spread the inoculum evenly over the whole membrane surface, using the sterile spreader avoiding any spillage from the membrane.

Leave the inoculated plates in a horizontal position for 15 minutes until the inoculum has soaked into the agar. Incubate the plates for 4 ± 1/2 h at 37°C with the membrane/agar surface uppermost. After this time, the membranes are transferred with inoculated side uppermost, to Tryptone Bile Agar plates.

Refer to suitable FIL-IDF/ISO standards for sample preparation, dilution process and interpretation of results.

References

- IDF-FIL Int. Standard 170A (1999) Milk and milk products - Enumeration of presumptive *Escherichia coli.* Part 3: Colony-count technique at 44°C using Membranes.
- ISO Standard 11866-2:2005. Milk and milk products - Enumeration of presumptive *Escherichia coli*. Part 2: Colony count technique at 44°C using membranes.
- ISO Standard 16649-1:2001. Microbiology of foods and feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Colony count at 44°C technique.
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- MURANO E.A. & J.A. HUDNALL (2001) Media, Reagents and stains. In Downes, F.P. & K. Ito (Eds.) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Mineral Modified Glutamate Agar Base

Art. No. 01-659

Quality control

Incubation temperature: 44°C / 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	-
Escherichia coli ATCC 25922	Productivity > 0.50	-
Escherichia coli ATCC 8739	Productivity > 0.50	-
Salmonella typhimurium ATCC 14028	Inhibited	-

Mineral Modified Glutamate Medium Base

Art. No. 02-656

Specification

Liquid medium for the selective enrichment of *E. coli* according to ISO Standard 16649-3:2005.

Formula* in g/L

Lactose	20,000
Dipotassium phosphate	1,800
Sodium formate	0,500
Magnesium sulfate	0,200
L-Cysteine	0,040
L(+)-Arginine	0,040
L(-)-Aspartic acid	0,048
Ammonium iron (III) citrate	0,020
Calcium chloride	0,020
Bromocresol purple	0,020
Thiamine	0,002
Nicotinic acid	0,002
Pantothenic acid	0,002

Final pH 6,7 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Single strength: Dissolve 11,35 g of powder in 1 L of distilled water. To this add 2,5 g of ammonium chloride (Art. No. AM0273) and 6,35 g of monosodium glutamate (Art. No. SO0400). Mix until totally dissolved, and heat only if necessary. Distribute 10 mL volumes into 16x160 mm tubes fitted with Durham tube. Sterilize in the autoclave at 116°C for 10 minutes or alternatively, heat to 100°C by flowing steam immersion for 30 minutes on three successive days.

Double strength: Dissolve 22,7 g of powder in 1 L of distilled water. To this add 5 g of ammonium chloride (Art. No. AM0273) and 12,7 g of monosodium glutamate (Art. No. SO0400). Mix until totally dissolved, and heat only if necessary. Distribute 10 mL volumes into 20x200 mm tubes fitted with Durham tube. Sterilize in the autoclave at 116°C for 10 minutes or alternatively, heat to 100°C by flowing steam immersion for 30 minutes on three successive days.

Note: The pH value is critical for medium performance. The sterilization process can affect the pH and care must taken to adjust the medium to give a final pH of 6,7.

Description

Glutamate-Lactose-Formate Broth was proposed in 1959 by Gray as alternative medium for the presumptive determination of coliforms in water. With several modifications, the medium has evolved to the current Minerals Modified Glutamate Medium (MMGM) and now is considered a valid alternative for the detection of coliforms and *Escherichia coli* in different types of samples.

In Great Britain this is the elective medium for the verification of faecal contamination in the chlorinated drinking water supplies. Several studies on the coliforms present in foods have shown the superiority of MMGM over other enrichment media as MacConkey Broth (Art. No. 02-611), Brilliant Green Bile Broth (Art. No. 02-041) or Lauryl sulfate Broth (Art. No. 02-108).

The 16649-3:2005 ISO Standard recommends the MMGM for the selective enrichment of *E. coli* and for the Most Probable Number enumeration.

Technique

The sample is prepared or diluted according to the established protocol and a series of 3,5 or 10 tubes are inoculated with any dilution. The double strength tubes are inoculated with 10 mL of sample and the single strength tubes are inoculated with 1 mL sample.

The 16646-3 ISO Standard states an incubation of the inoculated tubes at 37°C for 24 ± 2 hours. Tubes that changes the colour to yellow due to acid production (positive reaction) are then selected. A loop from the positive tubes is streaked onto TBX Agar (Art. No. 01-619) plates and incubating at 44°C for 20-24 hours. For the MPN result tubes are considered positive if blue or blue-green colonies on the TBX Agar, showing the presence of β-glucuronidase-positive *E. coli*.

If a coliform or *E. coli* presumptive MPN enumeration is to be performed, all tubes showing a colour change and gas production in 24-48 hours must be considered positive. Gas production can be detected in the Durham tube or by effervescence when the tube is tapped.

Limitations of the Procedure

- The results are influenced by the medium pH. Before inoculation it must be verified that the sterilization process has not changed the pH value.
- In spite of the selectivity of the medium, it is possible that some non-coliform organism can grow producing acid and gas. All the presumptive results must be confirmed before expressing them as "coliforms" or "*E. coli*".

References

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Mineral Modified Glutamate Medium Base

Art. No. 02-656

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- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO/TS 16649-3 (2005) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Part 3: Most probable number technique using 5-bromo-4-chloro-indolyl-β-D-glucuronide.
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- MANAFI, M. (2003) Media for the detection and enumeration of "total" Enterobacteriaceae, coliforms and *Escherichia coli* from water and foods. In "Handbook of Culture Media for Food Microbiology". J.E.L. Corry et al. (eds). Elsevier Science B. V. Amsterdam.
- MOUSSA, R.S., N. KELLER, G. CURIAT & J.C. de MAN ((1973) Comparison of five media for the isolation of coliform organisms from dehydrated and deep-frozen foods. J. Appl. Bact. 36:619-624.
- PHLS STANDING COMMITTEE on the BACTERIOLOGICAL EXAMINATION of WATER SUPPLIES (1968) A comparison of McConkey Broth, Teepol Broth and Glutamic Acid Media for the enumeration of coliforms organisms in water. J. Hyg. Camb. 66:67-82.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 19433	Inhibited	-
Enterobacter aerogenes ATCC 13048	Good	Gas (+) Yellow medium
Escherichia coli ATCC 25922	Good	Gas (+) Yellow medium
Salmonella typhimurium ATCC 14028	Good	Gas (-) Purple medium

Motility Indol Ornithine Fluid Medium (MIO)

Art. No. 03-422

Specification

Medium used for the demonstration of motility, indol production and the ornithine-decarboxylase activity of enterobacteria.

Formula* in g/L

Yeast extract	3,00
Gelatin peptone	10,00
Casein peptone	10,00
L-Ornithine HCl	5,00
Dextrose	1,00
Bromocresol purple	0,02
Agar	2,50

Final pH 6,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 31,5 g of powder in 1 L of distilled water. Bring to the boil and distribute into apporpriate tubes. Sterilize in the autoclave at 121°C for 15 minutes.

Description

Remove all dissolved air in the medium by heating the tubes in a boiling water bath and cooling them to room temperature. Taking the growth of primary isolation as the inoculum, inoculate the tubes by a single deep stab. Incubate aerobically at 35 ± 2°C for 18-24 hours.

Motility can be observed by the diffuse growth at the upper side of the stab; meanwhile the non-motile bacteria grow along the stab, producing a clear streak.

Ornithine decarboxylation is indicated by the presence of a dark purple colour throughout the tube. Negative reaction produces only a single purple band at the top, and the rest of the tube changes to yellow.

Indol production is verified after the addition of a few drops of Kovacs' Reagent (Art. No. RE0007) (shake gently). The presence of a red ring signifies the positive reaction, if the colour is yellow the reaction is negative.

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- McFADDIN, J.F. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkims. Baltimore. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Motility Indol Ornithine Fluid Medium (MIO)

Art. No. 03-422

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: Pure cultures using and inoculating needle

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	ORN (+) Mot (+) I (+)
Escherichia coli ATCC 8739	Good	ORN (+) Mot (+) I (+)
Klebsiella pneumonia ATCC13883	Good	ORN (-) Mot (-) I (-)
Salmonella typhimurium ATCC 14028	Good	ORN (+) Mot (+) I (-)
Proteus mirabilis ATCC 25933	Good	ORN (+) Mot (+) I (-)

Left: Uninoculated tube
Centre: *Escherichia coli* ATCC 25922
Right: *Salmonella typhimurium* ATCC 14028

"Detail"

Motility Nitrate Medium

Art. No. 03-612

Specification

Fluid medium used for the identification of *Clostridium perfringens* according to ISO standard 7937:2004.

Formula* in g/L

Meat extract	3,00
Peptone	5,00
Potassium nitrate	1,00
Bi-Sodium phosphate	2,50
Galactose	5,00
Agar	5,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 21,5 g of powder in 1 L of distilled water containing 5 mL of glycerol. Bring to the boil and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is produced according to the formulation of the US Food & Drug Administration and the ISO Standard 7937 for the identification of *Clostridium perfringens* in cosmetics and food.

Technique

The final containers, usually flat or standard tubes, are degassed by heating in a water bath at 100°C for 10 minutes. Then, they are cooled to 70-80°C and they are inoculated with a needle in the middle of the medium. The inocula must be obtained from black colonies growth on TSN Agar (Art. No. 01-195). The inoculated tubes are incubated at 37°C for 18-20 hours without any seal or reduced atmosphere.

If the growth in this medium appears at a deep of 5-7 mm from the surface this is evidence of anaerobiosis and the absence of motility is shown by a clear and limpid growth along the streak.

For verification of nitrate reduction, several drops of a mixture of Nitrate A Reagent (Art. No. 06-003) and Nitrate B Reagent (Art. No. 06-004) must be added. Development of a cherry red colour shows a positive nitrate to nitrite reduction.

Clostridium perfringens is an anaerobic, non-motile and nitrate-reducing microorganism.

References

- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
- ISO 7937 Standard (2004) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Clostridium perfringens* - Colony count. Technique.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Pure cultures using and inoculating needle

Microorganism	Growth	Remarks
Clostridium perfringens ATCC 13124	Good	Nitrate (+). Nonmotile
Clostridium perfringens ATCC 12917	Good	Nitrate (+). Nonmotile
Clostridium sporogenes ATCC 11437	Good	Nitrate (-). Motile

MRS Agar
Art. No. 01-135

Also known as

Lactobacilli MRS Agar

Specification

Solid culture medium for the isolation of lactobacilli, according to ISO standards 9332 and 15214 and IFU Methods 5, 7 and 9.

Formula* in g/L

Peptone proteose	10,00
Meat extract	8,00
Yeast extract	4,00
D(+)-Glucose	20,00
Sodium acetate	5,00
Triammonium citrate	2,00
Magnesium sulfate	0,20
Manganese sulfate	0,05
Dipotassium phosphate	2,00
Polysorbate 80	1,00
Agar	14,00

Final pH 6,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 66 g of powder in 1 L of distilled water. Bring to the boil slowly with gentle stirring until completely dissolved. Dispense into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat**.

Description

MRS Agar is a medium used for the cultivation of lactobacilli. It is a modification of a medium based on the highly nutritious properties of tomato juice. The addition of magnesium, manganese and acetate, together with polysorbate, provides an improved medium for the growth of lactobacilli, including very fastidious species such as *Lactobacillus brevis* and *Lactobacillus fermentum.*

The quality of the peptones in addition to the meat and yeast extracts, combine all the necessary growth factors that make MRS medium one of the best media for the cultivation of lactobacilli.

As the selectivity of this medium is low and contaminants tend to grow subculturing in a (double layer) solid medium, and then in broth is recommended to increase selectivity. In many cases, growth is encouraged by incubation in a CO_2 enriched atmosphere.

MRS medium is particularly recommended for the enumeration and maintenance of lactobacilli either by the MPN technique in broth, (Art. No. 02-135) or by inoculation on a plate, overlaying it with a second layer of molten medium. This technique overcomes the need for a CO_2 enriched atmosphere.

References

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- ISO Standard 15214 (1998) Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony count technique at 30°C.
- MAN, J.C. de, ROGOSA, M. & SHARPE, M. Elisabeth (1960) A medium for the cultivation of lactobacilli. J. Appl. Bact.; 23:130

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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MRS Agar

Art. No. 01-135

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h - 3 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Partial Inhibition	Incubate in a 5% CO_2 atmosphere
Lactobacillus sakei ATCC 15521	Productivity > 0.50	Incubate in a 5% CO_2 atmosphere
Lactobacillus lactis ATCC 19435	Productivity > 0.50	Incubate in a 5% CO_2 atmosphere
Lactobacillus fermentum ATCC 9338	Productivity > 0.50	Incubate in a 5% CO_2 atmosphere
Lactobacillus acidophilus ATCC 4536	Productivity > 0.50	Incubate in a 5% CO_2 atmosphere

Lactobacillus fermentum ATCC 9338

Uninoculated plate (Control)

Lactobacillus sakei ATCC 15521

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MRS Broth

Art. No. 02-135

Specification

Liquid culture medium for the isolation of lactobacilli, according to de Man, Rogosa and Sharpe and ISO standards.

Formula* in g/L

Proteose peptone	10,00
Meat extract	8,00
Yeast extract	4,00
D(+)-Glucose	20,00
Sodium acetate	5,00
Triammonium citrate	2,00
Magnesium sulfate	0,20
Manganese sulfate	0,05
Dipotassium phosphate	2,00
Polysorbate 80	1,00

Final pH 6,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 52 g of powder in 1 L of distilled water. Heat to dissolve completely and dispense into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat**.

Description

MRS Agar and Broth are media for the cultivation of lactobacilli, they are a modification of a medium based on the highly nutritious properties of tomato juice. The addition of magnesium, manganese and acetate, together with Polysorbate, has provided an improved medium for the growth of lactobacilli, including that of very fastidious species such as *Lactobacillus brevis* and *Lactobacillus fermentum.*

The quality of the peptones in addition to the meat and yeast extracts, combine all the necessary growth factors that make MRS medium one of the best media for the cultivation of lactobacilli.

As the selectivity of the medium is low and contaminants tend to grow subculturing in a (double layer) solid medium and then in broth is recommended to improve selectivity. In many cases, growth is encouraged by incubation in a CO_2 enriched atmosphere.

MRS media is particularly recommended for the enumeration and maintenance of lactobacilli either by the MPN technique (in broth) or on a plate by inoculation on a plate, overlaying it with a second layer of molten medium. This technique overcomes the need for a CO_2 enriched atmosphere.

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- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
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Storage

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MRS Broth

Art. No. 02-135

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 48 h - 3 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Partial inhibition	Incubate in a 5% CO_2 atmosphere
Lactobacillus sakei ATCC 15521	Good - very good	Incubate in a 5% CO_2 atmosphere
Lactobacillus lactis ATCC 19435	Good - very good	Incubate in a 5% CO_2 atmosphere
Lactobacillus fermentum ATCC 9338	Good - very good	Incubate in a 5% CO_2 atmosphere
Lactobacillus acidophilus ATCC 4536	Good - very good	Incubate in a 5% CO_2 atmosphere

Left: *Lactobacillus fermentum* ATCC 9338
Centre: *Escherichia coli* ATCC 25922
Right: Uninoculated tube (Control)

Mueller-Hinton Agar

Art. No. 01-136

CE IVD

Also known as

M-H Agar

Specification

Recommended medium used to perform Antibiotic and Sulphonamide sensitivity testing with pathogenic microorganisms from clinical specimens, according to the Kirby-Bauer and Ericsson methodology.

Formula* in g/L

Peptone	17,50
Beef infusion solids	2,00
Starch	1,50
Agar	17,00

Final pH 7,3 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 38 g of powder to 1 L of distilled water and let it soak. Bring to the boil to dissolve the medium completely. Sterilize in the autoclave at 121°C for 15 minutes.

Description

Mueller-Hinton Agar was originally designed for the primary isolation of meningococci and gonococci.

With the addition of blood it becomes an optimal medium for the growth of *Neisseria*. It is also more effective if reheated and turned into Chocolate Agar. **It should not be re-melted or reheated once blood has been added to it**.

Technique

For the culture of *Neisseria* the best results are obtained if incubation is carried out in a moist chamber with a CO_2 enriched atmosphere. If an anaerobic jar is not available, this environment can be obtained by placing the plates in a candle jar. The atmosphere inside the container is 5 to 8% CO_2 enriched.

Mueller-Hinton Agar has proved to be one of the most efficient media for use in anti-bacterial susceptibility testing. Without the addition of blood it can be used for sulfonamide sensitivity testing since it is free from most of its antagonists (nucleotides, etc.). If this type of assay is conducted, the zones of inhibition should be examined after 12-18 hours, before overgrowth occurs, since after 24 hours it can interfere with the sulphonamide sensitivity test. Using a small inoculum will help the early formation of zones of inhibition. The inoculum should be 100 to 300-fold smaller than that used in the testing of other antibiotics.

In 1970 the WHO proposed this medium for antibacterial sensitivity testing, and it has been widely used since then. Sensitivity testing can be conducted by a variety of techniques, both on solid and liquid media. The most commonly used method in routine work is that derived from Kirby-Bauer and recommended by the American Association of Clinical Pathologists.

The Kirby-Bauer method is a precise, semi-quantitative testing system. It uses Mueller-Hinton Agar and disks with a high antibiotic concentration. The inoculum is first standardized using a MacFarland standard, then the plate is inoculated with a swab dipped in the standardized suspension, and finally the disks are arranged properly equidistant from each other on the plate and then incubated (see the table).

Some authors suggest that the inoculum should be modified by introducing a double layer of inoculated medium. This system undoubtedly provides sharper and more defined zones of clearing of inhibition. Plates are incubated at 37°C overnight and then the zones of inhibition are measured. Results are reported in terms of Resistant, Moderately Resistant (Intermediate) and Sensitive strains (See table).

The Ericsson technique, which has been adopted in most European countries, uses a standardized culture medium (Mueller-Hinton), a standardized quantity per plate (25 mL on 9 cm diameter plates) and standardized inoculum concentration.

The fresh culture suspension used is incubated for 18 hours is in liquid medium and is then diluted accordingly. So as to ensure the appropriate amount of growth on the agar.

Suggested Dilutions:

- *Enterobacteria*- Pseudomonas: dilution of 1/300.
- *Staphylococcus* - *Enterococcus*: dilution of 1/300.
- *Streptococcus* - *Haemophilus*: dilution of 1/10.

The plate is seeded by flooding its surface. The excess inoculum is removed with a sterile pipette and the antibiotic disks are arranged properly on the plate. A pre-diffusion period of 30-60 minutes is allowed prior to incubation so that the antibiotic can slowly diffuse before growth. After incubation at 37°C for 12-18 hours, the zones of inhibition are measured and the Assay Regression Curves referenced. Results are reported in terms of Sensitive or Resistant or as Minimum Inhibitory Concentration (MIC) values.

The Ericsson technique undoubtedly offers more precision and reliability than the Kirby-Bauer. Nevertheless, the Kirby method, which is semi-quantitative, is much simpler and easier to perform in everyday practice. The Ericsson technique is highly recommended where high efficacy and precision are required.

Mueller-Hinton medium plates can be stored refrigerated in plastic bags for a month without affecting the results of the sensitivity testing. However, they should not be used if the medium shows any signs of dehydration.

Scharlau's Mueller-Hinton Agar fulfils the WHO requirements for microbial sensitivity tests and the basic characteristics are verified in every batch. Nevertheless some variation in results can sometimes occur. Please note the following factors that can be a source of variability:

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on the medium.

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Mueller-Hinton Agar

Art. No. 01-136

CE IVD

2. Factors such as: inoculum size, rate of growth, medium formulation and pH, length of incubation and incubation environment, disk content and drug diffusion rate, and measurement of endpoints can all affect the results.
   Therefore, strict adherence to protocol is required to ensure reliable results.
3. Disk diffusion susceptibility testing is limited to rapidly growing organisms. Drug inactivation may result from the prolonged incubation times required by slow growing organisms.
4. Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effects of sulphonamides and trimethoprim, causing zones of growth inhibition to be smaller or less distinct.
5. Variation in the concentration of divalent cations, primarily calcium and magnesium, affects results of amino-glycoside, tetracycline, and colistin tests with *Pseudomonas aeruginosa* isolates. A cation content that is too high reduces zone sizes, whereas a cation content that is too low has the opposite effect.
6. When Mueller-Hinton Medium is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2 to 3 mm smaller than those obtained with unsupplemented agar. Conversely, sheep's blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.
   Sheep's blood may cause indistinct zones or a film of growth within the zones of inhibition around sulphonamide and trimethoprim disks.
7. Mueller-Hinton Medium deeper than 4 mm may cause false-resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility results.
8. A pH value outside the range of 7,3 ± 0,1 may adversely affect susceptibility test results. If the pH is too low, amino-glycosides and macrolides will appear to lose potency; others may appear to have excessive activity.
   The opposite effects are possible if the pH is too high.
9. When Mueller-Hinton Medium is inoculated, no droplets of moisture should be visible on the surface or on the Petri dish cover.
10. Mueller-Hinton Medium should be inoculated within 15 minutes after the inoculum suspension has been adjusted.
11. The zone of inhibition diameters of some drugs, such as the macrolides, amino-glycosides and tetracyclines, are significantly altered by CO_2. Plates should not be incubated in an increased CO_2 atmosphere.

For further information on the performance of the antibiotic disk susceptibility test refer to the M2-A9 CLSI (formerly NCCLS) Monograph.

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Storage

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Mueller-Hinton Agar

Art. No. 01-136

CE IVD

Interpretation of inhibition zones of most common antibiotics according to the Kirby-Bauer Method

Antibiotic	Concentration	Diameter of the inhibition zone (in mm)		
		Resistant	*Moderately R.*	*Sensitive*
Ampicillin with S. aureus	10 mcg	20 or less	21 - 28	29 or more
Ampicillin	10 mcg	11 or less	12 – 13	14 or more
Bacitracin	10 iu	8 or less	9 – 12	13 or more
Cephaloridine	30 mcg	11 or less	12 - 15	16 or more
Cephalothin	30 mcg	14 or less	15-17	18 or more
Chloramphenicol	30 mcg	12 or less	13 -17	18 or more
Colistin	10 mcg	8 or less	9 - 10	11 or more
Doxycycline	30 mcg	12 or less	13 – 15	16 or more
Erythromycin	15 mcg	13 or less	14 – 17	18 or more
Gentamicin	10 mcg	12 or less	-	13 or more
Kanamycin	30 mcg	13 or less	14 – 17	18 or more
Lincomycin	2 mcg	9 or less	10 - 14	15 or more
Methicillin	5 mcg	9 or less	10 - 13	14 or more
Nalidixic, Acid	30 mcg	13 or less	14 – 18	19 or more
Neomycin	30 mcg	12 or less	13 - 16	17 or more
Nitrofurantoin	300 mcg	14 or less	15 - 16	17 or more
Novobiocin	30 mcg	17 or less	18 - 21	22 or more
Oleandomycin	15 mcg	11 or less	12 - 16	17 or more
Penicillin G	10 iu	20 or less	21 – 28	29 or more
Polymyxin B	300 iu	8 or less	9 - 11	12 or more
Streptomycin	10 mcg	11 or less	12 - 14	15 or more
Sulphonamide	300 mcg	12 or less	13 - 16	17 or more
Tetracycline	30 mcg	14 or less	15 - 18	19 or more
Vancomycin	30 mcg	9 or less	10 - 11	12 or more

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M

Mueller-Hinton Agar

Art. No. 01-136

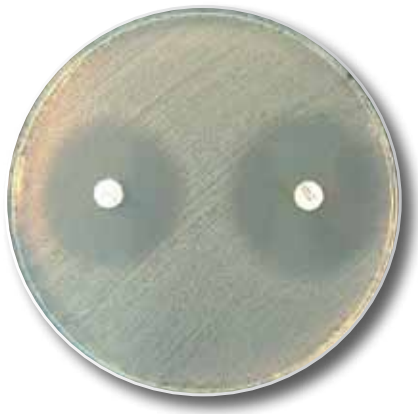
CE IVD

Quality control

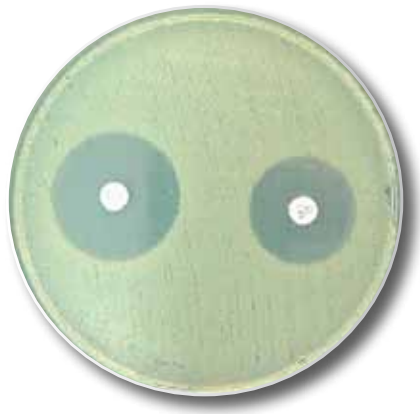
Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: Inoculate the entire agar surface and add antibiotic disks according to CLSI guidelines.

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Escherichia coli ATCC 25922	Good	-
Pseudomonas aeruginosa ATCC 27853	Good	-
Escherichia coli ATCC 35218	Good	-
Enterococcus faecalis ATCC 29212	Good	-

Pseudomonas aeruginosa ATCC 27853



Staphylococcus aureus ATCC 25923



Escherichia coli ATCC 25922

Mueller-Hinton Broth

Art. No. 02-136

Specification

Liquid version of the agar, recommended for studies of antibiotic MIC (Minimum Inhibitory Concentration).

Formula* in g/L

Peptone	17,50
Starch	1,50
Meat infusion Solids	2,00

Final pH 7,3 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 21 g of powder to 1 L of distilled water and dissolve completely. Distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

Mueller-Hinton Broth can be used in parallel with the agar when comparative studies are desired as well as when a broth with a high nutritive capacity is required. It is especially recommended for inoculum preparation for sensitivity assays.

In this medium, the presence of starch is very important, since it acts as a detoxifying agent against toxic substances if present in the sample and it also acts as a cell regenerator.

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- BARRY, A.L., M.D. COYLE, C. THORNBERRY, E.H. GARLACH & R.W. HAWKINSON (1979) Methodsof measuring zones of inhibition with Bauer-Kirby disk-susceptibility test. J. Clin. Microbiol. 10:885-889.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH)..

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 10 - 100 CFU (Productivity)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Escherichia coli ATCC 25922	Good	-
Pseudomonas aeruginosa ATCC 27853	Good	-
Escherichia coli ATCC 35218	Good	-
Enterococcus faecalis ATCC 29212	Good	-

M

Muller-Kauffmann Tetrathionate Broth Base

Art. No. 02-335

Also known as

MKTTn

Specification

Medium used for the selective enrichment of *Salmonellae*, according to ISO standards.

Formula* in g/L

Bile salts No. 3	4,78
Meat extract	4,30
Casein peptone	8,60
Sodium chloride	2,60
Calcium carbonate	38,70
Sodium thiosulfate (anhydrous)	30,50 (*1)

Final pH 8,0 ± 0,2 at 25°C

(*1) equivalent to 47,80 g/L Sodium thiosulfate 5 H_20

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 89,48 g of powder to 1 L of distilled water. Bring to the boil and let it cool to 40-45°C. Add 20 mL of iodine/iodide solution and 2 vials of the Brilliant Green-Novobiocin (Art. No. 06-017CASE or 06-017-LYO) selective supplement and distribute into sterile tubes.

Do not reheat. The complete medium must be used immediately; the base, without iodine or antibiotic, may be stored in the refrigerator until needed.

White precipitate is due to calcium carbonate and does not effect the broths performance.

Description

Tetrathionate Broth is a classic medium for the enrichment of enteric or intestinal pathogens, including all members of *Salmonella spp*., from heavily polluted samples, such as faeces, urine, waste water and others.

During preparation, when iodine is added, tetrathionate is produced from the sulfate, and this salt together with the bile salts in the medium, results in a strong inhibition of most of the normal intestinal bacteria, except for those which are capable of reducing tetrathionate, e.g. *Salmonellae*. Reduction reactions liberate sulphuric acid, which is neutralized by the carbonate, avoiding a decrease in the pH, which is harmful even for *Salmonellae*.

However, many *Proteus* species resist the bile salt concentration and, they may reduce tetrathionate. So, many authors recommend the addition of other inhibitors simultaneously, such as 0,1% Brilliant Green Solution (10 mL/L) and/or novobiocin at 40 mg/L.

Medium Base can be kept indefinitely in the refrigerator, but after the addition of inhibitors, efficacy of the medium decreases with time.

With refrigeration the MKTTn with brilliant green, novobiocin added remains effective for 2 months but only 48 hours at 37°C. Once the iodine solution is added it only remains effective for 40 hours.

Technique

Prepare the broth base, distribute it into tubes, sterilize and cool it. Add the brilliant green solution and store it in the refrigerator. If you are going to use the medium within 60 days, you may also add novobiocin. Iodine solution has to be added just before use. Do not reheat the medium after any of these additions. The usual technique consists of adding the sample to the medium (1:10) and then homogenizing it well. Incubate at 37°C for a period not longer than 48 hours, since after this time the medium loses its selectivity and the suppressed flora may also grow. Some authors suggest incubation at 43°C and observations after 18, 24 and 48 hours, but one can get better results if a sample is taken from the surface of the broth after 30-36 hours.

Take aliquots with a loop and inoculate onto the surface of a selective media such as SS Agar (Art. No. 01-555) or Hektoen Enteric Agar (Art. No. 01-216), etc.

Necessary supplements

Brilliant Green + Novobiocin Selective Supplement (Art. No. 06-017CASE / 06-017-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Brilliant green	5,00 mg
Novobiocin, sodium salt	20,00 mg

Ethanol-Distilled water (1: 20 Solvent)

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Muller-Kauffmann Tetrathionate Broth Base

Art. No. 02-335

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- U.S. PHARMACOPOEIA (2002) 25th ed. <61> Microbial Limits Test. US Pharmacopeial Convention Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

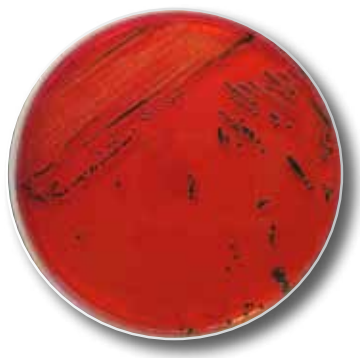
Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Inoculation according to ISO 6579 standard

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Recovery in TSA
Escherichia coli ATCC 8739	Inhibited	Recovery in TSA
Salmonella typhimurium ATCC 14028 or	Good	Recovery in XLD (Mixed cultures)
Salmonella enteritidis ATCC 13076	Good	Recovery in XLD (Mixed cultures)
Escherichia coli ATCC 25922 +	Inhibited	Recovery in XLD (Mixed cultures)
Pseudomonas aeruginosa ATCC 27853 +	Inhibited	Recovery in XLD (Mixed cultures)

Escherichia coli ATCC 8739
Total inhibition

Left: *Salmonella typhimurium* ATCC 14028 +
Centre: ***Pseudomonas aeruginosa*** ATCC 27853 +
Right: *Escherichia coli* ATCC 25922 (Mixed cultures)



Salmonella typhimurium ATCC 14028

Neutralizing Fluid

Art. No. 02-512

Specification

Liquid medium for neutralizing antimicrobials, according to the European Pharmacopoeia.

Formula* in g/L

Peptone	1,00
L-Histidine	1,00
Lecithin	3,00
Monopotassium phosphate	3,60
Disodium phosphate	7,20
Sodium chloride	4,30

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20,1 g of powder in 1 L of distilled water containing 30 mL of Polysorbate 80 (Art. No. TW0080). Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and homogenize the solution.

Description

Neutralizing Fluid is formulated according to the European Pharmacopoeia specification for the microbiological examination of non-sterile products. Its composition is the same as the general diluting solution for biological assays with the addition of polysorbate and lecithin as non toxic neutralizing agents.

However, the European Pharmacopoeia allows the concentration of inactivator to be changed depending on the preservative being neutralised. The following table provides guideline concentrations of inactivators according to the European Pharmacopoeia, that must be added aseptically to the neutralizing fluid once sterilized and cooled to 50°C or below.

References

- EUROPEAN PHARMACOPOEIA (2002) 4th ed. Supplement 4.7 § 2.6.13. Microbiological examination of Non-sterile products. (Test for specified organisms). Council of Europe. Strasbourg.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Inactivators of antimicrobial agents that can be added to Neutralizing Fluid and recommended concentrations

Anti Microbial Agent	Inactivator	Concentration
Phenolic Compounds	Sodium laurylsulphate	4 g/L
	Polysorbate 80 and Lecithin	30 g/L and 3 g/L
	Egg yolk	5-50 mL/L
Organo-mercurials	Sodium Thioglycollate	0,5-5 g/L
Halogenated Compounds	Sodium Thiosulfate	5 g/L
Quaternary Ammonium Compounds (QAC's)	Egg yolk	5-50 mL/L

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Neutralizing Fluid

Art. No. 02-512

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. (Productivity) at 0, 45 minutes and 3 h. (20 - 25ºC)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Satisfactory
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory
Candida albicans ATCC 10231	Good	No significant reduction
Escherichia coli ATCC 8739	Good	Satisfactory
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Bacillus subtilis ATCC 6633	Good	Satisfactory

Neutralizing Special Broth

Art. No. 02-583

WARNING

H: 3.4.S/1; H317

P: P261-P280-P321-P363-P333+P313-P501a

Specification

Liquid medium used for the neutralisation of preservatives in cosmetics and pharmaceutical products.

Formula* in g/L

Tryptone	5,00
Yeast extract	2,50
Dextrose	10,00
Sodium thioglycollate	1,00
Sodium thiosulfate	0,60
Sodium bi-sulfite	2,50
Lecithin	1,00
Bromocresol purple	0,04

Final pH 7,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 22,64 g of the powder in 1 L of distilled water containing 5 mL of Polysorbate 80 (Art. No. TW0080). Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This broth is a modification of the classical Dey-Engley Neutralizing Broth in which the concentration of lecithin and thiosulfate are lowered and the indicator increased to obtain an optimum balance between neutralization of preservatives and recuperation of stressed cells. Complete neutralization of preservatives and disinfectants is important because carry over of biocides can cause a false non-growth test result, as can an excess of neutralizing agents on the stressed cells. These are critical points to consider when evaluating a disinfectant or a preservative and being able to differentiate between bacteriostatic or bactericidal effects.

Tryptone provides the nitrogen and carbon sources for growth. Dextrose as a fermentable carbohydrate is the main source of energy and the yeast extract provides vitamins and cofactors required for microbial growth. Sodium thioglycollate neutralizes mercurials. Sodium thiosulfate neutralizes chlorine and iodine.

Sodium bi-sulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds. Polysorbate neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Bromocresol purple acts as a colorimetric indicator to show the production of acid due to the fermentation of dextrose.

References

- ATLAS, R.M. & L. C. PARKS (1993) Handbook of microbiological methods. CRC Press. Boca Ratón. Fla. USA.
- DEY, B.P. & F.B. ENGLEY, Jr. (1994) Neutralization of antimicrobial chemicals by recovery media. J. Microbiol. Methods. 19:51-58.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h (TSA)
Inoculum: 10-100 CFU. (Productivity) at 0,45 minutes and 3 h. (20 - 25°C)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Satisfactory
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory
Bacillus subtilis ATCC 6633	Good	Satisfactory
Escherichia coli ATCC 25922	Good	Satisfactory
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Candida albicans ATCC 10231	Good	Satisfactory

Nickerson Agar (Biggy Agar)

Art. No. 01-137

Also known as

Nickerson Agar; Bismuth Glycine Glucose Yeast Agar; Nickerson *Candida* Selective Agar

Specification

Solid medium for the isolation and identification of *Candida spp.*

Formula* in g/L

Yeast extract	1,00
Dextrose	10,00
Glycine	10,00
Sodium sulfite	3,00
Ammonium bismuth citrate	5,00
Agar	15,00

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 44 g of powder in 1 L of distilled water and bring to the boil. Dispense in tubes or Petri dishes, stirring the precipitate before pouring. **Do not autoclave. Avoid overheating.**

Description

Nickerson Agar is suitable for the isolation and identification of *Candida* species. The medium is made according to the general principles of Bismuth-Sulfite Agar. An inhibitory and differential medium using a high concentration of glycine for selectivity. This medium is highly inhibitory, and does not allow bacterial growth, however most *Candida spp.* grow freely and rapidly. Occasionally, tiny colonies of bacteria or highly repressed moulds may appear. Bacterial development may be totally prevented by adding neomycin sulfate 2 mcg/mL to the medium before dispensing. At this concentration the antibiotic will not affect the development or appearance of yeast.

The appearance of the colonies in this medium after an incubation of 48-72 hours at 30-35°C is as follows:

- *Candida albicans:* Creamy colonies, very convex, circular with very slight mycelial border and black or dark brown in colour. It has no metallic sheen or diffused pigment, even after 72 hours of incubation.
- *Candida tropicalis:* Acuminated colonies, creamy, irregular and with slight mycelial borders. Dark brown with black centre. After 72 hours of incubation the colonies may take on a metallic sheen and produce a diffused zone of pigment.
- *Candida krusei*: Big and plain colonies, with irregular borders. Brown colour, darker in the centre. A yellow halo appears around the colony.
- *Candida parakrusei*: Plain colonies, average size, irregular. Dark red centre and light red borders, but when the border is mycelial it looks yellow.
- *Candida pseudotropicalis*: Big and plain colonies, dark red colour with mycelial border.
- *Candida stellatoidea*: Average size plain colonies, dark brown colour, without mycelial development.
- *Rhodotorula*: Creamy convex colonies, with irregular border and colours ranging from pink to orange.
- Moulds in general: Restricted colonial growth and cottony appearance.

To maintain these colony characteristics it is important that the medium is freshly prepared and not reheated or overheated.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. BocaRaton. Fla. USA.
- FORBES, B.A., D.F. SAHM & A.S. WEISSFELD (1998) Bailey & Scott's Diagnostic Microbiology. Mosby. St Louis. MO. USA.
- ISENBERG, H.D. (1995) Clinical Microbiology Procedures Handbook. ASM Press. Washington. DC. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MacFADDIN, J.D. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. William & Wilkins. Baltimore. MD. USA.
- MURRAY, P.R., E.J. BARON, J.H. JORGENSEN, M.A. PFALLER & R.H. YOLKEN (Eds) (2003) Manual of Clinical Microbiology. 8th ed. ASM Press. Washington. DC. USA.
- NICKERSON (1953) Reduction of Inorganic substance by yeast I. Extracellular reduction of sulfite by species of *Candida* J. Inf. Dis 93:43.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Nickerson Agar (Biggy Agar)

Art. No. 01-137

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibited	Selectivity
Saccharomyces cerevisiae ATCC 9763	Partial inhibition	-
Candida albicans ATCC 10231	Productivity > 0.70	Light-dark brown
Candida albicans ATCC 2091	Productivity > 0.70	Light-dark brown

Candida albicans ATCC 2091

Uninoculated Plate

Candida albicans ATCC 10231

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Nitrate Broth

Art. No. 02-138

Specification

Liquid culture medium used for verifying the nitrate reducing ability of enterobacteria.

Formula* in g/L

Meat extract.....3,00
Peptone.....5,00
Potassium nitrate.....1,00
Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 9 g of powder in 1 L of distilled water, heating only if necessary to dissolve. Distribute into final containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Nitrate Broth is prepared according to the classical formula for testing nitrate reduction by enterobacteria, although it can also be used with other bacterial types.

Technique

Inoculate 2-3 tubes of broth with one loop of pure culture and incubate at 37°C, reading after 1 day, 2 days and 5 days. To each tube, add several drops of Nitrate A Reagent (Art. No. 06-003) and also Nitrate B Reagent (Art. No. 06-004). If all readings remain negative, it is recommended to investigate for the presence of nitrate by the addition of using zinc powder. Production of a red colour after the addition of zinc indicates unreduced nitrate and a negative result. The absence of colour indicates nitrate was reduced beyond nitrate and a positive result

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Rev. A. AOAC International Inc. Gaithersburg. MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 1.000 - 10.000 CFU

Microorganism	Growth	Remarks
Salmonella typhimurium ATCC 14028	Good	Nitrate (+)
Escherichia coli ATCC 25922	Good	Nitrate (+)
Escherichia coli ATCC 8739	Good	Nitrate (+)
Pseudomonas aeruginosa ATCC 27853	Good	Nitrate (+)

Left: *Escherichia coli* ATCC 25922
Centre: *Escherichia coli* ATCC 8739
Right: Negative nitrate (Control)

Nutrient Agar

Art. No. 01-140

Specification

Solid culture medium for general purpose use with less fastidious organisms according to 16266:2006 and EN12780:2002 ISO standards.

Formula* in g/L

Meat extract	1,00
Yeast extract	2,00
Peptone	5,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,4 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 28 g of powder in 1 L of distilled water and bring to the boil dissolving completely. Sterilize in the autoclave at 121°C for 15 minutes.

Description

Nutrient Agar is a simple medium based on meat infusions, complemented with yeast extract to reinforce its nutrient qualities as well as its growth factors. It is most suitable for general routine work and can support the growth of common organisms, even those considered somewhat fastidious with regard to nutrient requirements. The incorporation of sodium chloride allows for the addition of Blood if necessary, even though this is not an optimal medium for very fastidious organisms.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BRITISH STANDARD S41 (1934) Determining the Rideal-Walker Coefficient of Disinfectants. BSI London 9.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
- EUROPEAN NORME (EN) 12780:2002 Water Quality - Detection and enumeration of *Pseudomonas aeruginosa* by membrane filtration.
- ISO 16266 Standard (2006) Water Quality - Detection and enumeration of *Pseudomonas aeruginosa* - Method by membrane filtration.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Nutrient Agar

Art. No. 01-140

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Escherichia coli ATCC 8739	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Yersinia enterocolitica ATCC 9610	Productivity > 0.70	-

Enterococcus faecalis ATCC 19433

Salmonella typhimurium ATCC 14028

Nutrient Agar (APHA)

Art. No. 01-144

Specification

Solid culture medium for general purpose use according to ISO standards and APHA.

Formula* in g/L

Peptone	5,00
Meat extract	3,00
Agar	15,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 23 g of powder in 1 L of distilled water and bring to the boil. Dispense into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

APHA Nutrient Agar is a classical meat infusion medium. It is a very simple medium that can be used as a routine culture medium or as nutrient base to which growth factors can be added if necessary. This medium with the pH adjusted to 8.0 ± 0.2 is recommended for use as Assay Medium H in the Antibiotic Assay Chapter of the European Pharmacopoeia 6.0.

References

- APHA (1948) Standard Methods for the Examination of Dairy Products. Washington.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- EUROPEAN PHARMACOPOEIA 6.1 (2009) Antibiotic Assay. EDQM. Council of Europe. Strasbourg.
- ISO 6340 Standard (1995) Water Quality - Detection of *Salmonella spp.*
- ISO 6579 Standard (2002) Horizontal method for the detection of *Shigella spp.*
- ISO 6785 Standard (2001) Milk and milk products - Detection of *Salmonella spp.*
- ISO 8523 Standard (1991) General guidance for the detection of Enterobacteriaceae with pre-enrichment.
- ISO 10273 Standard (1994) General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica.*
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Nutrient Agar (APHA)

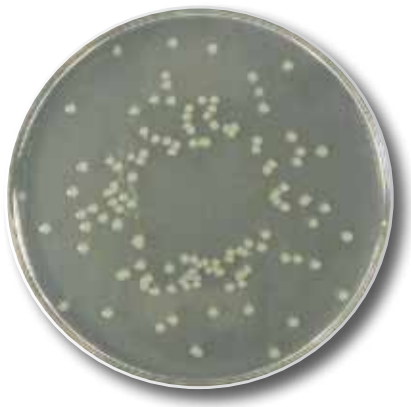
Art. No. 01-144

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Yersinia enterocolitica ATCC 9610	Productivity > 0.70	-

Bacillus subtilis ATCC 6633



Escherichia coli ATCC 25922

Nutrient Agar (ISO)

Art. No. 01-635

Specification

General purpose solid culture medium, according to 21528-1 and 21528-2 ISO standards.

Formula* in g/L

Peptone	5,00
Meat extract	3,00
Sodium chloride	5,00
Agar	15,00

Final pH at 25ºC, 7,3 ± 0,2

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 28 g of powder in 1 L of distilled water and bring to the boil. Dispense into suitable containers and sterilize in the autoclave at 121ºC for 15 minutes.

Description

By the addition of sodium chloride the present formulation is a modification of the classical formulation of APHA and other ISO standards. The inclusion of sodium chloride gives an osmotic pressure more appropriate for bacterial growth.

References

- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 21528 Standard (2004) Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 1: Detection and enumeration by MPN technique with pre-enrichment - Part 2: Colony-count method.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Yersinia enterocolitica ATCC 9610	Productivity > 0.70	-

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Nutrient Broth

Art. No. 02-140

Specification

A general purpose liquid culture medium for non-fastidious microorganisms, according to the British Pharmacopoeia.

Formula* in g/L

Meat extract	1,00
Yeast extract	2,00
Peptone	5,00
Sodium chloride	5,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 13 g of powder in 1 L of distilled water, heating if necessary to dissolve the medium. Distribute into containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Nutrient Broth is the liquid version of the solid medium. It is a classical meat infusion broth, that is useful for routine laboratory purposes Supplementation with yeast extract allows the growth of most common organisms. It is also suitable for the preparation of inocula, efficacy testing of biocides, as well as for the determination of the Phenol Coefficient etc.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BRITISH PHARMACOPOEIA (1968) 357.
- BRITISH STANDARD S41 (1934) Determining the Rideal-Walker Coefficient of Disinfectants. BSI London 9.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Good	-
Enterococcus faecalis ATCC 19433	Good	-
Bacillus subtilis ATCC 6633	Good	-
Escherichia coli ATCC 25922	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Yersinia enterocolitica ATCC 9610	Good	-

Nutrient Broth (APHA)

Art. No. 02-144

Specification

Liquid medium for the cultivation of non-fastidious microorganisms according to APHA and ISO standards.

Formula* in g/L

Meat peptone.....5,00
Meat extract.....3,00
Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 8 g of powder in 1 L of distilled water heating only if necessary. Dispense into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Nutrient Broth is the liquid version of Nutrient Agar, and is a classical medium for routine tasks with non fastidious microorganisms. It is the ideal medium for the subculture of bacteria, especially staphylococci, with a view to performing coagulase and other biochemical tests.

References

- APHA (1948) Standard Methods for the Examination of Dairy Products. Washington.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- ISO 8523 Standard (1991) General guidance for the detection of Enterobacteriaceae with pre-enrichment.
- ISO 6785 Standard (2001) Milk and milk products - Detection of *Salmonella spp.*
- ISO 6340 Standard (1995) Water Quality - Detection of *Salmonella species.*
- ISO 6579 Standard (2002) Horizontal method for the detection of *Salmonella*.
- ISO 10273 Standard (1994) General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica*.
- ISO 6579 Standard (2002) Horizontal method for the detection of *Shigella spp*.
- ISO 16266 Standard (2006) Water Quality - Detection and enumeration of *Pseudomonas aeruginosa* - Method by membrane filtration.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Pure cultures using an inoculating needle

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Good	-
Enterococcus faecalis ATCC 19433	Good	-
Bacillus subtilis ATCC 6633	Good	-
Escherichia coli ATCC 8739	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Yersinia enterocolitica ATCC 9610	Good	-

Orange Serum Agar

Art. No. 01-698

Also known as

OSA

Specification

Solid medium for the culture of aciduric organisms especially those associated with the spoilage of citrus products and their derivatives.

Formula* in g/L

Tryptone	10,00
Yeast Extract	3,00
Orange Serum	5,00
Dextrose	4,00
Disodium phosphate	3,00
Agar	17,00

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 42 g of powder in 1 litre of distilled water and boil to dissolve the agar. Distribute in suitable containers and autoclave for 15 min at 121°C. Avoid unnecessary overheating so as to minimise the darkening (caramelisation) and loss of gelification of the medium. It is recommended that the medium be used immediately after preparation.

Description

Orange Serum Agar was developed in the 1950's by Hays and coworkers for the detection, enumeration and isolation of spoilage microoganisms in fruit juices and products derived from citrus. Products with a low pH have microbial growth restricted to that of aciduric microorganisms. In a later study it was shown that Orange Serum Agar pH 5.4 was the most suitable medium for the isolation of lactic acid bacteria, especially (*Lactobacillus* and *Leuconostoc*) and yeasts that produce (buttermilk off-odour) in citrus fruits.

Orange Serum Agar is not a differential Agar but a culture medium in which the orange extract provides a favourable acidic environment in which aciduric microorganisms can be recovered including those damaged by food processing. Tryptone provides the main source of carbon and nitrogen, providing optimal growth conditions. Yeast Extract supplies Group B complex vitamins that stimulate growth and the phosphate provides an osmotic buffer for cell survival. Dextrose is a supplementary source of carbon and the agar is a solidifying agent.

Technique

The International Fruchtsaft-Union (IFU) recommends the use of Orange Serum Agar in several standardised methods, using the plate count method:

1. Prepare serial 10-fold dilutions of the sample using a suitable diluent such as (Buffered Peptone Water Art. No. 02-277).
2. Distribute aliquots of 1ml of the diluted sample in sterile Petri dishes.
3. Add 20ml of molten sterile medium cooled to 45°C, gently swirl the dish to mix the sample and medium properly.
4. Allow it to solidify and incubate at a 30 ± 1°C for 48 h before enumeration. If there is no growth extend the incubation to 5 days, reading daily before giving a negative result.

Generally the colonies of yeasts and moulds are distinguished by their morphology but those of aciduric bacteria need to be Gram stained and examined microscopically to be appropriately categorised.

References

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Orange Serum Agar

Art. No. 01-698

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 45 h - 5 days
Inoculum: 10-100 CFU. (according to standard ISO/TR 11133-1/2)

Microorganism	Growth	Remarks
Lactobacillus fermentum ATCC 9338	Productivity > 0.70	-
Saccharom-yces cerevisiae ATCC 9763	Productivity > 0.70	-

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Oxidation-Fermentation Fluid Medium Base (O/F Medium)

Art. No. 03-037

Also known as

O/F Enteric Medium; O/F Basal Medium according to Hugh & Leifson

Specification

Fluid medium used for determining the oxidative and/or fermentative metabolism of Gram negative bacilli.

Formula* in g/L

Casein peptone	2,00
Sodium chloride	5,00
Dipotassium phosphate	0,20
Bromothymol Blue	0,08
Agar	2,50

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 9,8 g of powder in 1 L of distilled water and bring to the boil. Add sugar in the desired concentration and distribute in fermentation tubes. Sterilize in the autoclave at 121°C for 15 min.

Description

Using this medium Hugh and Leifson were able to differentiate Gram negative bacteria into three categories: fermentative, oxidative and inactive. The organism to be studied is inoculated in two long narrow tubes (12x120 mm) by deep stab inoculation. One tube is covered with oil or a Vaseline® layer to induce an anaerobic environment that forces the strain to carry out fermentation.

Fermentative organisms produce a large amount of acid in both the tubes, and this is indicated by the yellow colouration of the Bromothymol Blue indicator. Bacteria that utilise an oxidative metabolic pathway carry out this reaction only in the tube without the oil/Vaseline. Inactive strains do not use sugars and therefore do not induce any change in either tube.

In some instances a slight blue colouration, probably due to alkalinization by peptone degradation, can occur.

Some authors have proposed the usage of just one tube for this assay, but the medium must be modified by solidifying (with 1,5% agar) and the addition of yeast extract and/or cystine. In these tubes the stab must be, at least, 8 cm deep.

Hugh and Leifson recommend simultaneous assay with glucose, lactose and sucrose of 1% concentration, adding the sugars, sterilised by filtration, to the medium.

References

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Oxidation-Fermentation Fluid Medium Base (O/F Medium)

Art. No. 03-037

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Pure cultures using an inoculating needle

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Poor	-
Pseudomonas aeruginosa ATCC 27853	Good	O / F: + / -
Escherichia coli ATCC 25922	Good	O / F: + / + Yellow medium
Salmonella typhimurium ATCC 14028	Good	O / F: + / + Yellow medium

Left: *Escherichia coli* ATCC 25922
Right: *Salmonella typhimurium* ATCC 14028

Pseudomonas aeruginosa ATCC 27853

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Oxford Agar Base

Art. No. 01-471

WARNING

H: 3.2/2; H315-3.3/2; H319
P: P280-P305+P351+P338-P321-P362-P332+P313-P337+P313

Specification

Solid, selective and differential medium for the detection, enumeration and isolation of *Listeria spp.* according to ISO standards 11290-1 and 11290-2.

Formula* in g/L

Tryptone	10,00
Lithium chloride	15,00
Proteose peptone	10,00
Sodium chloride	5,00
Yeast extract	3,00
Starch	1,00
Esculin	1,00
Ferric ammonium citrate	0,50
Agar	13,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 58,5 g of powder in 1 L of distilled water and let it soak. Bring to the boil and distribute 500 mL per flask. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and aseptically add the Oxford Agar Selective Supplement (Art. No. 06-127-LYO) to each flask. Mix well and pour into Petri dishes.

Note: Prepared medium (Agar + supplement) must be kept away from light, since it helps the production of acriflavine oxidised photocomplexes that can repress *Listeria* growth.

Description

Oxford Agar is a derivative of the original formulation used by Curtis *et al*, which had a high nutritive capability equivalent to Columbia agar with the addition of. Inhibitor agents helps reduce undesirable companion bacteria.

The current formulation retains the high capacity to support growth and inhibit both Gram negative and most Gram positive, bacteria including yeast. Thanks to the inhibitors incorporated in the selective supplement: cycloheximide, acriflavine, colistin, phosphomycin and cefotaxime and in a combination with lithium chloride the growth of all other bacteria except *Listeria* is inhibited.

Listeria colonies are easily recognizable since they hydrolyze esculin to free esculetin that reacts with the ferric ions and produces a dark precipitate around the colonies, which typically present as a grey-blue colour with a very dark core.

Technique

Although the selectivity of the medium is enough to allow the isolation and differentiation by direct surface inoculation, a previous dilution of the inoculum is advisable, using greater dilutions when the sample is highly polluted.

Most authors prefer one or two prior cultures in any of the primary enrichment media (UVM I, Art. No. 02-472 or Lovett, Art. No. 02-498) or secondary enrichment media (UVM II, Art. No. 02-472 or Fraser, Art. No. 02-496) before inoculating in Oxford Agar.

Incubation is carried out at 37°C, and after 24 hours typical colonies of *Listeria monocytogenes* are visible. However, it extending the incubation for another 20-24 hours is recommended in order to detect slow growing strains even though this may allow staphylococci or streptococci development.

Necessary supplements

Oxford Agar Selective Supplement (Art. No. 06-127-LYO)

Vial contents:

Necessary amount for 500 mL of complete medium.

Acriflavine	2,50 mg
Phosphomycin	5,00 mg
Sodium cefotaxime	1,00 mg
Colistin	10,00 mg
Cycloheximide	200,00 mg

Distilled water (Solvent)

References

- ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.
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- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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- VANDERZANT, C y D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Oxford Agar Base

Art. No. 01-471

WARNING

H: 3.2/2; H315-3.3/2; H319

P: P280-P305+P351+P338-P321-P362-P332+P313-P337+P313

Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 24 - 48 h

Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Listeria monocytogenes ATCC 19115	Productivity > 0.50	Esculin (+). Black medium
Listeria monocytogenes ATCC 19112	Productivity > 0.50	Esculin (+). Black medium
Listeria monocytogenes ATCC 7644	Productivity > 0.50	Esculin (+). Black medium
Listeria inocua ATCC 33090	Productivity > 0.50	Esculin (+). Black medium

Listeria monocytogenes ATCC 19114

Palcam Agar Base

Art. No. 01-470

Specification

Solid, selective and differential medium for the detection, enumeration and isolation of *Listeria spp.* according to ISO standards 11290-1 and 11290-2.

Formula* in g/L

Tryptone	23,00
Lithium chloride	15,00
Mannitol	10,00
Sodium chloride	5,00
Yeast extract	3,00
Starch	1,00
Esculin	0,80
Ferric ammonium citrate	0,50
Dextrose	0,50
Phenol red	0,08
Agar	13,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 72 g of powder in 1 L of distilled water and let it soak. Bring to the boil and distribute 500 mL per flask. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and aseptically add one vial of Palcam Agar Selective Supplement (Art. No. 06-110CASE or 06-110-LYO) to each flask. Mix well and pour into Petri dishes.

Note: Prepared medium (Agar + supplement) must be kept away from light, since it promotes the production of acriflavine oxidised photocomplexes that can repress *Listeria* growth.

Description

Palcam Agar is based on the formulation described initially by van Netten *et al.* which has a high selectivity and produces good colonial differentiation. Selectivity is achieved by the inclusion of lithium chloride, acriflavine, polymyxin B and ceftazidime, since they inhibit the growth of almost all the Gram negative and most of the Gram positive companion bacteria.

Listeria hydrolyze esculin to esculetin, which reacts with ferric ammonium citrate producing a dark precipitate and green-grey colonies with beige halos. If colonies of enterococci or staphylococci do grow on this medium they can be easily recognized, since they utilise mannitol and produce yellow colonies and haloes, contrasting with the cherry-red colour of medium.

However, when there are many *Listeria* colonies, the entire medium darkens, which can cause interference in differentiation. In these cases it is advisable to perform the inoculation with a more diluted sample.

Technique

Seed the Palcam Agar with growth from a primary enrichment broth (UVM I, Art. No. 02-472 or Lovett, Art. No. 02-498) or a secondary enrichment broth (UVM II, Art. No. 02-472 or Fraser, Art. No. 02-496). Incubate in a microaerophilic atmosphere for 48 hours at 37°C.

In these conditions, *Listeria* colonies have a size approx. 2 mm in diameter, and are green-grey in colour with a black core and halo. *Enterococcus* and *Staphylococcus* colonies are bigger, grey with a green-brown halo if they do not ferment mannitol and form yellow colonies with a yellow halo if they do. Presumptive *Listeria* colonies must be confirmed biochemically and serologically.

Necessary supplements

Palcam Agar Selective Supplement (Art. No. 06-110CASE/06-110-LYO)

Vial contents:

Necessary amount for 500 mL of complete medium.

Acriflavine	2,50 mg
Polymyxin B sulfate	5,00 mg
Sodium cephtazidime	10,00 mg

Distilled water (Solvent)

References

- ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press Boca Raton Florida.
- ISO 11290 standard (1996) Microbiology of food and animal feeding stuff. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1 - Detection method. Part 2 - Enumeration method.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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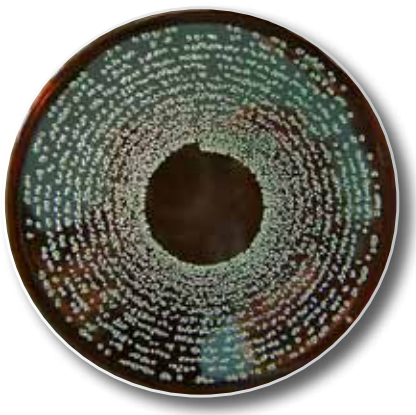
Palcam Agar Base

Art. No. 01-470

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 19433	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Listeria monocytogenes ATCC 19115	Productivity > 0.50	Esculin (+). Black medium
Listeria monocytogenes ATCC 19112	Productivity > 0.50	Esculin (+). Black medium
Listeria monocytogenes ATCC 7644	Productivity > 0.50	Esculin (+). Black medium
Listeria inocua ATCC 33090	Productivity > 0.50	Esculin (+). Black medium



Listeria monocytogenes ATCC 19112

Listeria monocytogenes ATCC 7644

Listeria monocytogenes ATCC 19114

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Phenol Red Broth Base

Art. No. 02-032

Specification

Liquid culture medium, suitable for sugar and other substrate fermentation studies.

Formula* in g/L

Casein peptone.....10,000
Sodium chloride.....5,000
Phenol red.....0,018
Final pH 7,40 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 15 g of powder in 1 L of distilled water. Add sugar in the desired concentration and distribute into suitable containers with Durham tubes. Sterilize in the autoclave at 121°C for 10 minutes. Pre heat the autoclave before putting the tubes into it to avoid sugar caramelization. Addition of some types of sugars may need a pH adjustment.

Description

Phenol Red Broth Base is a liquid version of the agar base for fermentation studies. Broth is preferred by many authors with the inclusion of Durham tubes, to verify gas production.
A sterile solution of sugar may be added after autoclaving the medium, or by adding impregnated discs to 10 mL of medium. Addition of some sugars may cause the acidification of the medium, in which case the original pH must be maintained by adding a few drops of 0,1 N NaOH.

For anaerobic bacteria, it is advisable to use freshly prepared medium, or place the medium in a boiling water bath for a few minutes, in order to eliminate dissolved oxygen. Many authors recommend the addition of 0,04% agar to avoid convection streams and subsequent incorporation of air.
The ISO standard 10273:1994 recommends adjustment of the final pH to 6,8 ± 0,2 to perform the auxonogram method for *Yersinia* identification.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- CRUIKSHANK, R. (1968) Medical Microbiology. 11th ed. Livingstone Ltd. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual 8th ed. Revision A. AOAC International. Gaithersburg. MD.
- ISO 10273 Standard (1994) General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica.*

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Pure culture (1000-10.000 CFU)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	Gas (-). Yellow medium
Bacillus subtilis ATCC 6633	Good	Gas (-). Yellowish medium
Enterococcus faecalis ATCC 19433	Good	Gas (-). Yellow medium
Escherichia coli ATCC 25922	Good	Gas (+). Yellow medium
Salmonella typhimurium ATCC 14028	Good	Gas (+). Yellow medium
Yersinia enterocolitica ATCC 9610	Good	Gas (-). Yellow medium

Left: *Escherichia coli* ATCC 25922
Centre: *Staphylococcus aureus* ATCC 25923
Right: Uninoculated tube (Control)

Phenylalanine Agar (PPA)

Art. No. 01-083

Also known as

PA Medium

Specification

Culture medium for enterobacteria, according to the Ewing *et al.* formulation.

Formula* in g/L

Yeast extract	3,00
DL-Phenylalanine	2,00
Di-sodium phosphate	1,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 26 g of powder in 1 L of distilled water and bring to the boil. Dispense in tubes or flasks and sterilize in the autoclave at 121°C for 15 minutes.

Description

This formulation corresponds to the solid form, proposed by Ewing *et al.* which is a modification of the medium developed by Buttiaux *et al.* achieving a long lasting green colour change that confirms a positive reaction.

The capacity to deaminate Phenylalanine oxidatively converting it to phenyl-pyruvic acid is a property of *Proteus spp* differentiating them from other enterobacteria. Phenyl-pyruvic acid is revealed by the presence of a characteristic greenish colour in the medium when it reacts with iron. Nowadays, this test and the urease production test, have great importance in the taxonomy of *Proteus spp.*

Technique

Inoculate the slant surface with plenty of inoculum, and incubate for 12-16 hours at 35 ± 2°C. Add 0,2 mL of 10% ferric chloride solution so that the solution floods the growth.

Phenyl-pyruvic acid (positive test) is shown by the development of a characteristic green-blue colour on the surface, after approximately 1 minute.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Inc.London.
- BUTTIAUX,R., R. OSTEUX, R. FRESNOY & J. MORIAMEZ (1954) Les propietés biochimiques du genre *Proteus*. Ann. Inst. Pasteur 87:357-386.
- EDWARDS and EWING (1973) Identification of Enterobacteriaceae. Burges Pub.Cod. Minneapolis. USA.
- ISENBERG, H.D. (1992) Clinical Microbiology Procedures Handbook. Vol I ASM Press Washington. DC. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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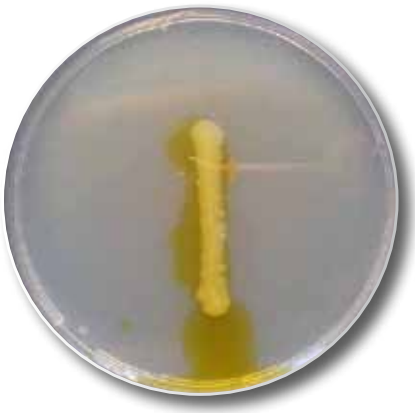
Phenylalanine Agar (PPA)

Art. No. 01-083

Quality control

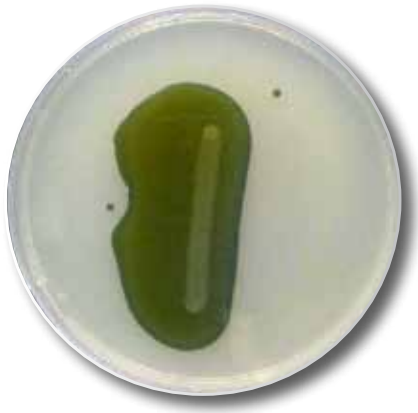
Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: Pure culture is inoculated by surface streaking

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	PPA (-) yellow
Salmonella typhimurium ATCC 14028	Good	PPA (-) yellow
Proteus mirabilis ATCC 43071	Good	PPA (+) dark green
Proteus mirabilis ATCC 29906	Good	PPA (+) dark green



Escherichia coli ATCC 25922
"Yellow reaction with 10% ferric Chloride solution"

" Growth without additive"



Proteus mirabilis ATCC 43071
"Dark green reaction with 10% ferric Chloride solution"

Phosphate-Buffered Peptone Water

Art. No. 02-568

Specification

Liquid non-selective pre-enrichment medium.

Formula* in g/L

Peptone	10,00
Sodium chloride	5,00
Disodium phosphate	3,50
Potassium phosphate	1,50

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 20 g of powder in 1 L of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is produced according the formulation of the *Schwezerisches Lebensmittelbuch* and is recommended for use in non-selective pre-enrichment for sub-lethally injured cells of the enterobacteria group in food or in others samples.

References

- BEKERS, H.J. (1987) Studies with salmonellae. J. Appl. Bact. 62:97-112.
- SCHWEIZERISCHES LEBENSMITTELBUCH (1992) 5th ed. Chapter 56A.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h (TSA)
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2) // Time: 0 and Time: 45 minutes

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Satisfactory recovery on TSA
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory recovery on TSA
Escherichia coli ATCC 8739	Good	Satisfactory recovery on TSA
Candida albicans ATCC 10231	Good	Satisfactory recovery on SAB
Salmonella typhimurium ATCC 14028	Good	Satisfactory recovery on TSA
Bacillus subtilis ATCC 6633	Good	Satisfactory recovery on TSA

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Plate Count Agar (PCA)

Art. No. 01-161

Also known as

Trypticase Glucose Yeast Agar; TGY; TGY Agar; Standard Methods Agar, SMA; SM Agar

Specification

Medium for aerobic plate counts by the surface inoculation method (standard Plate Count Agar) according to ISO 4833, 8552 & 17410 Standards and IFU No. 6.

Formula* in g/L

Casein peptone	5,00
Yeast extract	2,50
Dextrose	1,00
Agar	15,00

Final pH 7,0 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 23,5 g of powder in 1 L of distilled water. Dissolve by bringing to the boil with frequent stirring. Distribute into final containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

The Plate Count Agar formulation is according to that of Buchbinder *et al.* as recommended in their study of media for the plate count of microorganisms.

The original formulation of the standardized agar for dairy microbiology has been modified in order to avoid the addition of milk. This new composition allows the growth of most microorganisms without any further additions.

This medium's formulation is equivalent to that escribed by the 'Standard Methods for the Examination of Dairy products', the USP's 'Tryptone Glucose Yeast Agar', the 'Deutsche Landswirtchaft' and to the APHA and AOAC's Plate Count Agar. This is the medium of choice for the plate count of any type of sample.

Technique

Prepare ten fold serial dilutions of the sample and take 1 mL aliquots in duplicate from each dilution and put them into sterile Petri plates. Pour 20 mL approx. of sterile cooled medium (around 47°C) in each of the plates. Mix gently by swirling the plate in the form of a figure 8. Leave the plates undisturbed to solidify and incubate in an inverted position. The incubation time and temperature depend on the type of microorganism under study. For a general aerobic count, incubate for 3 days at 30°C. Taking readings after 24, 48 and 72 hours.

The plate count method proposed by the APHA consists of pouring the molten agar at 50°C on plates containing the diluted samples (pour plate technique). The final count is carried out after 48 hours of incubation at 32 -35°C.

For microorganisms with other temperature requirements, the following incubations have been suggested: 2 days at 32-35°C, 2-3 days at 45°C, 2 days at 55°C, 3-5 days at 20°C, 7-10 days at 5-7°C.

Sample dilutions are prepared with 1/4 Ringer's solution (Art. No. 06-073), Buffered Peptone Water (Art. No. 02-277), or Maximum Recovery Diluent (Art. No. 02-510) depending on their nature.

The poured plate count method is preferred to the spread plate technique, since it gives higher counts.

Nevertheless, the latter facilitates isolation and reseeding of the colonies.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BUCHBINDER, L., Y. BARIS & L. GOLDSTEIN (1953) Further studies on new milk-free media for the standard plate count of dairy products. Am. J. Public Health 43:869-872.
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- DIN 10192 (1971) Prüfungesbestimmungen für Milch und Milcherzeugnisse. Deutsche Landwirtsachft, Fachbereit und Ernahrung.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed., APHA, Washington.
- FIL/IDF Standards 3 (1958), 100, 101 (1981), 109 (1982) y 132 (2004).
- HORWITZ, W. (2000) Official Methods of Analysis. AOAC International. Gaithersburg.
- IFU Method No 6 (1996) Mesophilic, thermoduric and thermophilic bacteria: Spores Count. D-1 Mesophilic Aerobic Sporeforming bacteria: Spores count.
- ISO 4833 (2003) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count technique at 30ºC.
- ISO 8552 (2004) Milk- Estimation of psychrotrophic microorganisms. Colony count technique at 21ºC (Rapid method).
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 17410 (2001) Horizontal method for the enumeration of psychrotrophic microorganisms.
- MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA. Washington.

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Plate Count Agar (PCA)

Art. No. 01-161

• PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Staphylococcus aureus ATCC 6538	Productivity > 0.70	-
Listeria monocytogenes ATCC 19114	Productivity > 0.70	-
Yersinia enterocolitica ATCC 9610	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-

Staphylococcus aureus ATCC 6538

Escherichia coli ATCC 25922

Enterococcus faecalis ATCC 19433

Plate Count Modified Agar

Art. No. 01-329

Specification

Modified Plate Count Agar (with a lesser amount of agar), especially recommended for aerobic enumeration by the poured plate method.

Formula* in g/L

Casein peptone	5,00
Yeast extract	2,50
Dextrose	1,00
Agar	9,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 17,5 g of powder in 1 L of distilled water. Heat with constant stirring until boiling. Distribute in the suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Plate Count Modified Agar follows the same specifications as Plate Count Agar, except for a reduction in agar concentration. This modification provides better growth of colonies when using the poured plate method. As the medium is softer and hence the colonies can expand into the medium and appear larger.

Technique

Prepare ten-fold serial dilutions of the sample and take 1 mL in duplicate aliquots from each dilution and put them in sterile Petri dishes. Pour approx. 20 mL of sterile cooled medium (around 45°C) in each of the plates. Mix gently by swirling the plate in a figure 8. Leave the plates undisturbed to solidify and incubate in an inverted position. The incubation time and temperature depend on the type of microorganism under investigation. In general for an aerobic count, incubate for 3 days at 30°C. Checking the plates at 24, 48 and 72 hours.

The plate count method proposed by the APHA consists of the pour plate method i.e. pouring the molten agar at 50°C on plates containing the diluted samples. The final count is carried out after 48 hours of incubation at 32 -35°C.

For microorganisms with other temperature requirements, the following incubations have been suggested: 2 days at 32-35°C, 2-3 days at 45°C, 2 days at 55°C, 3-5 days at 20°C, 7-10 days at 5-7°C.

Sample dilutions are prepared with 1/4 Ringer's solution (Art. No. 06-073), Buffered Peptone Water (Art. No. 02-277) or Maximum Recovery Diluent (Art. No. 02-510) depending on their nature.

The poured plate count method is preferred to the surface inoculation method, since it gives higher counts, although the latter facilitates isolation and reseeding of the colonies.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BUCHBINDER, L., Y. BARIS & L. GOLDSTEIN (1953) Further studies on new milk-free media for the standard plate countof dairy products. Am. J. Public Health 43:869-872.
- CLESCERI, L.S., A.E.GREENBERG and A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed., APHA, AWWA, WPCF. Washington.
- DIN 10192 (1971) Prüfungesbestimmungen für Milch und Milcherzeugnisse. Deutsche Landwirtsachft, Fachbereit und Ernahrung.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed., APHA, Washington.
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- HORWITZ, W. (2000) Official Methods of Analysis. AOAC International. Gaithersburg.
- IFU Method No 6 (1996) Mesophilic, thermoduric and thermophilic bacteria: Spores Count. D-1 Mesophilic Aerobic Sporeforming bacteria: Spores count.
- ISO 4833 (2003) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count technique at 30°C.
- ISO 8552 (2004) Milk- Estimation of psychrotrophic microorganisms. Colony count techniqueat 21°C (Rapid method).
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 17410 (2001) Horizontal method for the enumeration of psychrotrophic microorganisms.
- MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA. Washington.
- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Plate Count Modified Agar

Art. No. 01-329

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Yersinia enterocolitica ATCC 9610	Productivity > 0.70	-

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Plate Count Skim Milk Agar

Art. No. 01-412

Specification

Solid medium for the plate count of milk and dairy products, according to DIN and FIL/IDF standards.

Formula* in g/L

Casein peptone	5,00
Yeast extract	2,50
Skimmed milk	1,00
Dextrose	1,00
Agar	10,50

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 20 g of powder in 1 L of distilled water and let it soak. Bring to the boil, constantly stirring. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium, with added milk, is more nutrient rich than other standard media; however, the opalescence of the medium makes early observations sometimes difficult.

Due to its lower agar concentration, it may be used for the pour plate method or the spread plate method.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BUCHBINDER, L., Y. BARIS & L. GOLDSTEIN (1953) Further studies on new milk-free media for the standard plate count of dairy products. Am. J. Public Health 43:869-872.
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- FIL/IDF Standards 3 (1958), 100, 101 (1981), 109 (1982) y 132 (2004).
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- IFU Method No 6 (1996) Mesophilic, thermoduric and thermophilic bacteria: Spores Count. D-1 Mesophilic Aerobic Sporeforming bacteria: Spores count.
- ISO 4833 (2003) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count technique at 30°C.
- ISO 8552 (2004) Milk- Estimation of psychrotrophic microorganisms. Colony count techniqueat 21°C (Rapid method).
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 17410 (2001) Horizontal method for the enumeration of psychrotrophic microorganisms.
- MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA. Washington.
- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Plate Count Skim Milk Agar

Art. No. 01-412

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Bacillus cereus var. mycoides ATCC 11778	Productivity > 0.70	-
Staphylococcus aureus ATCC 6538	Productivity > 0.70	-
Listeria monocytogenes ATCC 19114	Productivity > 0.70	-

Staphylococcus aureus ATCC 6538

Escherichia coli ATCC 25922

Listeria monocytogenes ATCC 19114

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Potato Dextrose Agar (Eur. Pharm.)

Art. No. 01-483

Also known as

PDA

Specification

Solid culture medium used for the detection and enumeration of yeast and moulds in food, specially recommended for dairy products and other samples, according to the Pharmacopeial Harmonized Methodology.

Formula* in g/L

Potato peptone	4,00 (1)
Glucose	20,00
Agar	15,00

Final pH 5,6 ± 0,2 at 25°C

(1) Equivalent to a 200 g Infusion from potatoes

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 39 g of powder in 1 L of distilled water and bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat.**

Description

Potato Dextrose Agar is a weakly selective medium for fungi due to its high sugar content and acidic pH. Pigment production and aerial mycelium development is enhanced by the potato peptone, especially in *Fusarium, Aspergillus* and *Penicillium* species.

The selectivity can be increased by adding antibiotics such as chloramphenicol or tetracycline, or by simply decreasing the pH to an acidic level. At pH 3,5 bacterial growth is almost totally inhibited without a significant effect on fungi. This acidification can be obtained by the aseptic addition of an adequate amount of organic acid to the medium after sterilization: 10-15 mL/L of a 10% sterile solution of tartaric or lactic acid is usually sufficient.

After its acidification the medium should not be overheated or reheated since it can hydrolyze the agar causing a pontential loss in the solidification property of the medium.

Technique

Distribute the diluted samples into sterile Petri plates. Pour over molten agar cooled to 45-50°C and gently mix to homogenize the mixture. After solidification, plates are incubated for 5-7 days at 20-25°C to permit the complete development of the fungal colonies.

The weak consistency of the agar due to its original acidity makes this medium inadequate for streaking.

References

- ATLAS R.M. (1995) Handbook of Microbiological Media for the Examination of Food. CRC Press. Boca Raton. Florida. USA.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
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- RICHARDSON, G. H. (1985) Standard Methods for the examination of dairy products 15th ed. APHA. Washington.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA
- VANDERZANT, C. & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. 3rd ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60 % RH).

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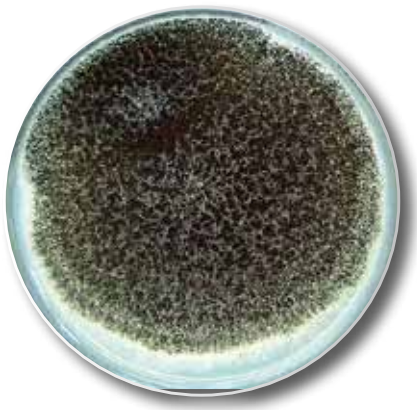
Potato Dextrose Agar (Eur. Pharm.)

Art. No. 01-483

Quality control

Incubation temperature: 20 - 25°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	48 h
Bacillus subtilis ATCC 6633	Fair to good	48 h
Candida albicans ATCC 10231	Productivity > 0.70	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	Black sporulation at 5 days



Aspergillus brasiliensis ATCC 16404

Candida albicans ATCC 10231

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Potato Dextrose Broth

Art. No. 02-483

Specification

Liquid culture medium for the maintenance and multiplication of yeast.

Formula* in g/L

Potato peptone.....4,00 (*1)
Glucose.....20,00
Final pH 5,6 ± 0,2 at 25°C
(*1) equivalent to a 200g Infusion from potatoes

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 24 g of powder in 1 L of distilled water, heating only if necessary. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Potato Dextrose Broth is the liquid version of the agar. This broth is mainly used to detect and enumerate yeast and moulds, since it does not contain any solidifying agent it may be acidified without altering its physical properties.
At pH 3,5, bacterial growth is totally inhibited without significant influence on fungi. This acidification may be achieved by the aseptic addition of an adequate amount of organic acid to the medium after sterilization:
10-15 mL/L of a 10% sterile solution of tartaric or lactic acid. This addition may also be made before sterilization, but it must be considered that in acidic conditions Maillard reactions are strong and hence the medium may turn slightly brownish.

References

- ATLAS R.M. (1995) Handbook of Microbiological Media for the Examination of Food CRC Press. Boca Raton. Fla.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington.
- RICHARDSON, G. H. (1985) Standard Methods for the examination of dairy products 15th ed. APHA. Washington. DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 20 - 25°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	-
Bacillus subtilis ATCC 6633	Good	-
Candida albicans ATCC 10231	Good	-
Saccharomyces cerevisiae ATCC 9763	Good	-
Aspergillus brasiliensis ATCC 16404	Good	-

Preston *Campylobacter* Agar Base

Art. No. 01-451

Also known as

Nutrient Agar no. 2

Specification

A general purpose medium-base that when appropriately supplemented can be used as a selective medium for *Campylobacter*.

Formula* in g/L

Meat Extract	10,00
Peptone	10,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,5 at 25ºC ± 0,2

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 40 g of the powder to 1 litre of distilled water and bring to the boil. Distribute in suitable containers and sterilize in an autoclave for 15 minutes at 121°C. For Preston *Campylobacter* Agar, cool to 45-50°C and add to each 500 ml of medium base

a) Lysed Blood in a proportion of 5% (v/v),
b) A vial of Growth Supplement for *Campylobacter* Art. No. 06-128-008 and
c) A vial of Selective Supplement for *Campylobacter* according to Preston (Art. No. 06-130-LYO) or a vial of Modified Selective Supplement for *Campylobacter* according to Preston (Art. No. 06-135-LYO). Mix carefully and pour into Petri dishes.

Description

Nutrient Agar no. 2 differs from the usual nutrient formulations in that the greater concentration of nutrients allows improved recovery of stressed or damaged microorganisms. This greater nutrient enrichment with and reducing atmosphere that is conferred by the growth supplement (Art. No. 06-128-008) makes it a very suitable medium for microaerophilic/capnophilic microorganisms and if, in addition, some of the appropriate inhibitor supplements are added it becomes a selective medium for *Campylobacter*.

Necessary supplements

Campylobacter Preston Selective Supplement (Art. No. 06-130-LYO)

Polymyxin B sulfate	2500,00 IU
Rifampicin	5,00 mg
Trimethoprim	5,00 mg
Cycloheximide	50,00 mg

Distilled water (Solvent)

Campylobacter Preston Modified Selective Supplement (Art. No. 06-135-LYO)

Vial Contents:
Necessary amount for 500 mL of complete medium.

Polymyxin B sulfate	2500,00 IU
Rifampicin	5,00 mg
Trimethoprim	5,00 mg
Amphotericin B sulfate	5,00 mg

Distilled water (Solvent)

References

- BOLTON, F.J. & L. ROBERTSON (1982) A selective medium for isolating *Campylobacter jejuni/coli* J. Clin. Pathol. 35:462-467.
- BOLTON, F.J., D. COATES, P.M. HINCHLIFFE & L. ROBERTSON (1983) Comparison of selective media for isolation of *Campylobacter jejuni/coli* J. Clin. Pathol. 36:78-83.
- CORRY, J.E.L., H. I. ATABAY, S.J. FORSYTHE & L.P. MANSFIELD (2003) Culture Media for the Isolation of *Campylobacters, Helicobacters* and *Arcobacters*, en Corry *et al.* (Eds) Handbook of Culture Media for Food Microbiology Chap 18 pgs 271-316. Elsevier Science B.V. Amsterdam.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

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Preston *Campylobacter* Agar Base

Art. No. 01-451

Quality control

Incubation temperature: 42°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 100-1000 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 1113-1/2)

Microorganism	Growth	Remarks
Campylobacter jejuni ATCC 29428	Good	Under microaerophilic atmosphere
E. coli ATCC 25922	Inhibited	Under microaerophilic atmosphere

Preston *Campylobacter* Broth Base

Art. No. 02-561

Also known as

Nutrient Broth no. 2

Specification

Liquid medium for general use, with a high concentration of nutrients.

Formula* in g/L

Meat Extract..... 10,00
Peptone..... 10,00
Sodium chloride.....5,00
Final pH 7,5 at 25°C ± 0,2

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 25 g of powder in 1 litre of distilled water and bring to the boil. Distribute in suitable containers and sterilize in an autoclave for 15 minutes at 121°C. To formulate Preston Broth for the Selective Enrichment of *Campylobacter*, cool to 45-50°C and add to each 500 ml of medium base:

a) Lysed Blood in a proportion of 5-7% (v/v),
b) A vial of Growth Supplement for *Campylobacter* (Art. No. 06-128-008) and
c) A vial of Selective Supplement of *Campylobacter* according to Preston (Art. No. 06-130-LYO) or a vial of Modified Selective Supplement for *Campylobacter* according to Preston (Art. No. 06-135-LYO). Mix carefully and distribute in bottles or tubes with a threaded cap, ensuring that the space between the liquid and the top is the minimum possible to maintain microaerophilic conditions. This means the preparation can be maintained refrigerated at (2-8°C) for a maximum period of 8 days.

Description

Nutrient Broth no. 2 is particularly rich in nutrients, allowing the growth of very small inoculates of certain fastidious microorganisms. Its formulation corresponds to the British Norm (formulation) for the determination of the Coefficient of Rideal-Walker in disinfectants, although in the latter it is used in double concentration. This medium supplemented with blood, antibiotics and reducing agents is used as a selective enrichment broth in the detection and isolation of *Campylobacter* from very contaminated samples.

Necessary supplements

Campylobacter Growth Supplement (Art. No. 06-128-008)

Vial Contents:
Necessary amount for 500 mL of complete medium.
Sodium pyruvate.....0,125 g
Sodium metabisulfite.....0,125 g
Ferrous sulfate.....0,125 g

Distilled water (Solvent)

Campylobacter Preston Selective Supplement (Art. No. 06-130-LYO)

Vial Contents:
Necessary amount for 500 mL of complete medium.
Polymyxin B sulfate.....2500,00 IU
Rifampicin.....5,00 mg
Trimethoprim.....5,00 mg
Cycloheximide.....50,00 mg

Distilled water (Solvent)

Campylobacter Preston Modified Selective Supplement (Art. No. 06-135-LYO)

Vial Contents:
Necessary amount for 500 mL of complete medium.
Polymyxin B sulfate.....2500,00 IU
Rifampicin.....5,00 mg
Trimethoprim.....5,00 mg
Amphotericin B sulfate.....5,00 mg

Distilled water (Solvent)

References

- BOLTON, F.J. & L. ROBERTSON (1982) A selective medium for isolating *Campylobacter jejuni/coli* J. Clin. Pathol. 35:462-467.
- BOLTON, F.J., D. COATES, P.M. HINCHLIFFE & L. ROBERTSON (1983) Comparative of selective media for isolation of *Campylobacter jejuni/coli* J. Clin. Pathol. 36:78-83.
- CORRY, J.E.L., H. I. ATABAY, S.J. FORSYTHE & L.P. MANSFIELD (2003) Culture Media for the Isolation of *Campylobacters*, *Helicobacters* and *Arcobacters*, en Corry *et al.* (Eds) Handbook of Culture Media for Food Microbiology Chap 18 pgs 271-316. Elsevier Science B.V. Amsterdam.
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

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Preston *Campylobacter* Broth Base

Art. No. 02-561

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Quality control

Incubation temperature: 42°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 10-1000 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Campylobacter jejuni ATCC 29428	Good	Under microaerophilic atmosphere
E.coli ATCC 25922	Inhibited	Under microaerophilic atmosphere

Purple Broth Base

Art. No. 02-663

Specification

Broth base used for the biochemical differentiation of *Shigella spp*, based on carbohydrate fermentation according to 21567:2004 ISO standard.

Formula* in g/L

Peptone	10,00
Meat extract	3,00
Sodium chloride	5,00
Bromocresol purple	0,04

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 18 g of the powder in 1 L of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 min. Cool and aseptically add the appropriate amount of carbohydrate substrate in order to reach a final concentration of 1%. After the addition verify the sterility. The complete medium can be stored refrigerated for 4 weeks.

Description

The peptone and meat extract act as nitrogen and growth factors sources. The sodium chloride maintains the osmotic pressure and the Bromocresol purple is a pH indicator. The main carbon and energy source is the carbohydrate substrate added. When the substrate is utilised by the microorganisms, the medium acidifies and the indicator turns from purple to yellow.

Technique

The 21567:2004 ISO Standard describes the inoculation of each of the prepared carbohydrate broths with a small inoculum and incubation at 37 ± 1°C for 24 ± 3 hours. A positive reaction when carbohydrate is fermented gives a change in the pH indicator from purple to yellow.

References

- EWING, W.H. & A.A. LINDBERG (1984) Serology of the *Shigella*. In "Methods in Microbiology". Ed. Bergan T. Vol 14. Acadeic Press.
- ISO 21567 (2004) Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella spp.*

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10 - 100 CFU. (Productivity) // (Sugar: Dextrose)

Microorganism	Growth	Remarks
Shigella flexneri ATCC 12022	Good	Yellow medium
Shigella sonnei ATCC 9290	Good	Yellow medium
Salmonella typhimurium ATCC 14028	Good	Yellow medium
Escherichia coli ATCC 8739	Good	Yellow medium

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Purple Maximum Recovery Diluent

Art. No. 02-631

Specification

Isotonic diluent for the maximum recovery of stressed microorganisms from acidic foods and animal feeding stuffs according to ISO standards.

Formula* in g/L

Peptone	1,00
Sodium chloride	8,50
Bromocresol purple	0,04

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 9,54 g of powder in 1 L of distilled water and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This formulation is recommended by the ISO Standards 6887-2, -3 and -4 (2003) for the dilution of samples of **acidic or very acidic foods** and animal feeding stuffs. Adjustment of pH after the addition of the sample can be performed without the use of a sterile probe. The addition of sufficient sterile sodium hydroxide solution is required to return the indicator to the initial colour.

Sodium chloride ensures isotonic conditions and the low peptone concentration ensures cellular growth in the short period (2-4 hours) of time required for the preparation of serial dilutions. The Bromocresol Purple is yellow at acidic pH and turns purple above 6,8.

Technique

According to the ISO method, the sample is diluted in a ratio 1:10 with the Purple Maximum Recovery Diluent and homogenized by a vortex mixer or Stomacher®. The pH is adjusted to neutral with a sterile solution of NaOH until the indicator turns purple. After a short period (10-15 min) of rest, a series of tenfold dilutions with the same diluent is prepared following standard procedures. Plates or tubes are inoculated from the different concentrations.

References

- ISO 6887-1:1999. Standard. Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for the microbiological examination - Part 1: General rules for the preparation of initial suspension and decimal dilutions.
- ISO 6887-2:2003. Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for the microbiological examination - Part 2: Specific rules for the preparation of meat and meat products.
- ISO 6887-3:2003. Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for the microbiological examination - Part 3: Specific rules for the preparation of fish and fishery products.
- ISO 6887-4:2003. Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for the microbiological examination - Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products and fish and fishery products.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Purple Maximum Recovery Diluent

Art. No. 02-631

Quality control

Incubation temperature: 25ºC ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. (Productivity) at 0, 45 minutes and 3 h. (20 - 25ºC)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Satisfactory
Enterococcus faecalis ATCC 29212	Good	Satisfactory
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory
Escherichia coli ATCC 8739	Good	Satisfactory
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Candida albicans ATCC 10231	Good	No significant reduction

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R2A Agar

Art. No. 01-540

Specification

Solid medium for the enumeration of heterotrophic microorganisms in treated waters.

Formula* in g/L

Proteose peptone	0,500
Casein hydrolysate (Tryptone)	0,500
Yeast extract	0,500
D(+)-Glucose	0,500
Starch	0,500
Sodium pyruvate	0,300
Dipotassium hydrogen phosphate	0,300
Magnesium sulfate (anhydrous)	0,024
Agar	15,000

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 18,1 g of powder in 1 L of distilled water and bring to the boil constantly stirring. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

R2A Agar was proposed in 1979 by Reasoner and Geldenreich and a few years later accepted by the APHA as an alternative medium for the enumeration of stressed cells in treated potable water.

The use of nutrient rich media like PCA or TSA allows the growth of most microbes, but does not permit the recuperation of stressed or chlorine resistant organisms. Using a medium like R2A with low nutrients in combination with a lower temperature and longer incubation time it is possible to induce the resuscitation of these damaged cells.

In R2A Agar the source of nitrogen is the peptone and Yeast Extract supplies the vitamins and growth factors. The source of carbon is dextrose and magnesium sulfate and potassium phosphate maintain the osmotic pressure. The starch is a detoxifier and sodium pyruvate increases the recuperation of stressed cells. The agar acts as gelling agent.

Technique

The water sample must be processed as quickly as possible. If it is not possible to process within the first 6 hours, the sample must be refrigerated, but not for more than 30 hours.

R2A Agar can be used for pour plates, streak plates or filtration. The pour plate method can affect the recovery capacity of the medium because due to thermal shock when mixing molten agar with the sample. The incubating at 35°C, an incubation period of 3-5 days is recommended. In most circumstances an incubation temperature of 20-25°C for 5-7 days is more effective. Plates must be protected agains dehydration.

References

- ATLAS, R.M. (1995) Handbook of Media for Environmental Microbiology. CRC Press. Boca Raton. Fla. USA.
- CLESCERI, L.S., A.E. GREENBERG and A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed. APHA Washington D.C. USA
- EATON, A.D., A.E. GREENBERG and L.S. CLESCERI (1995). Standard Methods for the Examination of Water and Wastewater. 19th ed. APHA Washington D.C. USA .
- EUROPEAN PHARMACOPOEIA. 6th ed. Suppl 6.3 (2009) General Monographs. Water for injections. (pg. 4339) EDQM. Council of Europe. Strasbourg
- GREENBERG, A.E., R.R TRUSSELL and L.S. CLESCERI (1985). Standard Methods for the Examination of Water and Wastewater. 16th ed. APHA-AWWA-WPCF. Washington D.C. USA.
- REASONER, D.J. and E.E. GELDREICH (1979) A new Medium for the enumeration and subculture of bacteria from potable water. Abstracts of Annual Meeting. ASM 79th Meeting. Paper #N7.
- Van SOETSBERGER, A.A. and C.H. LEE (1969) Pour plates or streak plates?. Appl. Microbiol. 18:1092 -1094.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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R2A Agar

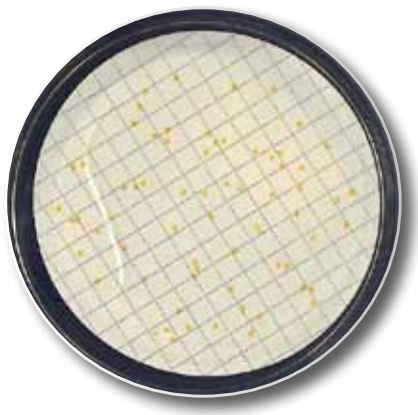
Art. No. 01-540

Quality control

Incubation temperature: 35°C / 20 - 28°C
Incubation time: 48 - 72 h / 5 - 7 days
Inoculum: 10-100 CFU. Spiral Plate Method / or Membrane Filter Method

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC 9027	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella abony NCTC 6017	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	-

Aspergillus brasiliensis ATCC 16404



Staphylococcus aureus ATCC 6538

Pseudomonas aeruginosa ATCC 9027

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Rappaport Vassiliadis Broth

Art. No. 02-379

Also known as

Rappaport Vassiliadis R10 Broth; RVS Broth

Specification

Liquid medium for the selective enrichment of *Salmonella* in foodstuffs and other samples, according to ISO and FIL-IDF standards.

Formula* in g/L

Soy peptone	4,500
Sodium chloride	7,200
Monopotassium phosphate	1,260
Dipotassium phosphate	0,180
Magnesium chloride	13,580
Malachite green	0,036

Final pH 5,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 26,8 g of powder in 1 L of distilled water, heating if necessary to help dissolve the powder. Dispense into test tubes or flasks and sterilize in the autoclave at 115°C for 15 minutes.

Description

The Rappaport Vassiliadis medium complies with the recommendations of the APHA for the examination of food.

This culture medium is a modification of the R10 Medium (from Rappaport et al.) or RV Broth (from Vassiliadis *et al.*) by van Schothorst & Renaud.

The modifications are an adjustment in the magnesium chloride concentration and the buffering capacity of the medium to aid pH maintenance during storage. It shows a higher selectivity towards *Salmonella* and produces better yields than other similar media, especially after preliminary enrichment and at an incubation temperature of 41 ± 0,5°C.

Malachite green, low pH and magnesium chloride inhibit the growth of microorganisms normally found in the intestine but do not affect the proliferation of most *Salmonellae*. As malachite green inhibits the growth of *Shigella*, other culture methods may need to be used to isolate this organism. The addition of soy peptone enhances the growth of *Salmonella*.

Technique

Inoculate the culture medium with the sample or material from a pre-enriched culture in Buffered Peptone Water (Art. No. 02-277) and incubate for up to 18-24 hours at 41 ± 0,5°C. Subculture from this broth onto selective culture media.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DOWNES, F.P. & K.ITO (2001) Compendium of Methods for the Examination of Foods. 4th ed. APHA. Washington. USA.
- FDA (Food and Drug Adminstrations) US (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC Internacional. Gaithersburg. MD. USA.
- FIL-IDF 93:2001 Standard. Milk and Milk Products. Detection of *Salmonella*. Brussels.
- HORWITZ, W. (2000) Oficial Methods of Analysis of AOAC International. Gaithersburg. MD. USA.
- ISO 6579:2002 Standard. Microbiology of Food and Animal Feeding Stuffs - Horizontal method for the detection of *Salmonella spp.* - Amendment 1 Annex D: Detection of *Salmonella* in animal faeces and in environmental samples from the primary production stage.
- ISO 6785:2001 Standard. Milk and Milk Products. Detection of *Salmonella*.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- RAPPAPORT, F., N. KONFORTI & B. NAVON (1956) A new enrichment medium for certain Salmonellae. J. Clin. Pathol. 9:261-266
- VAN SCHOTHORST, M. & A.M. RENAUD (1983) Dynamics of *Salmonellae* isolation with modified Rappaport's Medium (R10). J. appl. Bact. 54:209-215
- VASSILIADIS, P. (1983) The Rappaport Vassiliadis (RV) enrichment medium for the isolation of salmonellas: An overview. J. Appl. Bact. 54:69-76.
- VASSILIADIS, P., PATERAKI, EPAPAICONOMOU, N., PAPADAKIS, J.A.A., TICHOPOULOS, D. (1976) Noveau procédé d'enrichissement de *Salmonella*. Ann. Microbiol. (Inst. Pasteur) 127B (195-200).

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Rappaport Vassiliadis Broth

Art. No. 02-379

Quality control

Incubation temperature: 41°C ± 0,5
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
1. *Enterococcus faecalis* ATCC 29212	Total inhibition	Recovery in TSA
2. *Escherichia coli* ATCC 25922	Partial inhibition	Recovery in TSA
3. *Salmonella abony* NCTC 6017 + 6 + 7	Good	Recovery in XLD (Mixed cultures)
4. *Salmonella enteritidis* ATCC 13076 + 6 + 7	Good	Recovery in XLD (Mixed cultures)
5. *Salmonella typhimurium* ATCC 14028 + 6 + 7	Good	Recovery in XLD (Mixed cultures)
6. *Escherichia coli* ATCC 8739	Inhibited	Recovery in XLD (Mixed cultures)
7. *Pseudomonas aeruginosa* ATCC 27853	Inhibited	Recovery in XLD (Mixed cultures)

Left: Uninoculated Tube (Control)
Right: *Salmonella typhimurium* ATCC 14028 (24 h)

Left: Uninoculated Tube (Control)
Centre: *Salmonella typhimurium* ATCC14028 (48 h)
Right: *Salmonella enteritidis* ATCC 13076 (48 h)

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Rappaport Vassiliadis *Salmonella* Enrichment Broth

Art. No. 02-668

Specification

Selective liquid medium used for the enrichment of *Salmonella* according to the Pharmacopoeial Harmonised Method and ISO standard.

Formula* in g/L

Soy Peptone	4,500
Magnesium chloride · $6H_2O$	29,000
Sodium chloride	8,000
Dipotassium phosphate	0,400
Monopotassium phosphate	0,600
Malachite green	0,036

Final pH 5,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 42,5 g of powder in 1 L of distilled water. Warm gently if necessary. Distribute into suitable containers and sterilize in the autoclave at 115°C for 15 minutes.

Description

This culture medium is a modification of the Rappaport Vassiliadis Broth (Art. No. 02-379) with the composition adjusted to the formulation proposed by the European Pharmacopoeia in the Harmonized Methodology, and also to the requirements of the Japanese, and United States Pharmacopoeia.

Technique

Faecal specimens and water can be enriched directly on this medium. For pharmaceutical products, food and environmental specimens, a pre-enrichment step in Buffered Peptone Water (Art. No. 02-277 or 02-494) is recommended. Refer to suitable methodology (Pharmacopoeia or ISO Standard) for the incubation time and temperatures and confirmation subcultures and tests.

Precautions:

This medium should not be used if *Salmonella typhi* or *S. paratyphi* A is suspected.

To obtain optimum recovery, the enrichment broth must be incubated at 42 ± 1°C.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiology Media. CRC Press Inc. London.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- ISO Standard 6340 (1995) Water Quality. Detection of *Salmonella* species.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- PETERZ, M., C. WIBERG & P. NORBERG (1989) The effect of incubation temperature and magnesium chloride concentration on growth of *Salmonella* in home-made and commercially available dehydrated Rappaport Vassiliadis broths. J. Appl. Bact. 66:523-528.
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- VASSILIADIS, P., C.H. MAVROMMATI, M. EFSTRATIOU & G. CHROMAS (1985) A note on the stability of Rappaport Vassiliadis enrichment medium. J. Appl- Bact. 59:143-145.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Rappaport Vassiliadis *Salmonella* Enrichment Broth

Art. No. 02-668

Quality control

Incubation temperature: 30 - 35°C
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Inhibited	-
Escherichia coli ATCC 25922	Partial inhibition	-
Salmonella typhimurium ATCC 14028	Good	-
Salmonella abony NCTC 6017	Good	-

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Rappaport Vassiliadis Modified Semisolid Medium Base

Art. No. 03-376

Also known as

MSRV

Specification

Semisolid medium used for the isolation of motile strains of *Salmonella*.

Formula* in g/L

Tryptose	4,590
Casein peptone	4,590
Sodium chloride	7,340
Potassium dihydrogen phosphate	1,470
Magnesium chloride anhydrous	10,930
Malachite green	0,037
Agar	2,700

Final pH 5,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 31,6 g of powder in 1 L of distilled water. Heat in a boiling water bath until completely dissolved.

Cool to 50°C and add 20 mg/L of Novobiocin Selective Supplement (06-147-LYO). **Without autoclaving or reheating,** homogenize and pour plates. Keep plates in a cool place to allow the gel to settle (1 hour aprox.). Handle with care as the medium is semisolid and may spill. It is recommended keeping MSRV plates in the dark, at (2-8°C).

Description

The Modified Semisolid Rappaport Vassiliadis Medium Base is formulated according to DeSmedt et al. This formulation shows improved efficiency over traditional enrichment methodologies.

The rapid migration of motile strains of *Salmonella* in the semisolid medium allows early detection due to the production of a halo of growth around the inoculation zone.

Other competitive motile organisms are inhibited by novobiocin, malachite green and the high concentration of magnesium chloride.

The low concentration of agar produces a very soft and fragile gel which, at the temperature of incubation (42°C), allows the motile strains of *Salmonella* to move easily and quickly.

Technique

1. Three drops (~ 0,1 mL) of a pre-enrichment culture are inoculated in three different spots on the dry surface of the Agar plate at room-temperature.
2. Incubate the plates aerobically in an upright position for **no longer than 24 hours** at 42°C.
3. The formation of a turbid or opaque halo around the initial inoculation zone shows the presence of motile *salmonellae*.
4. To confirm the purity of the isolation and to carry out confirmative identification tests, samples from the outer border of the halo can be used.
5. To prevent false negative results due to the absence of motile strains of *Salmonella* in the samples it is advisable to simultaneously perform a standard enrichment procedure in liquid medium.

Necessary supplements

Novobiocin Selective Supplement (Art. No. 06-147-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Novobiocin, sodium salt ..... 20,00 mg

Distilled water (Solvent)

References

- De SMEDT, J.M., R. BOLDERDJIK, H. RAPPOLD & D. LAUTENSCHLAEGER (1986) Rapid *Salmonella* detection in foods by motility enrichment on a Modified Semisolid Rappaport Vassiliadis Medium. J. Food Protect. 49:510-514.
- De SMEDT, J.M. & R. BOLDERJIK (1987) Dynamics of *Salmonella* Isolation with Modified Semisolid Rappaport Vassiliadis Medium. J. Food Protect. 50:658-661.
- HOLBROOCK, R., J.M. ANDERSON, A.C. BAIRD-PARKER, L.M. DODDS, D. SAWHNEY , S.H. STRUCHBURY & D. SWAINE (1989) Rapid detection of *Salmonella* in food: A convenient two-day procedure. Lett. Appl. Microbiol. 8:139-142.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Rappaport Vassiliadis Modified Semisolid Medium Base

Art. No. 03-376

Quality control

Incubation temperature: 42°C ± 0,5
Incubation time: 24 - 48 h
Inoculum: Pre-enrichment 4 h and inoculate 3 drops on the surface of the plate.

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Inhibited	-
Salmonella enteritidis ATCC 13076	Good	Medium turns yellow-white. Motility+
Salmonella typhimurium ATCC 14028	Good	Medium turns yellow-white. Motility+

Uninoculated plate (Control)

Salmonella typhimurium ATCC 14028
Motility (+)

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Reinforced Clostridial Agar

Art. No. 01-289

Also known as

RCA

Specification

Solid medium for the cultivation and enumeration of clostridia and other anaerobic bacteria.

Formula* in g/L

Casein peptone	10,00
Yeast extract	3,00
Meat extract	10,00
Dextrose	5,00
Sodium chloride	5,00
Sodium acetate	3,00
Soluble starch	1,00
Cysteine	0,50
Agar	15,00

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 52,5 g of powder in 1 L of distilled water and bring to the boil constanty stirring. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Reinforced Clostridial Agar was originally described by Hirsch and Grinstead to enhance the growth of small inoculums and achieve a higher clostridial count. Later, Barnes and Ingram used the medium to develop vegetative cells in assays of *Clostridium perfringens.* Barnes also used this medium to count clostridia in food; moreover other authors used this medium in enumeration assays of *C. thermoscharolyticum* in sugar, the study of intestinal flora, and for bacterial counts in human or animal faeces, etc. Tartera et al. (1999) modified it by the addition of antibiotics for the isolation and counting of phages infecting Bacteroides. Later this medium was adopted in the 10705-4:2001 ISO Standard.
For enumeration by the MPN method, the liquid version is preferred.

Technique

Material to be examined is ground in a grinder or Stomacher®, and a dilution bank is prepared. From each of the dilutions, take an aliquot and add to plates or tubes, pour the molten medium at 50°C over the sample. Let it solidify. Incubate for a time and temperature suitable to the Microorganism. An anaerobic environment can be achieved in tubes by covering with oil immediately after the Reinforced Clostridial Medium is solidified. If plates are used, they must be incubated in an anaerobic atmosphere.
Muñoa and Parés added a filter sterilized solution of Nalidixic acid 0,02 g/L, Polymyxin 0,025 g/L, Kanamycin sulfate 0,05 g/L, Sodium iodoacetate 0,025 g/L and triphenyl-tetrazolium HCl 0,025 g/L to obtain a selective and differential medium for bifidobacteria in water and wastewater.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Boca Raton Fla. USA.
- HIRSCH, A. & E. GRINSTEAD (1954) Methods for the Growth and Enumeration of Anaerobic Sporeformers from Cheese, with Observations on the Effect of Nisin.
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- MUÑOA, F.J., R. PARÉS (1988) Selective medium for isolation and enumeration of *Bifidobacterium spp.* Appl. Environm. Microgiol 54:1715-1718.
- TARTERA, C., R. ARAUJO, T. MICHEL & J. JOFRE (1992) Culture and decontamination methods affecting enumeration of phages infecting *Bacteroides fragilis* in sewage. Appl. Environm. Microbiol. 58:8:2670 -2673.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Reinforced Clostridial Agar

Art. No. 01-289

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity).

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Inhibited	Anaerobiosis
Staphylococcus aureus ATCC 25923	Good	Anaerobiosis
Escherichia coli ATCC 25922	Good	Anaerobiosis
Clostridium perfringens ATCC 13124	Good	Gas(+) Anaerobiosis
Clostridium perfringens ATCC 10543	Good	Gas(+) Anaerobiosis

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Reinforced Clostridial Medium (Eur. Pharm.)

Art. No. 03-289

Also known as

RCM, Reinforced Medium for Clostridia

Specification

Fluid medium for the cultivation and enumeration of clostridia by the MPN Technique according to the Pharmacopoeial Harmonised Method.

Formula* in g/L

Casein peptone	10,00
Yeast extract	3,00
Meat extract	10,00
Dextrose	5,00
Sodium chloride	5,00
Sodium acetate	3,00
Soluble starch	1,00
Cysteine	0,50
Agar	0,50

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 38 g of powder in 1 L of distilled water and bring to the boil constantly stirring. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Reinforced Clostridial Agar was originally described by Hirsch and Grinstead to enhance the growth of small numbers and achieve a higher clostridial count. Later, Barnes and Ingram used the medium to develop vegetative cells in assays of *Clostridium perfringens*. Barnes also used this medium to count clostridia in food; moreover other authors used this medium in enumeration assays of *C. thermoscharolyticum* in sugar, the study of intestinal flora, and for bacterial counts in human or animal faeces, etc.

For enumeration by the MPN method, the liquid version is the preferred one.

Muñoa and Parés added a filter sterilized solution of nalidixic acid 0,02 g/L, polymyxin 0,025 g/L, kanamycin sulfate 0,05 g/L, sodium iodine-acetate 0,025 g/L and triphenyl-tetrazolium HCl 0,025 g/L to obtain a selective and differential medium for bifidobacteria in water and wastewater. Tartera et al use it with the addition of antibiotics (BPRM Broth) for the isolation and enumeration of bacteriophages from bacteroides. This technique was adopted in the 10705-4:2001 ISO standard.

Technique

Material to be examined is ground in a mill or Stomacher®, and a decimal dilution bank prepared. From each the dilutions, an aliquot is added to a Petri dish or tube, and molten medium is poured, at 50°C, over each sample.

Let solidify and incubate at 30-55°C (depending on the suspected microorganism) for 1-10 days. An anaerobic environment can be achieved in tubes by covering with Oil immediately after the Reinforced Clostridial Medium is solidified. If plates are used, they must be incubated in an anaerobic atmosphere.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Boca Raton. Fla. USA.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- HIRSCH, A. & E. GRINSTEAD (1954) Methods for the Growth and Enumeration of Anaerobic Sporeformers from Cheese, with Observations on the Effect of Nisin.
- INGRAM, M. & E.M BARNES (1956) A simple modification of the deep shake tube for counting anaerobic bacteria. Lab. Practice 5, 4:145.
- ISO 10705-4 Standard (2001) Water Quality - Detection and enumeration of bacteriophages infectin *Bacteroides fragilis*.
- MUÑOA, F.J. & R. PARÉS-FARRÁS (1988) Selective medium for isolation and enumeration of *Bifidobacterium spp*. Appl. Environm. Microbiol. 54:1715-1718.
- TARTERA, C., R. ARAUJO, T. MICHEL & J. JOFRE (1992) Culture and decontamination methods affecting enumeration of phages infecting *Bacteroides fragilis* in sewage. Appl. Environm. Microbiol. 58:8:2670 -2673.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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R

Reinforced Clostridial Medium (Eur. Pharm.)

Art. No. 03-289

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. (Productivity) // Anaerobic conditions

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Escherichia coli ATCC 25922	Good	-
Pseudomonas aeruginosa ATCC 27853	Inhibited	-
Clostridium perfringens ATCC 13124	Good	Gas (+)
Clostridium sporogenes ATCC 19404	Good	Gas (D)*
Clostridium perfringens ATCC 10543	Good	Gas (+)

* D (Gas production doubtful/poor)

Ringer Powder

Art. No. 06-073

Specification

Isotonic solution for the cellular suspensions.

Formula* in g/L

Sodium chloride	2,250
Potassium chloride	0,105
Calcium chloride	0,120
Sodium bicarbonate	0,050

* Adjusted and /or supplemented as required to meet performance criteria

Directions

To obtain an isotonic solution for eukaryotic cells, dissolve 10 g of powder in 1 L of distilled water. To obtain an isotonic solution for prokaryotic cells, dissolve 2,5 g of powder in 1 L of distilled water.
Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Ringer saline solution is an isotonic medium which is more balanced than a simple sodium chloride saline solution, and its formulation permits autoclaving without producing any precipitation.

Technique

For routine work with bacteria the solution is diluted one in four (Ringer 1/4), and is employed to prepare cell suspensions or dilution banks.
To dilute food samples or substances that have undergone thermal treatment, it is more advisable to use Peptone Water (Art. No. 03-156) for the dilutions, since the Peptone Water acts as a revitalizer.

References

- ANONYMOUS (1937) Bacterial Tests for Graded Milk. Memo 139-Foods. Dept. of Health and Social Security. London.
- DAVIS, J.G. (1956) Laboratory Control of Dairy Plant. Dairy Industries Ltd., London.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C
Incubation time: 24 h
Inoculum: 10-100 CFU. (Productivity) at 0,45 minutes and 3 h. (20 - 25°C)

Microorganism	Growth	Remarks
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Staphylococcus aureus ATCC 6538	Good	Satisfactory
Escherichia coli ATCC 8739	Good	Satisfactory
Candida albicans ATCC 10231	Good	No significant reduction
Pseudomonas aeruginosa ATCC 9027	Good	Satisfactory

R

Rose Bengal Agar

Art. No. 01-301

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

Also known as

Rose Bengal Chloramphenicol Agar; RBC Agar; Rose Bengal-Malt Extract Agar

Specification

Solid and selective medium for the isolation of yeasts and moulds from the environment and food products.

Formula* in g/L

Peptone	5,00
Dextrose	10,00
Potassium phosphate	1,00
Magnesium sulfate	0,50
Rose bengal	0,05
Chloramphenicol	0,10
Agar	15,00

Final pH 7,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 32 g of powder in 1 L of distilled water and bring to the boil stirring constantly. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Rose Bengal Agar is a selective medium used to detect and enumerate moulds and yeasts in food samples. In addition the nutritional requirements for moulds and yeasts, this medium also contains Rose Bengal, which apart from turning the yeast a pink colour, facilitates counting, by reducing the luxuriant growth of moulds such as *Rhizopus* and *Neurospora*. This makes it is easier to detect other slower growing moulds.

The chloramphenicol included in the formulation inhibits bacterial growth, but does not interfere with the growth of fungi.

Technique

After making a dilution bank, take 0,1 mL from each dilution and inoculate on Rose Bengal Agar plates with a Drigalsky Loop or glass spreader. Should the pour plate method be preferred, take 1 mL from each dilution and put it in an empty Petri dish. Pour the molten medium at 50°C and homogenize it by gently swirling the plate in the shape of a figure 8. Incubate at 22°C for 5 days enumerate the fungi.

After making a dilution bank, take 0,1 mL from each dilution and inoculate with a Drigalsky Loop or glass spreader on Rose Bengal Agar plates. Should the massive seed method be preferred, take 1 mL from each dilution and put it in an empty plate. Pour the molten medium at 50°C and homogenize it by gently moving the plate in an eight (8) shape. Incubate at 22°C for 5 days and proceed to enumerate the fungi.

Limitations:

- The low concentration of antibiotic that contains the culture medium can be expected that the growth of certain strains of bacteria is inhibited only partially.
- This medium is photo-sensible. Do not expose this medium to the light since photo-degradation of Rose Bengal produce compounds toxic to fungi.
- The prepared medium or ready-to-use plates haven a shelf-life of seven days at 4 ± 2°C in the dark."

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- CLESCERI, L.S., A.E. GREENBERG y A.D. EATON (1998) Standard Methods for the examination of water and wastewater. 20th ed. APHA. Washington DC.
- DOWNES, F.P. y K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington DC.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MARSHALL, R.T. (1993) Standard methods for the examination of dairy products. 16th ed. APHA, Washington DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Rose Bengal Agar

Art. No. 01-301

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

Quality control

Incubation temperature: 25 - 30°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Total inhibition	-
Escherichia coli ATCC 8739	Total inhibition	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.50	Black sporulation (5 days)
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.50	-
Candida albicans ATCC 10231	Productivity > 0.50	-
Penicillium aurantiogriseum ATCC 16025	Productivity > 0.50	White sporulation (8 days)

Saccharomyces cerevisiae ATCC 9763

Aspergillus brasiliensis ATCC 16404

Candida albicans ATCC 10231

Sabouraud Chloramphenicol Agar

Art. No. 01-166

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

Specification

Solid culture medium for the isolation of fungi.

Formula* in g/L

Casein peptone	5,00
Meat peptone	5,00
D(+)-Glucose	40,00
Chloramphenicol	0,50
Agar	15,00

Final pH 5,6 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 65,5 g of powder in 1 L of distilled water and bring to the boil. Distribute into final containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat or reheat** the medium since it will affect it's solidification.

Description

This culture medium differs from the classical Sabouraud Agar only by the addition of chloramphenicol. This thermostable antibiotic has a broad antibacterial spectrum which ensures the selective isolation of fungi from highly contaminated samples, such as exudates, faeces, nails and hair.

References

- AJELLO, L. (1957) Cultural Methods for Human Pathogenic Fungi J. Chron. Dis. 5:545-551.
- GEORGE, L.K., AJELLO, L. y PAPAGEORGE,C. (1954) Use of Cycloheximide in the Selective Isolation of Fungi Pathogenic to Man. J. Lab. Clin. Med, 44 (422-428).
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- PAGANO, J. LEVIN, J.D. y TREJO, W. (1957-58) Diagnostic Medium for Differentiation of Species of *Candida*. Antibiotics Annual,137-143.
- SABOURAUD, R. (1910) Les Teignes. Masson, Paris.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Sabouraud Chloramphenicol Agar

Art. No. 01-166

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

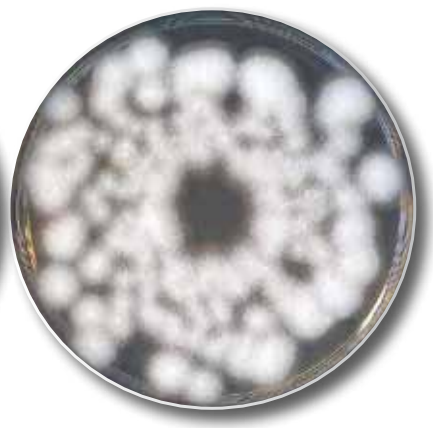
Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited	Selectivity
Escherichia coli ATCC 8739	Inhibited	Selectivity
Aspergillus brasiliensis ATCC 16404	Productivity > 0.50	Growth and black sporulation
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.50	-
Candida albicans ATCC 10231	Productivity > 0.50	-
Penicillium aurantiogriseum ATCC 16025	Good	Growth and yellow-green sporulation

Candida albicans ATCC 10231

Saccharomyces cerevisiae ATCC 9763



Penicillium aurantiogriseum ATCC 16025

Sabouraud Dextrose Agar

Art. No. 01-165

Specification

Solid medium for the enumeration and cultivation of fungi according to the Pharmacopeial Harmonised Method and ISO standard.

Formula* in g/L

D(+)-Glucose	40,00
Meat peptone	5,00
Casein peptone	5,00
Agar	15,00

Final pH 5,6 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 65 g in 1 L of distilled water and bring to the boil stirring frequently. Distribute into final containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat** the medium as its acidic pH may partially hydrolyze the agar. Alternatively, if the European Pharmacopoeia formulation is desired, add, 50 mg/L of chloramphenicol prior to sterilization (Art. No. 06-118CASE or 06-118-LYO) and then immediately before its use, add aseptically, 0,10 g/L of benzyl-penicillin sodium salt and 0,10 g/L of tetracycline (Art. No. 06-115CASE or 06-115-LYO).

Description

Sabouraud Dextrose Agar is a modification of the classical Sabouraud medium for the cultivation of fungi. This new formula helps to maintain the morphology of fungi, providing a reliable medium for both cultivation and differentiation.

Its selectivity is due to a low pH and a high glucose concentration, which together with incubation at a relatively lower temperature (25-30°C) favours the growth of fungi while discouraging that of bacteria.

The mixture of peptones employed has been selected to provide the fungi with all their nitrogen requirements.

Since Sabouraud medium's low pH can partially hydrolyze the agar, only the required amount should be prepared and it should not be re-melted. Any overheating will also diminish its gelling capacity.

Should a higher selectivity be required, a variety of inhibitors or selective agents may be added after sterilization, while the medium is still in the molten form. It can also be made differential by adding suitable indicator agents. Some of the inhibitory and differential mixtures most commonly used are listed below:

- Penicillin: at 20.000 u/L, for bacterial inhibition.
- Penicillin and Streptomycin: at 20.000 u/L and 40.000 u/L used for the isolation of *Histoplasma* in dogs.
- Penicillin and Neomycin: at 20.000 u/L and 40 mg/L for bacterial inhibition.
- Streptomycin and Chloramphenicol: at 40 mg/L and 500 mg/L, for the isolation of *Trichophyton verrucosum*.
- Colistin, Novobiocin and Cycloheximide: at 8 mg/L, 0.1 mg/L and 30 mg/L, for the isolation of *Candida albicans*.
- Potassium Tellurite: at 150 mg/L, used for the primary isolation of fungi from scales and scabs.
- Cupric Sulfate, Crystal Violet and Brilliant Green: at 500 mg, 2 mg and 5 mg each, for bacterial inhibition.
- Triphenyltetrazolium chloride (TTC): at 100 mg/L, is the basis of a Pagano-Levin medium for the isolation of *Candida albicans*, which remains non-pigmented, among other pink coloured pathogenic yeasts.

Necessary supplements

Chloramphenicol Selective Supplement (Art. No. 06-118CASE / 06-118-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Chloramphenicol..... 25,00 mg

Distilled water (Solvent)

Oxytetracycline Selective Supplement (Art. No. 06-115CASE/06-115-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Oxytetracycline HCl..... 50,00 mg

Distilled water (Solvent)

References

- AJELLO, L. (1957) Cultural Methods for Human Pathogenic Fungi J. Chron. Dis. 5:545-551.
- COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- GEORGE, L.K., AJELLO, L. y PAPAGEORGE,C. (1954) Use of Cycloheximide in the Selective Isolation of Fungi Pathogenic to Man. J. Lab. Clin. Med, 44 (422-428).
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- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 13681 Standard. (1995). Enumeration of Yeasts and Moulds. Colony Count Technique.
- PAGANO, J. LEVIN, J.D. and TREJO, W. (1957-58) Diagnostic Medium for Differentiation of Species of *Candida*. Antibiotics Annual,137-143.
- SABOURAUD, R. (1910) Les Teignes. Masson, Paris.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

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Sabouraud Dextrose Agar

Art. No. 01-165

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 20 - 25°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	48 h
Bacillus subtilis ATCC 6633	Good	48 h
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	Growth & black sporulation at 4 days
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-
Penicillium aurantiogriseum ATCC 16025	Good	Growth and sporulation at 8 days

Saccharomyces cerevisiae ATCC 9763

Candida albicans ATCC 10231

Penicillium aurantiogriseum ATCC 16025

Sabouraud Broth

Art. No. 02-165

Also known as

Antibiotic Medium 13. AM13. Medium 13.

Specification

Liquid medium for fungal isolation according to the Pharmacopeial Harmonised Method.

Formula* in g/L

Casein peptone	5,00
Meat peptone	5,00
D(+)-Glucose	20,00

Final pH 5,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 30 g of powder in 1 L of distilled water, heating only if necessary. Dispense into suitable containers and sterilize in a preheated autoclave for 15 minutes at 121°C. **Avoid overheating**, since it may brown the glucose.

Description

This medium is especially adapted to the culture of fungi and acidophilic bacteria.

Sabouraud USP Broth is formulated according to the US Pharmacopoeia, US NF and the 21 CFR guidelines. In the latest editions of these methods Tryptone Soy Broth may be used for sterility checking in pharmaceutical products for injection. This formulation is similar to Antibiotic Medium No. 13 by Grove and Randall and the 21 CFR guideline.

This medium is not selective, but the low pH inhibits the growth of non acidophilic microorganisms. Special measures must be taken while reconstituting and heating the medium due to this strong acid reaction and the high content of glucose. It is important to preheat the autoclave and thereby reach the sterilization temperature as soon as possible otherwise the glucose becomes caramelized turning the medium dark and reducing its effectiveness.

Technique

This medium is recommended for use in many tests and assays, but for a long time has been the medium of choice for verifying the sterility of sterile pharmaceutical products.

The efficacy of the medium and absence of fungistatic products is verified by using *Candida albicans* positive control. A loop of a 1:1000 dilution of a fresh 24 hour culture is added to the control tube and incubated appropriately. Sterility testing must be carried out in a controlled and verified medium. To check fungistatic activity of a product, prepare an control culture inoculum as mentioned above and inoculate two series of tubes of Sabaroud Broth as follows:

a) To one set of tubes add a specified amount of product to be tested. This is the test series.
b) To another series add only the inoculum. And incubate both series of tubes simultaneously.
c) Incubation of both series of tubes must be carried out at 22°C for 10 days. After this period compare the results.

If the assay series has less growth than the control series, the product has fungistatic activity. If the growth is equal or more, then it has no fungistatic properties. For the quantitative assay of fungistatic activity, perform the assay with several series of different concentrations until a point of equal growth in both control and test series.

References

- AJELLO, L. (1957) Cultural Methods for Human Pathogenic Fungi J. Chron. Dis. 5:545-551.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC. USA.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MARSHALL, R.T. (1992) Standard methods for the examination of dairy products. 16th ed. APHA. Washington. DC. USA.
- SABOURAUD, R. (1910) Les Teignes. Masson. Paris.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.
- USP 33 - NF 28 (2011) <81> Antibiotics - Microbial Assays. USP Corp. Inc. Rockville. MD. USA

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Sabouraud Broth

Art. No. 02-165

Quality control

Incubation temperature: 20 - 25°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	-
Escherichia coli ATCC 8739	Good	-
Aspergillus brasiliensis ATCC 16404	Good	5 days, black sporulation
Candida albicans ATCC 10231	Good	-
Saccharomyces cerevisiae ATCC 9763	Good	-

Sabouraud Oxytetracycline Agar Base (OGYEA)

Art. No. 01-275

Also known as

Oxytetracycline-Glucose-Yeast Extract Agar; OGY; OGYE Agar Base

Specification

Solid culture medium for the total enumeration of moulds and yeast.

Formula* in g/L

Glucose	20,00
Yeast extract	5,00
Agar	15,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 40 g of powder in 1 L of distilled water and let it soak for a few minutes. Distribute into suitable containers and sterilize in the autoclave for 15 minutes at 121°C. Cool to 50°C and add oxytetracycline (Art. No. 06-115CASE or 06-115-LYO) to obtain a 0,1 mg/mL concentration.

Description

This formulation of the classical Sabouraud Medium differs from others as it has no peptone and has a neutral pH. It has a high oxytetracycline concentration that will provide almost total inhibition of bacteria.

Technique

Some authors suggest an inoculum of 1 mL to prepare a series of suitable dilutions, in duplicate Seed petri dishes using the pour plate method. Incubate at 22-25°C for 5 days with intermittent observations after 3 days incubation.

Necessary supplements

Oxytetracycline Selective Supplement (Art. No. 06-115CASE/06-115-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Oxytetracycline HCl	50,00 mg

Distilled water (Solvent)

References

- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. APHA. Washington DC. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- ISO 13681 Standard (1995) Enumeration of Yeasts and Moulds. Colony Count Technique.
- MacFADDIN, J.F. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. William & Wilkins. Baltimore. MD. USA.
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- MOSSEL, D.A.A. A.M.C. KLEYNEN-SEMMELING, H.M. VINCENTIE, H. BEERENS & M. CATSARAS (1970) Oxytetracycline-Glucose-Yeast Extract Agar for selective enumeration of moulds and yeasts in foods and clinical material. J. Appl. Bacteriol. 33:454-457.
- SABOURAUD, R. (1910) Les Teignes. Masson, Paris.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30°C and <60 %RH).

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Sabouraud Oxytetracycline Agar Base (OGYEA)

Art. No. 01-275

Quality control

Incubation temperature: 25°C ± 2,0
Incubation time: 3 - 5 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited	-
Escherichia coli ATCC 8739	Inhibited	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.50	-
Candida albicans ATCC 10231	Productivity > 0.50	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.50	Black sporulation (5 days)

Saccharomyces cerevisiae ATCC 9763

Aspergillus brasiliensis ATCC 16404

S

Salmonella-Shigella Agar (SS Agar)

Art. No. 01-555

CE IVD

Specification

Differential and selective solid medium for the isolation of *Salmonella* and some *Shigella* species from clinical specimens, foods, etc.

Formula* in g/L

Meat extract	5,00000
Peptone	5,00000
Lactose	10,00000
Bile salts	5,60000
Sodium citrate	10,00000
Sodium thiosulfate	8,50000
Ferric citrate	1,00000
Brilliant green	0,00033
Neutral red	0,02500
Agar	15,00000

Final pH 6,90 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 60, 1 g of the powder in 1 L of distilled water. Bring to the boil, with frequent agitation and allow it to simmer gently dissolving the agar. **Do not autoclave.** Cool to 50°C, mix well and pour into sterile Petri dishes.

Description

SS Agar is a highly selective agar used for the isolation of *Salmonella* and *Shigella* species from very contaminated samples.

Selectivity is obtained by a high concentration of bile salts and brilliant green, which inhibits the growth of Gram-positive bacteria. The growth of other Gram-negative flora is highly repressed due to the presence of citrate and thiosulfate. Some coliforms may still grow on this medium. Differentiation between pathogenic species and coliforms is achieved by the colour change of the pH indicator (neutral red). Lactose fermenters produce a pink or red coloured medium and colonies, while non-fermenting species form colourless colonies and turn the medium yellow. Should any species produce H_2S, it is easily detected by the black precipitate of ferrous sulfide, which turn the colonies black.

The peptone and the meat extract are capable of inducing the growth of most pathogenic species, nevertheless some *Shigella* are very fastidious and may grow poorly.

Technique

If it is suspected that organisms might have been damaged and the viability of the microorganisms is poor i.e. (processed food, faeces from the patients under antibiotic treatment, etc.) it is advisable to proceed with a prior enrichment in Selenite-Cystine Broth Base (Art. No. 02-602) or Tetrathionate Broth Base (Art. No. 02-033/Art. No. 02-335). After enrichment, inoculate SS Agar plates heavily with the specimen and proceed in the same way as with other specimens on a less selective medium, such as Brilliant Green Agar (Art. No. 01-203) or MacConkey Agar (Art. No. 01-118).

Incubate the inoculated plates at 37°C for 18-24 hours. The presumptive colonies should then be sub-cultured on differential media to be identified biochemically or serologically.

Appearance of the colonies after 24 hours on SS Agar:

- *Shigella*: Colourless, transparent and flat.
- *Salmonella* (Non H_2S producers): Colourless, transparent and flat.
- *Salmonella* (H_2S producers): Black or black centred, flat, with transparent borders.
- *Proteus*: Similar appearance as *Salmonella* colonies, but smaller in size.
- *Escherichia coli*: If they grow, they are small, convex and pink or red coloured.
- Coliforms (in general): Large, opaque, smooth and white or pink in colour.

References

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Food. 4th ed. APHA. Washington. DC.
- GRAY, L.D. (1995) *Escherichia*, *Salmonella*, *Shigella* and *Yersinia*. In Murray, Baron, Pfaller Tenover & Yolken (eds) Manual Clinical Microbiology. 6th ed. ASM Washington DC.
- HORWITZ,W.(2000) Official Methods of Analysis 17th ed. AOAC International. Gaithersburg. MD.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- LEIFSON, E. (1935) New culture media based on sodium deoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol., 40.581.
- WINN, W., S. ALLEN, W. JANDA, E. KONEMAN, G. PROCOP, P. SCHRECKENBERGER & G. WOODS (2006) Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams & Wilkins. Philadelphia.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Salmonella-Shigella Agar (SS Agar)

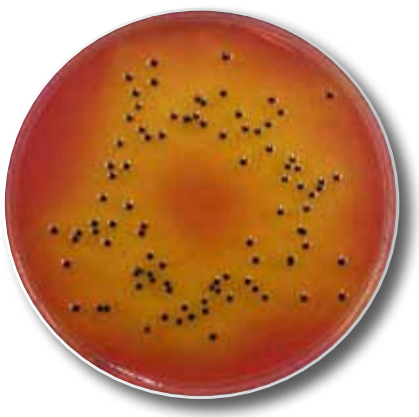
Art. No. 01-555

CE IVD

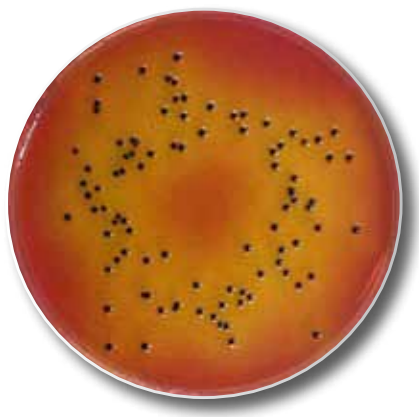
Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Partial inhibition	48 h (poor)
Escherichia coli ATCC 25922	Total inhibition	-
Salmonella abony NCTC 6017	Productivity > 0.50	Colourless colonies with black centre (H_2S+)
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Colourless colonies with black centre (H_2S+)
Salmonella enteritidis ATCC 13076	Productivity > 0.50	Colourless colonies with black centre (H_2S+)
Shigella flexneri ATCC 12022	Productivity > 0.30	Colourless colonies with transparent centre (H_2S-)



Salmonella typhimurium ATCC 14028



Salmonella enteritidis ATCC 13076

S

Selenite Broth Base

Art. No. 02-598

CE IVD

Also known as

Selenite F Broth

Specification

Liquid medium used for the enrichment of *Salmonella* and *Shigella* from clinical specimens and other products according to ISO standards.

Formula* in g/L

Peptone	5,00
Lactose	4,00
Potassium phosphate	10,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 19 g of powder in 1 L of distilled water and add 4 g of sodium biselenite (Art. No. SO0160). Homogenize and bring to the boil. Distribute in suitable containers. This a thermolabile medium therefore **do not overheat**. Use immediately. **Do not autoclave**.

Description

Selenite Broth is formulated according to an original formulation by Leifson for the selective enrichment of *Salmonellae* from very contaminated samples.

Enrichment is especially effective during the first 12 hours of cultivation, since in this period only *Salmonellae*, some *Proteus* and some strains of Pseudomonas tend to grow. For this reason, it is advisable not to extend the enrichment phase and to proceed quickly to selective medium, either liquid or solid. According to Bänffer, the efficacy of the medium is improved notably if enrichment is performed at 43°C. Presence of a red precipitate in the medium before inoculation indicates that there was overheating in which case the selective properties of the medium are reduced.

Technique

For normal assays or experiments, an incubation at 37°C for a period not exceeding 18 hours is recommended, since within this period growth enhancement of pathogens is achieved, but after 24 hours this effect seems to diminish and the growth of accompanying organisms may mask the growth of *Salmonella*.

Presence of abundant sample residues may also inactivate the selective property of the medium, e.g. faeces and or egg powder. In these cases, it is better to make a 1:10 dilution and let the bigger particles separate by settling to the bottom of the dilution tube, and then inoculate the Selenite Broth with an aliquot of the sample supernatant. Maintaining a proportion of 1:10 between the sample and the medium.

It has been demonstrated that for *Salmonella* isolation from faeces, that results are improved if the enrichment medium is incubated at 43°C. However this procedure does not work for the isolation of *Salmonella typhi*. For this microorganism it is recommended to enrich using Selenite Mannitol Broth at 37°C.

When the starting material is urine, the best procedure is to use Selenite Cystine Broth in double concentration, and to inoculate it with an equal volume of urine.

Sub-culturing must always be carried out after 6 hours of incubation but before 24 hours. Most authors recommend the simultaneous use of another enrichment broth, such as Muller-Kauffmann Tetrathionate Broth Base (Art. No. 02-335).

References

- ANDERSON, K. & H. KENNEDY (1965) Comparison of selective media for the isolation of *Salmonellae*. J. Clin. Path. 18:747-749.
- ATLAS, R.M., LC. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BÄNFFER, J.R. (1971) Comparison of the isolation of *Salmonellae* from human faeces by enrichment at 37°C and 43°C. Zbl. Bakt. I Orig. 217:(35-40).
- DIN - Standard 10160: Untersuchung von Fleisch u. Fleischerzneugissen. Nachweiss von *Salmonella* (Referenzverfahren).
- ISO 6785 Standard (2002) Milk and Milk products - Detection of *Salmonella spp*.
- ISO 6340 Standard (1995) Water Quality Detection of *Salmonella spp*.
- LEIFSON, E. (1936) A new Selenite Selective Enrichment media for the Isolation of Typhoid and Paratyphoid (*Salmonella*) Bacilli. Am.J.Hyg. 24:423-432.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Selenite Broth Base

Art. No. 02-598

CE IVD

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Inoculation with mixed cultures

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Total inhibition	Recovery in TSA
Escherichia coli ATCC 8739	Total inhibition	Recovery in TSA
Salmonella typhimurium ATCC 14028 + (1) + (2)	Good	Recovery in XLD (Mixed cultures)
Salmonella enteritidis ATCC 13076 + (1) + (2)	Good	Recovery in XLD (Mixed cultures)
Escherichia coli ATCC 25922 (1)	Inhibited	Recovery in XLD (Mixed cultures)
Pseudomonas aeruginosa ATCC 27853 (2)	Inhibited to poor	Recovery in XLD (Mixed cultures)

Salmonella typhimurium ATCC 14028 (Excellent)
Pseudomonas aeruginosa ATCC 27853

Left:
Salmonella typhimurium ATCC 14028
Pseudomonas aeruginosa ATCC 27853
Escherichia coli ATCC 25922
Right:
Salmonella enteritidis ATCC 13076
Pseudomonas aeruginosa ATCC 27853
Escherichia coli ATCC 25922

Total inhibition
Escherichia coli ATCC 25922

Selenite Cystine Broth Base

Art. No. 02-602

CE IVD

Specification

Liquid medium used for the enrichment of *Salmonella* and *Shigella* from clinical specimens and other products according to ISO standards.

Formula* in g/L

Peptone	5,00
Lactose	4,00
Potassium phosphate	10,00
L-Cystine	0,01

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 19,01 g of powder. in 1 L of distilled water and add 4 g of sodium biselenite (Art. No. SO0160). Homogenize and bring to the boil. Distribute in suitable containers. Tis is a thermolabile medium therefore **do not overheat**. Use immediately. **Do not autoclave**.

Description

Selenite Cystine Broth has been developed according to Leifson's formulation with the addition of L-Cystine to comply with FDA and APHA specifications; since it was shown that the medium performed better in a reduced CO_2 atmosphere.

It is essentially an enrichment medium for *Salmonella* found in food or pathological materials, such as faeces or urine. It is used as an initial step prior to isolation on selective media such as SS Agar (Art. No. 01-555) or Hektoen Agar (Art. No. 01-216).

Technique

For normal samples incubation at 37°C for a period not exceeding 18 hours is recommended, since within this period an enhanced growth of pathogens is achieved, but after 24 hours this effect seems to diminish and the growth of accompanying organisms may mask the growth of *Salmonella*.

Appearance of a red precipitate before inoculation indicates overheating of the medium, in which case the selective properties are significantly reduced.

Presence of abundant sample residues may also inactivate the selective property of the medium, e.g. faeces and or egg powder. In these cases, it is better to make a 1:10 dilution to allow the bigger particles to separate out by settling to the bottom of the dilution tube, and then inoculate Selenite Cystine Broth with an aliquot of the diluted sample supernatant. Maintaining a proportion of 1:10 between the sample and the medium.

When isolation of *Salmonella* from faeces is required, the results are better if the enrichment medium is incubated at 43°C. However this procedure does not work for the isolation of *Salmonella typhi*. For this microorganism enrichment in Mannitol-Selenite Broth at 37°C is recommended.

When the starting material is urine, the best procedure is to use Selenite Cystine Broth in double concentration, and to inoculate it with an equal volume of urine.

Sub-culturing must always be carried out after 6 hours of incubation but before 24 hours. Most authors recommend the simultaneous use of another enrichment broth, such as Muller-Kauffmann Tetrathionate Broth Base (Art. No. 02-335).

References

- ANDERSON, K. & H. KENNEDY (1965) Comparison of selective media for the isolation of *Salmonellae*. J. clin. Path. 18:747-749.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
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- DOWNES, F. P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. DC. USA.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. A.O.A.C. International. Gaithersburg. VA. USA.
- ISO 6785 Standard (2002) Milk and Milk Products - Detection of *Salmonella spp*.
- ISO 6340 Standard (1995) Water Quality - Detection of *Salmonella spp*.
- LEIFSON, E. (1936) A new Selenite Selective Enrichment Media for the Isolation of Typhoid and Paratyphoid (*Salmonella*) *Bacilli*. Am. J. Hyg. 24:423-432.
- U.S. PHARMACOPOEIA (2008) 31th ed. § <61> Microbial Limits Tests. The US Pharmacopeial Convention. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Selenite Cystine Broth Base

Art. No. 02-602

CE IVD

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Inoculation with mixed cultures

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Total inhibition	Recovery in TSA
Escherichia coli ATCC 25922	Total inhibition	Recovery in TSA
Salmonella typhimurium ATCC 14028 + (1) + (2)	Good	Recovery in XLD (Mixed cultures)
Salmonella enteritidis ATCC 13076 + (1) + (2)	Good	Recovery in XLD (Mixed cultures)
Escherichia coli ATCC 8739 (1)	Inhibited	Recovery in XLD (Mixed cultures)
Pseudomonas aeruginosa ATCC 27853 (2)	Inhibited to poor	Recovery in XLD (Mixed cultures)

Salmonella typhimurium ATCC 14028 (Excellent)
Pseudomonas aeruginosa ATCC 25668 (Good)

Left: Salmonella typhimurium ATCC 14028
Centre: Escherichia coli ATCC 25922
Right: ***Pseudomonas aeruginosa*** ATCC

Total inhibition
Escherichia coli ATCC 25922

Sensitivity Test Agar (STA)

Art. No. 01-655

Specification

Solid inhibitor-free culture medium for antibiotic susceptibility testing.

Formula* in g/L

Peptone	18,00
Yeast extract	3,50
Starch	0,60
Sodium chloride	5,00
Disodium citrate	1,00
Adenine sulfate	0,01
Guanine HCl	0,01
Uracil	0,01
Xanthine	0,01
Aneurine HCl	0,01
Uridine	0,10
Special agar	15,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 43,2 g of the powder in 1 L of distilled water. Bring it to the boil and distribute it into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. Allow it to cool to 45-47°C and add either 6% defibrinated blood or 7% lysed blood according to preference. Mix well and pour into Petri dishes.

Description

Sensitivity Test Agar was originally proposed by Bechtle and Scherr in 1958, but its current formula was modified by Stokes from a Report on the antibiotic sensitivity test trial of the Association of Clinical Pathologists.

STA is an inhibitor-free medium. It is very rich and includes various nucleotides to enable fastidious microorganisms to be tested. The type of agar used as a solidifying agent is selected due to its low content of Ca^{2+} and Mg^{2+} ions which can be released into the medium on heating and interfere with antibiotic diffusion.

A certain proportion of common pathogens require nutritional supplementation for their growth. It may be necessary to add defibrinated, lysed or 'chocolated' blood for some microorganisms.

References

- A.C.P. Committee (1965) Report on the antibiotic sensitivity test trial organized by the Bacteriology Committee of the Association of Clinical Pathologists. J. Clin Pathol. 18:1-15.
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- HANUS, F.J., J.G SANDS & E.O. BENNETT (1967) Antibiotic activity in the presence of agar. Appl. Microbiol. 15:31-34.
- STOKES, E.J. (1968) Clinical Bacteriology. 3rd ed. E. Arnold Pub. Ltd. London.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: Inoculate the entire agar surface and add antibiotic disks according to CLSI

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Pseudomonas aeruginosa ATCC 27853	Good	-
Escherichia coli ATCC 25922	Good	-
Escherichia coli ATCC 35218	Good	-
Enterococcus faecalis ATCC 29212	Good	-

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Shigella Broth

Art. No. 02-662

Specification

Liquid culture medium used for the selective enrichment of *Shigella spp* according to ISO standard 21567:2004.

Formula* in g/L

Casein peptone.....20,00
Dextrose.....1,00
Potassium hydrogen phosphate.....2,00
Potassium dihydrogen phosphate.....2,00
Sodium chloride.....5,00
Polysorbate 80.....1,50
Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 31,5 g of powder in 1 L of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45°C and aseptically add novobiocin to obtain a final concentration of 0,5 mcg/mL. The complete medium must be used on the day of preparation. The broth base without antibiotic can be stored in the refrigerator for 4 weeks.

Description

Shigella Broth is a liquid culture medium that complies with ISO standard 21567:2004 for the detection of *Shigella spp* in food and animal feeding stuffs. It is used in the preparation and dilution of the sample and as the selective enrichment step. It is a highly nutritive medium (peptone and glucose), with a strong phosphate buffer. The toxic-neutralizing and surfactant capacity of the polysorbate allows good growth of *Shigella*. The concentration of novobiocin used does not inhibit *Shigella* but inhibits the companion flora.

Technique

Prepare a 1:10 dilution of the sample in *Shigella* Broth with novobiocin and homogenize. A portion of 25 g or mL of the sample diluted in 225 mL of broth is recommended. Readjustment of the pH may be necessary after the addition of food to the broth.

The inoculated medium is incubated in anaerobic conditions with loose caps at 41,5 ± 1°C for 16-20 hours before streaking solid media with different selectivity levels: Art. No. 01-118, MacConkey Agar (Low Selectivity); Art. No. 01-552, XLD Agar (Medium Selectivity) and Art. No. 01-216, Hektoen Enteric Agar (High Selectivity).

The FDA proposes a slightly different technique: For *S. sonnei* the incubation temperature is 44°C and the novobiocin concentration is 0,5 µg/mL and for all other species the antibiotic concentration is increased to 3 µg/mL whilst the incubation temperature is lowered to 42°C.

References

- ATLAS, R.M. (1995) Handbook of Microbiological Media for the Examination of Food. C.R.C Press. Boca Raton. Fla. USA.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 21567:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella spp.*
- VAN DER ZEE, H. (2003) Media for the isolation of *Shigella spp*. In "Handbook of Culture Media for Food Microbiology" (Corry *et al.* Eds.) Elsevier-Sci B.V. Amsterdam.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 41°C ± 0,5
Incubation time: 16 - 20 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO 11133-1/2)

Microorganism	Growth	Remarks
Shigella flexneri ATCC 12022	Good	Recovery in XLD
Shigella sonnei ATCC 9290	Good	Recovery in XLD
Salmonella typhimurium ATCC 14028	Good	Recovery in XLD
Escherichia coli ATCC 8739	Good	Recovery in XLD
Enterococcus faecalis ATCC 29212	Inhibited	Recovery in XLD

SIM Medium

Art. No. 03-176

Also known as

Suplhite Indol Motility

Specification

Differential medium for motility, H_2S production and indol formation.

Formula* in g/L

Yeast extract	10,00
Casein Peptone	10,00
Meat peptone	6,00
Ferric-ammonium sulfate	0,20
Sodium thiosulfate	0,20
Agar	3,70

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 30 g of powder in 1 L of distilled water. Heat to boiling point and dispense into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This classical medium was originally developed to distinguish several types of enterobacteria, on the basis of the motility test, detection of indol and H_2S production.

It is a semisolid or fluid medium, and so the motile microorganisms can move freely. At the same time, the sulphur containing amino acids and the presence of thiosulfate allow those microorganisms that are able to, produce sulfides to do so, this then reacts with iron and produce black precipitates which in turn make the medium darker. The amount of thiosulfate present in medium does not affect the motility mechanisms, instead it ensures H_2S production by those microorganisms that are not able to produce it from cystine or cysteine.

Finally, the medium allows the production of indol from tryptophan present in the peptone, which can be easily detected with the addition of Kovacs' Reagent (Art. No. RE0007) (directly or with extraction) or with paper strips impregnated with the reagent.

Technique

The recommended technique is to stab inoculate 2/3 of the distance to the bottom in the centre of the medium from a pure culture (or from an isolated colony). After an incubation period of 16-18 hours at 37°C, observe the stab. Non-motile microorganisms produce growth only in the stab, whereas motile ones may be easily detected by their displacement which is indicated by turbidity in the medium.

Production of H_2S is indicated by a blackening of the media. A large amount of FeS will blacken the entire medium, while a small amount may only cause blackening around the stab.

Despite the fact that many authors suggest an extraction of indol by mixing the surface of the culture medium with chloroform, if Kovacs' Reagent (Art. No. RE0007) is employed, then this is not necessary and the observations can be made by pouring a few drops of reagent on the surface of the medium. A positive test, will produce a colour change of purple/red in the interphase, whereas a negative test will produce no colour change. Chloroform extraction may give erroneous results, since the appearance of colour must be observed immediately after the addition of the reagent. However if it is delayed by more than 30 seconds, the test must be disregarded.

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- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton Fla. USA.
- BLAZEVIC, D. J. (1968) Improved Motility Indol Medium. Appl. Microbiol. 16(4), 668-671.
- BULMASH, J.M. & FULTON, M. (1964) Discrepant Test for Hydrogen Sulfide. J. Bact. 88 (6) 1813.
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- MaCFADDIN, J. (1985) Media for isolation-cultivationidentification-maintenance of medical bacteria. Vol 1. William & Wilkins. Baltimore. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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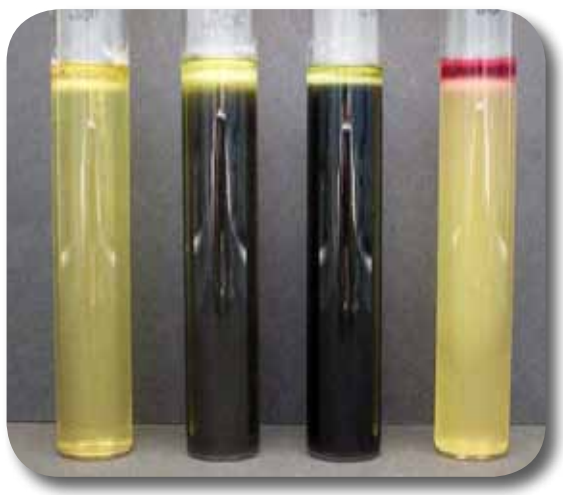
SIM Medium

Art. No. 03-176

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: Pure cultures deep inoculum stabbing with a straight needle

Microorganism	Growth	Remarks
Proteus mirabilis ATCC 12453	Good	H_2S (+) Mot (+) Indol (-)
Escherichia coli ATCC 8739	Good	H_2S (-) Mot (+) Indol (+)
Escherichia coli ATCC 25922	Good	H_2S (-) Mot (+) Indol (+)
Salmonella abony NCTC 6017	Good	H_2S (+) Mot (+) Indol (-)
Salmonella typhimurium ATCC 14028	Good	H_2S (+) Mot (+) Indol (-)
Shigella flexneri ATCC 12022	Good	H_2S (-) Mot (-) Indol (-)
Pseudomonas aeruginosa ATCC 9027	Good	H_2S (-) Mot (+) Indol (-)



First: *Pseudomonas aeruginosa* ATCC 9027
Second: *Salmonella abony* NCTC 6017
Third: *Salmonella typhimurium* ATCC 14028
Fourth: *Escherichia coli* ATCC 25922

Simmons Citrate Agar

Art. No. 01-177

Specification

Solid medium for verifying citrate utilization by enterobacteria according to the ISO standard 10273.

Formula* in g/L

Magnesium sulfate	0,20
Monoammonium phosphate	1,00
Dipotassium phosphate	1,00
Sodium citrate	2,00
Sodium chloride	5,00
Bromothymol blue	0,08
Agar	15,00

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 24 g of powder in 1 L of distilled water. Bring to the boil. Dispense in tubes and sterilize in the autoclave at 121°C for 15 minutes. Allow to solidify with a long slant.

Description

Simmons Citrate Agar is the solid version of the classical Koser citrate medium, and can be used in plates format as well as in slanted tubes. Slant tubes can be inoculated by surface streaking or by a deep stab.
Although it was originally described as an isolation and identification medium for certain fungi, Edwards and Ewing recommended it for the IMViC (Indol, Methyl red, Vogues Proskauer and Citrate) test. It has the advantage over Koser's medium that readings can be made by the indicator colour change, instead of the turbidity of the medium, which is sometimes difficult to detect.

Technique

To ensure an accurate result use an inoculum as small as possible and unsure the medium is freshly prepared, because if it is very dry, a false result (colour change) may appear, even before inoculation, especially at the bottom of the slant.
The basis of this medium is in the capacity of microorganisms to use citrate as a sole carbon source and ammonium compounds as the only nitrogen source for their growth. Among enterobacteria, these properties are possessed by the following genera: *Enterobacter*, *Klebsiella*, *Serratia*, *Citrobacter* and some species of *Salmonella* such as *S. schottumelleri, S.typhimurium*, *S.arizona* etc. *Escherichia*, *Shigella*, *Salmonella typhi* and *S.paratyphi* are unable to growon this media.
Although the test result must be read as growth proceeds, the presence of an indicator makes it easier, as citrate degradation results in an alkaline reaction, which is indicated by the indicator turning an intense blue. This is evident even when the growth is at an early stage.

References

- APHA-AWWA-WEF (1998) Standard Methods for the examination of water and wastewater. APHA. Washington DC. USA.
- FDA (Food and Drug Adminstrations) (1998). Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg Md. USA.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburgs. Md. USA.
- ISO 10273 Standard. (1994) General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica*.
- SIMMONS J.S. (1926) A culture medium for differentiating organisms of typhoid-colon aerogenes group and for isolating certain fungi. J.Inf. Dis. 39:209.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Simmons Citrate Agar

Art. No. 01-177

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: Pure culture is inoculated by surface streaking

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 27853	Good - very good	Blue medium
Enterobacter aerogenes ATCC 13048	Good - very good	Blue medium
Escherichia coli ATCC 25922	Inhibited	Green medium
Sjigella flexneri ATCC 12022	Inhibited	Green medium
Salmonella typhimurium ATCC 14028	Good - very good	Blue medium
Salmonella abony NCTC 6017	Good - very good	Blue medium
Escherichia coli ATCC 8739	Inhibited	Green medium

Escherichia coli ATCC 25922

Uninoculated Plate

Salmonella typhimurium ATCC 14028

Slanetz Bartley Agar Base

Art. No. 01-579

WARNING

H: 3.1.O/4; H302-4.1.C/3; H412

P: P273-P264-P270-P301+P312-P330-P501a

Also known as

m-Azide Agar; m-Enterococcus Agar; m-Slanetz Enterococcus Agar

Specification

Differential selective medium for the detection and enumeration of enterococci according to ISO standard 7899-2:2000.

Formula* in g/L

Tryptose	20,00
Yeast extract	5,00
Dextrose	2,00
Potassium phosphate	4,00
Sodium azide	0,40
Agar	12,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 43,4 g of powder in 1 L of distilled water and bring to the boil. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and add 10 mL/L of 1% TTC sterile solution (Art. No. 06-023). Mix well and distribute into sterile Petri dishes immediately.

Description

This formulation, without TTC allows sterilization in the autoclave without the development of a pink colour due to formazan which is formed as a result of the partial thermal-reduction of TTC. This modification is more tedious in its preparation but provides a colourless medium, making the results easier to read and the colonies are more sharply defined.

Technique

For the membrane filtration technique, take 100 mL of a well mixed water sample, and pass it through a sterile membrane filter. Then wash with 30 mL of sterile water to rinse the funnel of the filtering system.

Using sterile forceps, transfer the membrane aseptically to the culture medium contained in a Petri dish, making sure that the filter surface faces upwards. Close the lid and invert the plate. Incubate at 37°C for 48 hours. The developed colonies that appear red or purple in colour must be considered as enterococci, since these bacteria reduce Triphenyltetrazolium-HCl to an insoluble formazan which is red in colour. The secondary or accompanying Gram negative bacteria are inhibited by sodium azide.

For food samples, from a decimal dilution bank of the sample, spread 0,1 mL of the dilutions onto the plated medium using a Drigalsky loop. Incubation and examination is then carried out in the same way as in the membrane filtration technique.

References

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
- ISO 7899-2:2000 Standard. Water Quality. Detection and enumeration of enterococci by membrane filtration method.
- LACHICA, LV.F. and P.A. HARTMAN (1968) Two improved media for isolating and enumerating enterococci in certain frozen foods. J. appl. Bact. 31:151-156.
- SLANETZ, L.W. and BARTLEY, C.H. (1957) Numbers of enterococci in water, sewage and faeces determined by the membrane filter technique with an improved medium. J. Bact. 74:591-596.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Slanetz Bartley Agar Base

Art. No. 01-579

WARNING

H: 3.1.O/4; H302-4.1.C/3; H412

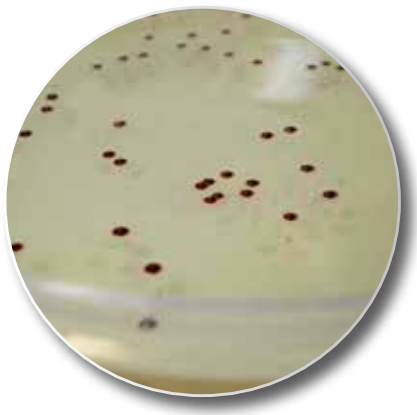
P: P273-P264-P270-P301+P312-P330-P501a

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity)/1.000-10.000 CFU (Selectivity). Spiral Plate Method (or Membrane Filter Method)

Microorganism	Growth	Remarks
Bacillus cereus var. mycoides ATCC 11778	Inhibited	Selectivity
Escherichia coli ATCC 25922	Inhibited	Selectivity
Enterococcus faecalis ATCC 29212	Productivity > 0.50	Dark red colonies
Enterococcus faecalis ATCC 19433	Productivity > 0.50	Dark red colonies

Enterococcus faecalis ATCC 19433



Enterococcus faecalis ATCC 29212
"Detail"

Enterococcus faecalis ATCC 29212

Soybean Casein Lecithin Polysorbate 80 Medium

Art. No. 02-613

Also known as

SCDLP 80 Broth; TSB + Lecithin + Polysorbate 80

Specification

Non-selective liquid medium suitable for detecting microbial contamination in cosmetics, according to the ISO Standards 18415, 18416, 21149 and 21150.

Formula* in g/L

Casein peptone	17,00
Soybean peptone	3,00
Sodium chloride	5,00
Di-potassium phosphate	2,50
Dextrose	2,50
Lecithin	1,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 30 g of the powder in 1 L of distilled water containing 7 mL of Polysorbate 80. Heat if necessary.
Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

The composition of this medium corresponds to the classical Tryptic Soy Broth (TSB) with the addition of Lecithin and Polysorbate 80. In this way the culture medium combines the optimal growth characteristics of TSB with the antimicrobial, neutralizing action of the lecithin and the Polysorbate 80. Polysorbate also helps to emulsify the hydrophobic phase of the cosmetic and protects the stressed microorganisms.

Technique

This medium is used as an enrichment broth in the first step of microbial detection.
Refer to the specific standard method for the appropriate procedure.

References

- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 18415 Standard (2007) Cosmetics - Microbiology - Detection of specified and non-specified microorganisms.
- ISO 18416 Standard (2007) Cosmetics - Microbiology - Detection of *Candida albicans*.
- ISO 21149 Standard (2006) Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria.
- ISO 21150 Standard (2006) Cosmetics - Microbiology - Detection of *Escherichia coli*.
- ISO 22717 Standard (2006) Cosmetics - Microbiology - Detection of Pseudomonas *aeuruginosa*.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	-
Staphylococcus aureus ATCC 6538	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Escherichia coli ATCC 8739	Good	-
Pseudomonas aeruginosa ATCC 27853	Good	-

Sporulating Agar (USP Antibiotic Medium 32)

Art. No. 01-069

Also known as

AK 2 Agar; Arret & Kirschbaum Agar, AK Agar; USP Antibiotic Medium 32

Specification

This formulation is according to the Arret and Kirshbaum formulation, adopted by US, FDA for the preparation of spore suspensions for antibiotic assay.

Formula* in g/L

Gelatin peptone	6,00
Casein peptone	4,00
Yeast extract	3,00
Meat extract	1,50
Dextrose	1,00
Manganous sulfate	0,30
Agar	15,00

Final pH 6,6 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 30,8 g of powder in 1 L of distilled water. Bring it to the boil stirring constantly. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Sporulation Agar is made according to the original formulation of Arret and Kirshbaum, and later adopted by the FDA for the preparation of *Bacillus subtilis* ATCC 6633 spore suspensions.

To prepare the suspension, suspend growth in 10 mL of 1/4 Ringer's Solution (Art. No. 06-073). With this suspension, inoculate the surface of a Roux flask containing 300 mL of solidified and controlled Sporulation Agar.

The Roux flask must be incubated at 35°C for 5 days. Growth is harvested with 50 mL of sterile Ringer's Solution, aided by a Drigalsky loop or sterile crystal balls (pearls) if necessary. The suspension obtained is carefully centrifuged at 5000 rpm for 15 minutes. Discard the supernatant liquid and suspend the pellet in a fresh volume of Ringer's Solution. Put it in a boiling water bath at 70°C for 30 minutes. This suspension is active for up to 6 months if refrigerated. Depending on the objective of the experiment, it can be standardized by turbidimetry.

References

- ARRET & KIRSCHBAUM (1959) J. Milk Food Tech. 22:329.
- SANCHO, GUINEA, PARES (1980) Microbiología Analítica Básica. Ed. JIM. Barcelona.
- USP 31 - NF 26 (2008) <81> Antibiotic Microbial Assays. US Pharmacopeial Convention Inc. Rockville. MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: Inoculate the entire agar surface

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	Spore present
Bacillus cereus var. mycoides ATCC 11778	Good	Spore present
Bacillus cereus ATCC 10876	Good	Spore present
Staphylococcus aureus ATCC 25923	Good	-
Escherichia coli ATCC 8739	Good	-

S

Stuart Ringertz Transport Medium

Art. No. 03-454

CE IVD

WARNING
H: 3.4.S/1; H317
P: P261-P280-P321-P363-P333+P313-P501a

Specification

Medium used for the maintenance and transport of pathogenic specimens or fastidious microorganisms from clinical or other origins.

Formula* in g/L

Sodium glycerophosphate	10,000
Sodium thioglycolate	1,000
Calcium chloride	0,100
Methylene blue	0,002
Agar	8,000

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 19 g of powder in 1 L of distilled water and bring to the boil. Distribute in tubes or flasks and close with an air tight cap in such a way that the medium forms a vertical column of 7-10 cm. Sterilize in the autoclave at 121°C for 15 minutes and cool quickly in the vertical position.

Description

The growth of microorganisms in this medium is restricted by the total lack of nitrogen, but they remain alive and inactive for a long periods thanks to the buffering and protective effect of glycerophosphate. Thioglycolate provides a reducing environment which is aided and maintained by the low concentration of agar, which prevents the occurrence of convection streams and restricts oxygen diffusion. Progressive oxidation of the medium can be seen by the change in colour the methylene blue, which acts as an *Eh* (redox) indicator.

Technique

The sample is placed directly inside the tube, taking care that it is beneath the blue band. If the sample is taken with a swab, it is advisable to impregnate it with a suspension of active carbon (activated charcoal) before putting it into the transport medium.
The sample must always be in the centre of the medium and beneath the blue band that indicates oxidation. If the depth of the blue band is bigger than half of the medium, do not use the tube.

References

- RINGERTZ, O. (1960) A modified Stuart medium for the transport of gonococcal specimens. Acta Path. Microbiol. Scand. 48:105-112.
- STUART, R.D. (1959) Transport medium for specimens in public health bacteriology. Publ. Hlth. Rep. 74:431-438.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Swabs wiped with microbial suspensions of McFarland 0,5 concentration (20 - 25°C for 24 h)

Microorganism	Growth	Remarks
Salmonella typhimurium ATCC 14028	Good	Satisfactory
Shigella sonnei ATCC 9290	Good	Satisfactory
Klebsiella pneumoniae ATCC 10031	Good	Satisfactory
Streptococcus pneumoniae ATCC 49619	Good	Satisfactory

TCBS Agar

Art. No. 01-567

Also known as

Cholera Medium TCBS

Specification

Solid medium for the selective isolation of *Vibrio parahaemolyticus* according to the ISO standard 8914.

Formula* in g/L

Proteose peptone	10,00
Yeast extract	5,00
Sodium citrate	10,00
Sodium thiosulfate	10,00
Ox bile	8,00
Sucrose	20,00
Sodium chloride	10,00
Ferric citrate	1,00
Thymol blue	0,04
Bromothymol blue	0,04
Agar	14,00

Final pH 8,6 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 88 g of powder in 1 L of distilled water and bring to the boil. Boil for 1 minute. Cool to 45-50°C and pour into sterile Petri dishes. **Do not autoclave**.

Description

TCBS Agar is universally accepted as the medium of choice for differential isolation of enteropathogenic *vibrios*, whilst inhibiting all the accompanying organisms. This formulation provides high growth of *Vibro cholerae* and *V. parahaemolyticus. V. alginoliticus* and NAG-vibrios.

Enterobacteria are strongly inhibited by high concentrations of citrate, thiosulfate, bile and sodium chloride.

Although some enteric bacteria may also grow in this medium, their colony morphology is quite different to that of *Vibrio spp*.

The organisms that can be confused with vibrios are some biotypes of *Proteus* and Pseudomonas. There are some resistant enterococci which may form exceptionally small and yellow colonies on this medium.

Usually, colonies are selected or chosen and then identified with primary tests [oxidase reactions in Kligler Iron Agar (Art. No. 01-103), MRVP Broth (Art. No. 02-207), and antibiotic sensitivity test] before performing serological identification and phage typing.

Due to its high selectivity, the medium can be seeded with large inoculum of pathological material. Once solidified and cooled, the medium is turbid, but the observations are not affected.

This medium is very thermolabile and so it must not be autoclaved, overheated or re-melted.

Colonial appearance on TCBS Agar after 24 hours at 37°C:

- *Vibrio alginolyticus* and *Vibrio cholerae*: Large, yellow colonies.
- *Vibrio parahaemolyticus*: Small, yellow, without halo and with a green core.
- *Streptococcus faecalis*: Very small and convex, yellow with yellow halo.
- Enterobacteria generally: Small and transparent.
- Pseudomonas, Aeromonas, *Proteus*: meaf medium size and blue.
- Some strains of *Vibrio cholerae* and *Vibrio parahaemolyticus* carry out delayed sucrose fermentation so they produce medium sized colonies, and are colourless or dirty yellow with a dark nucleus.

Technique

After making a dilution bank, take 0,1 mL from each dilution and inoculate, with a Drigalsky Loop or glass spreader, Rose Bengal Agar plates.

Should the pour plate method be preferred, take 1 mL from each dilution and put it in an empty plate. Pour in the molten medium at 50°C and homogenize it by gently moving the plate in a figure eight (8) shape. Incubate at 35°C for 24-48 h and proceed to enumeration.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media CRC Press. BocaRaton. Fla. USA.
- BHATTACHARYA, M.K., S.K. BATTACHARYA, S. GARG, P.K. SAHA, D. DUTTA, G.B. NAIR, B.C. DEB & K.P. DAS (1993) Outbrek of *Vibrio cholerae* non-01 in India and Bangladesh. Lancet, 341:1345-1347.
- DOWNES, F.P. & K. ITO (2001). Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
- FORBES, B.A., D.F SAHM & A.S. WEISSFELD (Eds) (1998) Bailey & Scott's Diagnostic Microbiology 10th ed. Mosby. St. Louis, MO. USA.
- HORWITZ, W. (Ed) (2000) Official Methods of Análisis of AOAC Internacional. 17th ed. Gaithersburg. MD. USA
- ISO 8914 STANDARD (1990) General guidance for the detection of *Vibrio parahaemolyticus*.
- KOBAYASHI, T., ENOMOTO, S. SAKAZARI, R. and KUWAHARA, S. (1963) A new selective medium for patogenic vibrios: TCBS (modified Nakanishi Agar) Jap. J.Bact. 18:387.
- MacFADDIN, J.F. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins. Baltimore. MD. USA.
- MURRAY, P.R., E.J. BARON, J.H. JORGENSEN, M.A. PFALLER & R.H. YOLKEN (Eds) (2003) Manual of Clinical Mcrobiology 8th ed. ASM Press. Washington. DC. USA.
- PASCUAL ANDERSON, MªRª (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- US FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual 8th ed. Revision A. AOAC International Inc. Gaithersburg. MD. USA.

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TCBS Agar

Art. No. 01-567

Storage

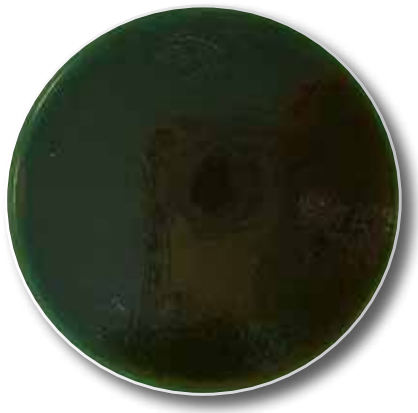
For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

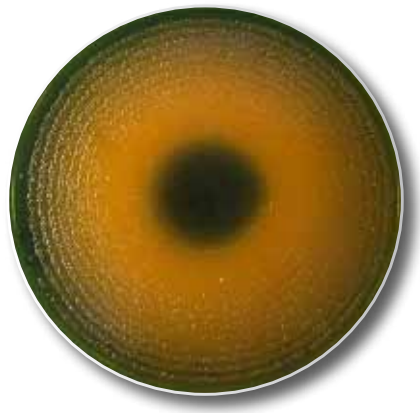
Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: Streak isolation

Microorganism	Growth	Remarks
Vibrio parahaemolyticus ATCC 17802	Good	Blue-green colonies
Vibrio algynoliticus ATCC 17749	Good	Yellow colonies
E. coli ATCC 25922	Inhibited	-

Vibrio parahaemolyticus ATCC 17802



Uninoculated plate (Control)



Vibrio alginolyticus ATCC 17749

www.scharlab.com
scharlab@scharlab.com

Tetrathionate Bile Brilliant Green Broth Base

Art. No. 02-629

Specification

Liquid medium for the selective enrichment of *Salmonella* in the examination of pharmaceutical products.

Formula* in g/L

Peptone	8,60
Bile	8,00
Sodium chloride	6,40
Calcium carbonate	20,00
Brilliant green	0,07

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 43,07 g of the powder in 1 L of distilled water and heat just to boiling. Add 20 g/L of potassium tetrathionate (Sigma-Fluka, Art. No. 60593), homogenize and adjust the pH so that after heating it is 7,0 ± 0,2. **Do not re-heat. Do not autoclave.** The broth is turbid, green with a white sediment of calcium carbonate. Any undissolved sediment should be homogenously mixed before distributing into final containers.

Description

This medium complies with the specifications for Broth Medium I as is described in the European Pharmacopoeia 5.0 for *Salmonella* testing (2.6.13). Peptone is the source of nitrogen and carbon. Bile inhibits non-enteric bacteria and brilliant green specifically inhibits Gram-positive accompanying microflora. Tetrathionate suppresses the development of enterobacteria but not *Salmonella* that can grow almost alone in this medium. Potassium tetrathionate is a very unstable product that can not be mixed with the dehydrated medium base because its addition reduces the shelf-life to less than one year.

Calcium carbonate neutralizes the sulphuric acid released due to the tetrathionate-reduction by *Salmonella*.

Technique

The product to be analysed is prepared as described in the Eur. Pharm. general method for the microbiological examination of non sterile products (§ 2.6.12) but using Tryptic Soy Broth (Art. No. 02-200) as a diluent and pre-enrichment broth, which is incubated at 35 ± 2°C for 18-48 hours.

Transfer 1 mL of the pre-enrichment culture to 10 mL of Tetrathionate Bile Brilliant Green Broth and incubate at 41-43°C for 18-24 hours. Subculture on at least 2 different agar media chosen from Deoxycholate Citrate Agar (Art. No. 01-056), XLD Agar (Art. No. 01-211) and Brilliant Green Agar (Art. No. 01-203).

The presumptive presence of *Salmonella* is indicated by the growth of colonies with typical appearance on the plating media. The identity of *Salmonella* must be verified by carrying out appropriate biochemical and serological confirmatory tests.

References

- EUROPEAN PHARMACOPOEIA (2005) 5th ed. § 2.6.13. Microbiological Examination of Non-Sterile Products (Test for Specified Microorganisms). EDQM. Council of Europe. Strasbourg.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Mixed culture

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Recovery on TSA
Escherichia coli ATCC 8739	Inhibited	Recovery on TSA
Salmonella typhimurium ATCC 14028 or	Good	Recovery on XLD (Mixed cultures)
Salmonella enteritidis ATCC 13076 +	Good	Recovery on XLD (Mixed cultures)
Escherichia coli ATCC 25922 +	Inhibited	Recovery on XLD (Mixed cultures)
Pseudomonas aeruginosa ATCC 27853 +	Inhibited	Recovery on XLD (Mixed cultures)

T

Thioglycolate Broth

Art. No. 02-186

CE IVD

WARNING

H: 3.4.S/1; H317

P: P261-P280-P321-P363-P333+P313-P501a

Also known as

USP Alternative Thioglycolate Medium

Specification

Medium used for the cultivation of anaerobic pathogenic microorganisms in clinical specimens and in sterility test of viscous or cloudy products.

Formula* in g/L

Peptone from casein	15,00
Yeast extract	5,00
Dextrose	5,50
Sodium chloride	2,50
Sodium thioglycolate	0,50
L-Cystine	0,50

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 29 g of powder in 1 L of distilled water, heating if necessary to dissolve. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. This culture medium should always be freshly prepared or heated at 100°C for 10 minutes before use.

Description

Thioglycolate Broth also know as Alternative Thioglycolate Medium was formulated and recommended by the USP, NF, NIH and FDA.
It is used for sterility testing of biological products or samples of turbid appearance where Fluid Thioglycolate Medium (Art. No. 03-187) is not suitable because of its viscosity. The formula of Thioglycolate Broth is the same as Thioglycolate USP Fluid Medium without resazurin and agar. The media must be freshly prepared, boiled, sterilised, cooled and used within 4 hours.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.1. Sterility. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburg. MD. USA.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- USP 33 - NF 28 (2011) <71> Sterility Tests. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Thioglycolate Broth

Art. No. 02-186

CE IVD

WARNING

H: 3.4.S/1: H317

P: P261-P280-P321-P363-P333+P313-P501a

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	-
Bacillus subtilis ATCC 6633	Good	-
Pseudomonas aeruginosa ATCC 9027	Good	-
Escherichia coli ATCC 8739	Good	-
Clostridium sporogenes ATCC 19404	Good	Anaerobic conditions
Candida albicans ATCC 10231	Good	48 h
Aspergillus brasiliensis ATCC 16404	Good	48 h

Left: Uninoculated tube (Control)
Right: *E. coli* ATCC 8739

Thioglycolate Fluid Medium

Art. No. 03-187

WARNING

H: 3.4.S/1; H317

P: P261-P280-P321-P363-P333+P313-P501a

Also known as

FTM; F Thio M

Specification

Fluid medium used for sterility testing according to the Eur. Pharm., USP, FDA, and for the cultivation of microaerophilic and anaerobic organisms.

Formula* in g/L

Peptone from casein	15,000
Yeast extract	5,000
Dextrose	5,500
Sodium chloride	2,500
Sodium thioglycolate	0,500
L-Cystine	0,500
Resazurin	0,001
Special agar	0,750

Final pH 7,1 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 30 g of powder in 1 L of distilled water; slowly bring to the boil, stirring until completely dissolved. Distribute into final containers and sterilize in the autoclave at 121°C for 15 minutes. Mix well and cool to room temperature.

Description

Thioglycolate Fluid Medium is a standard medium formulated and recommended by the European Pharmacopoeia, USP, APHA and FDA.
The reducing agents thioglycolate and L-Cystine ensure anaerobiosis which is adequate even for fastidious anaerobes. The -SH groups of these substances also inactivate arsenic, mercury and other heavy metal compounds. Thioglycolate media are thus suitable for the examination of materials which contain heavy metals or heavy metal preservatives.
In the present formulation a special agar with a high viscosity but a very low turbidity is used. A very slow cooling is recommended to prevent stratification. The higher viscosity of the fluid thioglycolate medium prevents rapid uptake of oxygen. Any increase in the oxygen content is indicated by the redox indicator sodium resazurin which changes colour to pink.

Technique

Inoculate the culture medium with the sample material taking care that the sample reaches the bottom of the tube.
Incubate for at least 14 days at the optimal temperature.
Anaerobes grow in the lower part of the culture medium container.

Precautions and Limitations of the Procedure

- Store the prepared medium away from light at room temperature.
- If more than 30% of the medium is pink prior to use reheat once at 100°C to drive off absorbed oxygen.
- Do not reheat the medium more than once; continued reheating gives rise to toxicity.
- Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
- Some glucose-fermenting organisms which are able to reduce the pH of the medium to a critical level may not survive in this medium. Early sub-culture is necessary to isolate these organisms.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Food. 3rd ed. A.P.H.A. Washington. DC.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.1. Sterility. Harmonised Method. EDQM. Council of Europe. Strasbourg.
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- MaCFADDIN, J.F. (1985) Media for Isolation-cultivation-identification-maintenance of medical bacteria. Vol. I. Williams & Wilkins. Baltimore. MD. USA.
- USP 33 - NF 28 (2011) <71> Sterility Tests. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Thioglycolate Fluid Medium

Art. No. 03-187

WARNING

H: 3.4-S/1: H317

P: P261-P280-P321-P363-P333+P313-P501a

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h - 14 days
Inoculum: 10-100 CFU. (Productivity)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	Aerobic and anaerobic zone
Escherichia coli ATCC 8739	Good	Aerobic and anaerobic zone
Bacillus subtilis ATCC 6633	Good	Only in aerobic zone
Clostridium sporogenes ATCC 19404	Good	Only in anaerobic zone
Pseudomonas aeruginosa ATCC 9027	Good	Only in aerobic zone
Candida albicans ATCC 10231	Good	Only in aerobic zone

First: Uninoculated tube (Control)
Second: *Escherichia coli* ATCC 8739
Third: ***Pseudomonas aeruginosa*** ATCC 9037
Fourth: *Clostridium sporogenes* ATCC 19404

Todd-Hewitt Broth

Art. No. 02-191

Specification

Liquid culture medium for the propagation of β-haemolytic streptococci and for serological typing studies.

Formula* in g/L

Meat extract	10,00
Casein peptone	20,00
Dextrose	2,00
Sodium bicarbonate	2,00
Sodium chloride	2,00
Disodium phosphate	0,40

Final pH 7,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 36,4 g of powder in 1 L of distilled water, heating if necessary. Distribute into suitable containers and sterilize by autoclaving at 121°C for 15 minutes.

Description

This classical formulation has been modified to achieve optimal results in the growth and production of haemolysins, which are not inhibited due to the high buffer composition of the medium. Many official organisations, such as APHA, have recommended this medium for use in the epidemiologic studies of group A streptococci as well as other pathogenic microorganisms. Some authors recommend this broth supplemented with antibiotics (Nalidixic acid and Gentamicin or Colistin) as a selective enrichment broth for *Streptococcus agalactiae* in female genital specimens.

With the addition of 15g/L of agar, the solid form of this medium becomes an excellent substrate for the production of capsules in streptococci.

References

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc.London
- FORBES, B.A., D.F. SAHM y A.S. WEISSFELD (1998) Bailey & Scott's Diagnostic Microbiology. 10ª Ed. Mosby Inc., St Louis, Missouri. USA
- MacFADDIN, J. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. Vol. 1. Williams & Wilkins. Baltimore. USA.
- TODD, E.W. and HEWITT, L.F. (1932) A new culture medium for the production of antigenic streptococcal haemolysin.J. Path. Bact. 35:973-974
- WINN, W.C., S.D. ALLEN, W.M. JANDA, E.W. KONEMAN, G.W. PROCOP, P.C. SCHRECKENBERGER y G.L. WOODS (2006) Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Lippincott, William & Wilkins, Baltimore, MD, USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 10-100 CFU (Productivity).

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 19433	Good	-
Staphylococcus aureus ATCC 25923	Good	-
Streptococcus pyogenes ATCC 19615	Good	-
Streptococcus pneumoniae ATCC 49619	Good	-

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Triple Sugar Iron Agar (TSI Agar)

Art. No. 01-192

Specification

Differential medium for the identification ofenterobacteria, according to ISO standards 6579, 6785 and 10272.

Formula* in g/L

Peptone	20,000
Meat extract	3,000
Yeast extract	3,000
Lactose	10,000
Sucrose	10,000
Dextrose	1,000
Sodium chloride	5,000
Ferric ammonium citrate	0,300
Sodium thiosulfate	0,300
Phenol red	0,025
Agar	12,000

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 64,6 g of powder in 1 L of distilled water and bring to the boil. Dispense into tubes and sterilize at 121° C for 15 minutes. Leave to solidify with short slants and good butts.

Description

TSI Agar is a modification of the classical Kliger's agar. 1% sucrose has been added to this medium to differentiate *Proteus* and *Hafnia* (sucrose positive) from *Salmonella* and *Shigella* (sucrose negative).

Sugar degradation with acid formation is detected by turning an indicator (phenol red) to yellow, whereas alkalinization turns it to purple. When only glucose is degraded, the acid production is weak and is evaporated on the surface, so the indicator may be re-oxidised producing an alkaline surface (red) and an acid butt (yellow). If lactose or sucrose is degraded, acid production is intense and the entire medium (surface and butt) turns yellow. Gas production is detected by the formation of bubbles and occasionally cracks in the agar.

Hydrogen sulfide production, from thiosulfate or sulphured amino-acids from peptones, is detected by the formation of black FeS precipitate when the medium reacts with iron salts.

Use the medium in slanted tubes with a good butt and a short slant. Inoculate by streaking on the surface and stabbing deeply. It is advisable to use tubes with cotton plugs, in order to allow a re-oxidation of the indicator. If screw caps are used, they must be loose. See the following page for the table of reading (observations) and interpretation of results in TSI Agar.

References

- DIN Standards. Nachweiss von *Salmonella*. Referenzeverfahren DIN 10160 (Fleisch und Fleischerzenguissen) and DIN 10181 (Milch).
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the microbiological examination of Foods. 4th ed. APHA. Washington DC. USA.
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- ISO 6579 Standard (2002) Microbiology of foods and animal feeding stuffs - Horizontal method for the detection of *Salmonella spp*.
- ISO 6785 Standard (2001) Milk and milk Products - Detection of *Salmonella spp*.
- ISO 10272 Standard (1995) Microbiology of foods and animal feeding stuffs - Horizontal method for the detection of thermotolerant *Campylobacter*.
- ISO 21567 Standard (2004) Microbiology of food and animal feeding stuffs.- Horizontal method for the detection of *Shigella spp*.
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- US PHARMACOPOEIA (2002) <61> Microbial Limit Tests. 25th ed. US Phamacopeial Convention Inc. Rockville. Md. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Triple Sugar Iron Agar (TSI Agar)

Art. No. 01-192

Genus and species	Butt	Surface	H_2S Production
Escherichia coli	AG	A	-
Enterobacter aerogenes	AG	A	-
Enterobacter cloacae	AG	A	-
Citrobacter freundii	AG	A	+
Klebsiella pneumoniae	A/AG	R/K	-
Alcaligenes faecalis	R/K	R/K	-
Proteus vulgaris	AG (1)	A	+
Proteus mirabilis	AG (1)	K/A	-
Morganella morganii	AG (1)	R/K	-
Providencia	A/K	R/K	-
Salmonella typhi	A	R/K	+ (2)
Salmonella typhimurium	AG	R/K	+
Salmonella enteritidis	A/G	R/K	+
Salmonella choleraesuis	A/G	R/K	-
Shigella spp.	A	R/K	-
Pseudomonas aeruginosa	R/K	R/K	-

INTERPRETATION

Key	*Colour and appearance*	*Butt*	*Surface*
A	Yellow	Glucose fermentation and acid production	Lactose and/or sucrose fermentation and acid production
G	Yellow with bubbles or cracks	Gas production from glucose	
K	Deep red	No sugar fermentation. Formation of alkaline products	No sugar fermentation. Formation of alkaline products
R	Orange red original (No change)	No fermentation of glucose	No fermentation of lactose or sucrose
H_2S	Blackened	H_2S production	
	Not blackened	No H_2S production	

NOTES

(1) Some strains can be A without gas formation

(2) Only at the top of the column and sometimes only a ring after 48 hours

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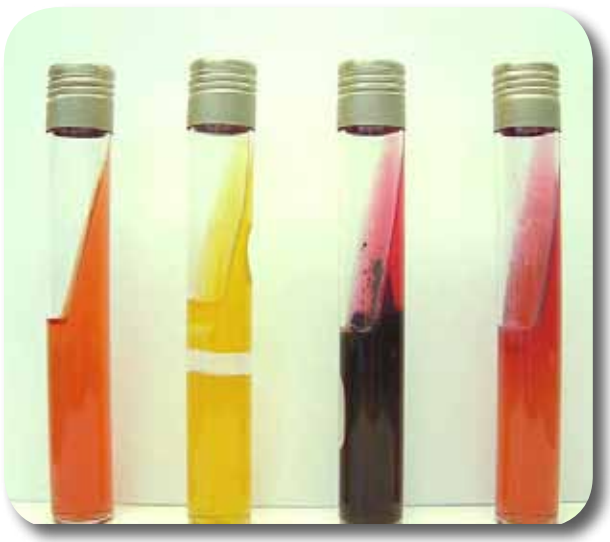
Triple Sugar Iron Agar (TSI Agar)

Art. No. 01-192

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: Stab the butt and streak the slant

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Good	Slant:A; Butt:K; G(-); H_2S (-)
Shigella flexneri ATCC 12022	Good to very good	Slant:A; Butt:K; G(-); H_2S (-)
Proteus mirabilis ATCC 43071	Good to very good	Slant:A; Butt:K; G(-); H_2S (-)
Escherichia coli ATCC 25922	Good to very good	Slant:A; Butt:K; G(-); H_2S (-)
Salmonella typhimurium ATCC 14028	Good to very good	Slant:A; Butt:K; G(-); H_2S (-)
Salmonella abony NCTC 6017	Good to very good	Slant:A; Butt:K; G(-); H_2S (-)
Shigella sonnei ATCC 9290	Good to very good	Slant:A; Butt:K; G(-); H_2S (-)



First: Uninoculated tube;
Second: *E. coli* ATCC 25922;
Third: *Salmonella typhimurium* ATCC 14028;
Fourth: *Shigella sonnei* ATCC 9290

Tryptic Soy Agar (TSA) (Eur. Pharm.)

Art. No. 01-200

Also known as

Casein Soybean Digest Agar

Specification

General purpose solid medium containing animal and plant peptone according to Pharmacopeial Harmonised Method and ISO standards.

Formula* in g/L

Casein peptone	15,00
Soy peptone	5,00
Sodium chloride	5,00
Agar	15,00

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Mix 40 g of powder in 1 L of distilled water. Let it soak and bring to the boil to dissolve the agar. Sterilize in the autoclave at 121°C for 15 minutes.

Description

TSA is a widely used medium containing two peptones which support the growth of a wide variety of organisms, even that of very fastidious ones such as *Neisseria, Listeria, Brucella,* etc. It is frequently used for routine diagnostic purposes due to its reliability and its easily reproducible results.

The following list includes some of its most common applications:

1. Sensitivity testing, either by the Kirby-Bauer system or by following the WHO guidelines. Both systems recommend the use of the Mueller-Hinton Agar (Art. No. 01-136) for verification purposes.
2. The medium provides, with added blood, perfectly defined haemolysis zones, while preventing the lysis of erythrocytes due to its sodium chloride content.
3. It can be used for the preparation of an exceptionally nutrient 'chocolate´ agar, thanks to the richness of its peptones.
4. In a reducing environment or with a CO_2 enriched atmosphere, it provides an excellent medium for the isolation of *Brucella and Neisseria*. It may be made selective by using additives.
5. Most streptococci grow in this medium though clear differences can be observed from one species to another.
6. Tryptic Soy Agar can be used as a selective medium for the count of urine samples although differentiation must be done on selective differential media.
7. Several tests for the differentiation and identification of staphylococci can be performed on this medium, provided suitable additives are used.
8. Yeast, particularly *Candida* species, can grow in this medium forming very characteristic colonies.
9. Chromogenic pseudomonads frequently produce pigmentation on TSA and are therefore easily recognized.
10. A vast bibliography documents its applications in the food industry.
11. It has been frequently used in the Health industry to produce antigens, toxins, etc...
12. Its simple and inhibitor-free composition makes it suitable for the detection of antimicrobial agents in food and other products.
13. A balanced and high nutrient value together with a lack of fermentable carbohydrates make this medium ideal for maintaining bacterial strains.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Food, 4th ed, ASM, Washington D.C.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD.
- HORWITZ, W. (2000) Official Methods of Analysis of AOAC INTERNATIONAL, 17th ed. Gaithersburg, MD. USA.
- ISO 9308-1 Standard (2000) Water Quality. Detection and enumeration of *E. coli* and coliform bacteria. Membrane filtration method.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 22717 Standard (2006) Cosmetics. - Microbiology. - Detection of *Pseudomonas aeruginosa*.
- PASCUAL ANDERSON, MªRª (1992) Microbiología Alimentaria. Díaz de Santos S.A., Madrid.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptic Soy Agar (TSA) (Eur. Pharm.)

Art. No. 01-200

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h - 6 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Staphylococcus aureus ATCC 6538	Productivity > 0.70	-
Escherichia coli ATCC 8739	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	48 h
Salmonella abony NCTC 6017	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC 9027	Productivity > 0.70	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	6 d (Black sporulation)

Bacillus subtilis ATCC 6633

Escherichia coli ATCC 8739

Staphylococcus aureus ATCC 6538

Tryptic Soy Broth (TSB) (Eur. Pharm.)

Art. No. 02-200

Also known as

Digest Broth

Specification

Highly nutrient liquid medium for general purpose use, formulated according to Pharmacopeial Harmonised Method.

Formula* in g/L

Casein peptone	17,00
Soya peptone	3,00
Sodium chloride	5,00
Dipotassium phosphate	2,50
Dextrose	2,50

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 30 g of powder in 1 L of distilled water. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

The Tryptic Soy Broth was initially developed for the cultivation of very fastidious microorganisms without the addition of serum, blood or any other enrichment agent.

As a general purpose culture medium it supports the growth of most organisms, both aerob and facultative anaerobes, even if their requirements are high. Due to its high vitamin content *Brucella*, *Pasteurella* and *Streptococcus* are perfectly viable, moreover a CO_2 enriched atmosphere can further fenhance growth.

In anaerobic conditions this broth will grow *Bacteroides* and *Clostridium* species. For this purpose, the best results can be obtained by adding 0.3% agar and 0.05% sodium azide for *Clostridium*.

Tryptic Soy Broth's superior growth-promoting properties make it particularly suitable for tube dilution methods for antibiotic sensitivity testing.

The broth can be used for bile solubility testing in pneumococci, and also used for catalase and coagulase assays and for the preparation of hypersaline broths.

It is a most suitable medium for the preparation of antigens and toxins in bacteria, moulds and yeasts.

TSB is used as a primary enrichment medium for food examination. In the dairy industry it is employed for testing resazurine reduction.

The medium **is not suitable** for maintenance purposes since carbohydrate fermentation liberates many acids which may threaten the organism's viability. Therefore, though it allows the growth of streptococci and *Neisseria*, these species tend to die if repeatedly sub-cultured in this medium. Such fastidious organisms are best maintained on Cystine Tryptone Fluid Medium (CTA) or even TSA (Art. No. 01-200).

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Food, 4th ed. ASM. Washington. DC.
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PASCUAL ANDERSON, MªRª (1992) Microbiología Alimentaria. Díaz de Santos S.A., Madrid.

- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.
- USP 33 - NF 28 (2011) <71> Sterility Tests. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30°C and <60% RH).

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Tryptic Soy Broth (TSB) (Eur. Pharm.)

Art. No. 02-200

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	-
Staphylococcus aureus ATCC 6538	Good	-
Escherichia coli ATCC 8739	Good	-
Pseudomonas aeruginosa ATCC 9027	Good	-
Candida albicans ATCC 10231	Good	-
Salmonella abony NCTC 6017	Good	-
Clostridium sporogenes ATCC 19404	Good	Anaerobic conditions

Left: *Salmonella abony* NCTC 6017
Centre: *Escherichia coli* ATCC 8739
Right: Uninoculated tube (Control)

Left: Uninoculated tube(Control)
Right: *Clostridium sporogenes* ATCC 19404

Tryptic Soy Broth Irradiated

Art. No. 02-575

Also known as

Irradiated TSB

Specification

General liquid medium, sterilized by gamma-radiation to suit "media fill" tests in the pharmaceutical industry.

Formula* in g/L

Casein peptone	17,00
Soya peptone	3,00
Sodium chloride	5,00
Dipotassium phosphate	2,50
Dextrose	2,50

Final pH 7,3 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Aseptically dissolve 30 g in 1 L of sterile distilled water and use as per standardized media fill run.

a) For liquid filling tests: Suspend 30 g in 1 L of sterile distilled water and mix well to dissolve. Use for liquid fill validation procedures.

b) For solid filling tests: Use powder for dry-fill validation procedures. Ensure that the final concentration of the medium is 30 g of Irradiated TSB suspended in 1 L of sterile distilled water.

Description

The Irradiated Tryptone Soy Broth (Irradiated TSB) is the classical TSB culture medium sterilized by a gamma-irradiation process making it appropriate for validation of aseptic filling processes in the pharmaceutical industry (Media-Fill Test).

The sterility of the dehydrated culture medium is verified according to the methodology as described in the pharmacopoeia. It is subjected to exactly the same conditions as the pharmaceutical product, including filling and closing to ensure that there is no microbial contamination occurring during the process.

The gamma-irradiation treatment is carried out according to the Annex B of the ISO 11137 Standard, and warrants a 25 kGy absorption by the medium, which is enough to suppress any vegetative and/or spore from of microorganisms present in the powder without modification of the medium's performance. All batches of Irradiated TSB are checked for sterility and performance.

Technique

All the conditions and data for the validation of aseptic filling process can be consulted in the ISO Standard 13408 -1:1998 in the chapters devoted to the methods of preparation of sterile products in several Pharmacopoeias.

References

- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- ISO Standard 11137:1995. Sterilization of health care products-Requirements for validation and routine control - Radiation Sterilization. (Annex B).
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- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO Standard 13408-1:1998. Aseptic processing of health care products-Part 1: General requirements.
- US PHARMACOPOEIA 28 / NATIONAL FORMULARY 23 (2005) General Chapters § <71> Sterility Tests y § <1208> Sterilization and Sterility Assurance of Compendial Articles.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Tryptic Soy Broth Irradiated

Art. No. 02-575

Quality control

Incubation temperature: 35°C ± 2.0
Incubation time: 24 h-5 days
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	-
Staphylococcus aureus ATCC 6538	Good	-
Clostridium sporogenes ATCC 19404	Good	In anaerobic conditions
Escherichia coli ATCC 8739	Good	-
Salmonella abony NCTC 6017	Good	-
Candida albicans ATCC 10231	Good	-
Aspergillus brasiliensis ATCC 16404	Good	Black sporulation at 5 days

Left: *Escherichia coli* ATCC 8739
Centre: *Salmonella sp*. NCTC 6017
Right: Uninoculated tube (Control)

Left: Uninoculated Tube (Control)
Right: *Clostridium sporogenes* ATCC 19404

Tryptic Soy Broth Modified

Art. No. 02-691

Also known as

TSBm

Specification

Liquid culture medium used for the selective enrichment of Enterohaemorrhagic *Escherichia coli* (EHEC) in food, according to the ISO standard 16654:2001.

Formula* in g/L

Tryptone	17,00
Soy peptone	3,00
Dextrose	2,50
Bile salts No.3	1,50
Sodium chloride	5,00
Bi-potassium phosphate	4,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 33 g in 1 L of distilled water. Distribute in volumes of 500 mL/container and sterilize in the autoclave at 121°C for 15 minutes. Cool to 50°C and aseptically add a vial of Novobiocin Selective Supplement (Art. No. 06-139-LYO) to each 500 mL of sterile medium. Homogenize and distribute into the final containers.

Description

The actual formulation of the medium complies with the European Norm EN-ISO 16654:2001, horizontal method for the detection of *Escherichia coli* O157.

The modification of the classical Tryptic Soy Broth was proposed in 1987 by Doyle and Schoenli by adding 1,5 g/L of bile salts No.3 to eliminate the non-enteric bacteria and the selectivity is reinforced by the addition of an antibiotic to inhibit the growth of Gram positive microbiota.

A broth with the same composition but with a higher (1,5%) concentration of salt and lesser (0,1 mg/L) novobiocin was used in the isolation and culture of *Shigella* from food samples.

The European methodology (EN, ISO, UNE, CCFRA, DIN, etc.) uses novobiocin at 20 mg/L concentration, but American methods (FDA, BAM, AOAC) pefers a mixture of antibiotics: cefixime 0,05 mg/L to suppress the growth of *Proteus spp.*; cefsulodine 10 mg/L to inhibit Aeromonas and Pseudomonas and vancomycin 8 mg/L to eliminate Gram positive bacteria. Such medium was proposed in 1995 by Weagant and collaborators. It is also known as “EHEC Broth” or EEE Broth”.

Technique

The European Methodology proposes four steps in the *Escherichia coli* O157 detection:

1. Enrichment of the sample by incubation at 41,5°C ± 1°C in TSBm with antibiotic(s) for 6 hours followed by a supplementary period of 12-18 hours.
2. Separation and Concentration with immunomagnetic particles.
3. Isolation and presumptive identification from the immunomagnetic particles onto selective solid media.
4. Confirmation by cultural, biochemical and serological methods.

Precautions:

A rigorous control of enrichment incubation temperature is recommended because above 42°C the growth rate of serotype O157 decreases dramatically.

Necessary supplements

Novobiocin Selective Supplement (Art. No. 06-139-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Novobiocin, sodium salt	10,00 mg

Distilled water (Solvent)

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiology Media. CRC Press Inc. London.
- BAYLIS, C.L., (editor) (2007) Method 3.4.1:2007. Detection of *E. coli* O157: Cultural Technique Incorporating Immunomagnetic Separation. Manual of Microbiological Methods for the Food and Drinks Industry. 5th ed. CCFRA. United Kingdom.
- BENNET, A.R., S. MacPHEE & R.P. BETTS (1996) The isolation and detection of *Escherichia coli* O157 by use of immunomagnetic separation and immunoassay procedures. Letters in Appl. Microbiol. 22:237-243.
- DIN 10167 (2001) Nachweis von *Escherichia coli* 0157: im Lebensmitel. Deutsches Institut für Normung.
- DOYLE, M.P. & J.L. SCHOENI (1987) Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394-2396.
- FDA (Food and Drug Adminstrations) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. USA.
- HEUVELINK, A.E. (2003) Review of media for the isolation of diarrhoeagenic *Escherichia coli.* Handbook of Culture Media for Food Microbiology. J.E.L. Corry *et al* (eds). Elsevier Science B.V. Amsterdam.

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Tryptic Soy Broth Modified

Art. No. 02-691

- HITCHINS, A.D., P. FENG, W.D WATKINS, S.R. RIPPEY & L.A. CHANDLER (1995) *Escherichia coli* and the Coliform Bacteria.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO Standard 16654 (2001) Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Escherichia coli.* O157.
- SCOTTER, S., M. ALDRIDGE & K. CAPPS (2000) Validation of a method for the detection of *E. coli* O157: H7 in foods. Food Control. 11:85-95.
- VARNAM, A.H. & M.G. EVANS (1991) Food Borne Pathogens: An Illustrated Text. Manson Publishing. United Kingdom.
- WEAGANT, S.D., J.L. BRYANT & K.G. JINNEMAN (1995) An improved rapid technique for isolation of *Escherichia coli* O157:H7 from foods. J. Food Protect. 58:7-12.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Inhibited	Recovery on TSA
Enterococcus faecalis ATCC 29212	Inhibited	Recovery on Sorbitol Agar
E.coli ATCC 35150	Good	Recovery on Sorbitol Agar
E.coli ATCC 8739	Poor to good	Recovery on Sorbitol Agar
E.coli ATCC 25922	Poor to good	Recovery on Sorbitol Agar
Pseudomonas aeruginosa ATCC 27853	Poor	Recovery on Sorbitol Agar

Tryptic Soy Broth without Dextrose

Art. No. 02-227

Specification

Liquid culture medium used for the production of spores of *Geobacillus stearothermophilus* for the inhibitory substances test in food according to FDA-BAM.

Formula* in g/L

Casein peptone	17,00
Soya peptone	3,00
Sodium chloride	5,00
Dipotassium phosphate	2,50

Final pH 7,3 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 27,5 g of powder in 1 L of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

TSB w/o Dextrose is produced according to the formulation from Bacteriological Analytical Manual (BAM) of Food and Drug Administration (FDA) for the production of spores of *Geobacillus stearothermophilus* used to determine the presence of inhibitory substances in milk and dairy products.
This medium is not recommended for sugar fermentation studies because the amount of fermentable carbohydrates in the soy peptone.

References

- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MATURIN, L.J. (1998) Inhibitory substances in milk. Qualitative Method II: B. *stearothermophilus* disk assay. In: FDA (Food and Drug Adminstrations) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International Inc. Gaithersburg. VA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	-
Bacillus cereus var. mycoides ATCC 11778	Good	-
Bacillus cereus ATCC 10876	Good	-
Geobacillus stearothermophilus ATCC7953	Good	-

Tryptone Bile Agar

Art. No. 01-526

Also known as

TBA

Specification

Selective solid medium for the rapid enumeration of *Escherichia coli* in meat products and in water according to ISO standards 6391 and 9308-1.

Formula* in g/L

Tryptone	20,00
Bile salts No. 3	1,50
Agar	15,00

Final pH 7,20 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 36,5 g of powder in 1 L distilled water and bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium is formulated according to the Anderson & Baird-Parker Direct Plating Method (DPM) for the rapid enumeration of *Escherichia coli* in raw meats. The method is based on the characteristically production of indol from tryptophane when the bacteria growth at 44°C over a cellulose acetate membrane in the surface of the Tryptone Bile Agar.

The International Commission on Microbiological Standards for Food noticed that DPM was less variable and offered a better recovery and rapidity than the MPN method for frozen samples of meat. The ISO Standard 6391:1997 also uses this medium for *E. coli* enumeration.

Indol producing microorganisms other than *E. coli* are inhibited by the bile salts and the incubation temperature. In sugar-rich samples indol production can be inhibited due to the sugar concentration interfering with tryptophanase synthesis.

Technique

The method used by Anderson and Baird-Parker and by ICMSF is as follows:

1. A cellulose acetate membrane of 0,45 µm pore size is placed on the surface of the medium.
2. 0,5 mL of the sample dilution (with Tryptone Water Art. No. 03-156) is spread on the membrane and the plates are incubated in an upright position at 44 ± 1°C for 18-24 hours.
3. After incubation the membrane is immersed in indol reagent using the Petri dish cover as a container and exposed to direct sunlight for 5 minutes or to the Wood lamp for 10 minutes.
4. The indol positive colonies turn reddish in colour (pink to deep red).
5. The results are expressed as number of *Escherichia coli* per g or mL of sample.

References

- ANDERSON, J.M. & A.C. BAIRD-PARKER (1975) Appl. Bact. 39:111-117. ICMSF (1979) International Commission on Microbiological Specifications for Foods Can. J. Microbiol. 25:1321-1327.
- ISO 9308-1 Standard (2000) Water Quality - Detection and enumeration of *E.coli* and coliform bacteria. Membrane filtration method.
- ISO 6391 Standard (1997) Meat and meat products - Enumeration of *Escherichia coli* - Colony-count technique at 44°C using membranes.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptone Bile Agar

Art. No. 01-526

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibition	24 h
Escherichia coli ATCC 8739	Productivity > 0.50	Indol (+)
Escherichia coli ATCC 25922	Productivity > 0.50	Indol (+)
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Indol (-)

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Tryptone Bile Glucuronic Agar (TBX Agar)

Art. No. 01-619

Specification

Selective and differential solid medium for the detection and enumeration of β-glucuronidasepositive *Escherichia coli* according to ISO standards 16649-1 and -2.

Formula* in g/L

Tryptone	20,000
Bile salts No. 3	1,500
X-β-D-glucuronide	0,075
Agar	15,000

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 36.5 g of the powder in 1 L of distilled water and heat to boiling with continuous stirring until total dissolution. Dispense into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Escherichia coli is the only coliform that possesses β-D-glucuronidase and can be easily differentiated from other coliforms that do not show this enzymatic activity. There are some strains of *E. coli* (less than 3-4% of the total population) that are β-D-glucuronidase negative.

E. coli absorbs the chromogenic substrate (X-β-D-glucuronide) and the bacterial enzyme β-D-glucuronidase splits the bond between the chromophoric X-fraction and the β-D-glucuronide.

The free X-fraction dyes the *E. coli* cells and produces a blue-green colony.

The high content in bile salts of the medium inhibits the growth of accompanying Gram positive bacteria and the high incubation temperature (44°C) inhibits Gram-negative bacteria other than *E. coli.*

Technique

1. Direct inoculation (Pour plate technique)

Transfer 1 mL of test sample to a sterile Petri dish aseptically, and repeat the procedure with further dilutions. Inoculate two plates per dilution. Pour 15 mL of melted and cooled (44-47°C) TBX Agar into each Petri dish. Mix carefully and allow the mixture to solidify. The time between the distribution of the inoculum and pouring the medium should not exceed 15 minutes.

Invert the inoculated plates and incubate them at 44°C for 18-24 h. If the presence of stressed cells is suspected incubate for an initial period of 4 h at 37°C and then raise the incubation temperature to 44°C. The total incubation time should not exceed 24 h and the incubation temperature should not exceed 45°C.

2. Membrane incubation (Resuscitation technique)

No special membranes are recommended. Any sterile and non-inhibitive membrane made of cellulose acetate or mixed esters of cellulose, with 0.45 µm to 1.2 µm pore size and 85 mm diameter can be used.

2.1. Resuscitation

Aseptically place a membrane on the dried surface of each of two plates of Mineral-Modified-Glutamate Agar (Art. No. 01-571) with care to avoid trapping air bubbles. Add 1 mL of the test sample to the centre of each membrane and spread the inoculum evenly over the whole membrane surface. Repeat the procedure for each dilution of the sample.

Leave the inoculated plates at room temperature for 15 minutes until the inoculum has soaked into the agar. Incubate the plates at 37°C for 4 ± 1 h.

2.2. Transfer to the selective medium

After the resuscitation period, transfer the membranes from the resuscitation medium to the plates of TBX Agar using sterile forceps, taking care to avoid trapping air bubbles beneath the membrane. Do not touch nor disturb the membrane surface. Incubate the plates for 18-24 h at 44°C (and not more than 45°C).

3. Results

The β-D-glucuronidase-positive *Escherichia coli* produces blue-green colonies. Some strains (3-4% of the total population) of *E. coli* lack the glucuronidase enzyme and produce colourless colonies. Some stressed cells of *E. coli* are unable to grow at 44°C and produces no colonies.

References

- DELISLE, G.L. & A. LEY (1989) Rapid detection of *E. coli* in urine samples by a new chromogenic β-glucuronidase assay. J. Clin. Microbiol. 27:778-779.
- ISO Standard 16649 (2001) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*. Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-Dglucuronide.
- OGDEN, I.D. & A.J. WATT (1991) An evaluation of fluorogenic and chromogenic assays for the direct enumeration of *E. coli*. Letters in Appl. Microbiol. 13:212-215.
- SCHWEIZERISCHES LEBENSMITTELBUCH (2005) Kap. 56 Mikrobiologie, Bundesamt für Gesundheit. Direktionsbereich Verbraucherschutz. Bern.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptone Bile Glucuronic Agar (TBX Agar)

Art. No. 01-619

Quality control

Incubation temperature: 35 / 44°C / ± 1,0
Incubation time: 4 - 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method or Spiral Plate Method

Microorganism	Growth	Remarks
C.freundii ATCC 43864	Inhibited (poor)	-
Enterococcus faecalis ATCC 19433	Inhibited (poor)	Selectivity
Escherichia coli ATCC 25922	Productivity > 0.70	Dark-green colonies
Escherichia coli ATCC 11775	Productivity > 0.70	Dark-green colonies
Salmonella typhimurium ATCC 14028	Productivity > 0.70	Colourless colonies
Salmonella abony NCTC 6017	Productivity > 0.70	Colourless colonies

Escherichia coli ATCC 25922

Salmonella abony NCTC 6017

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Tryptone Glucose Extract Agar (TGE Agar)

Art. No. 01-082

Also known as

Colony Count Agar; Trypticase Glucose Extract Agar

Specification

Plate count medium for milk and dairy products, according to standard Methods for the Examination of Dairy Products.

Formula* in g/L

Meat extract	3,00
Tryptone	5,00
D(+) Glucose	1,00
Agar	15,00

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 24 g of powder in 1 L of distilled water. Heat to the boil with constant stirring. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Tryptone Glucose Extract Agar was adopted as an alternative to Nutrient Agar according to APHA (Art. No. 01-144) and Nutrient Agar according to British Pharmacopoeia (Art. No. 01-140) for bacteria enumeration in milk, being a complement to Plate Count Agar (Art. No. 01-161).

Technique

For enumeration purposes the poured plate Method is preferred, with incubation at 30-32°C for 48 hours. If the dilution is more than 10% it is advisable to add milk to the medium. To do this, prepare a suspension of skimmed milk (Art. No. 06-019) separately, and sterilize it for 10 minutes at 118°C.

Autoclaving must be as short as possible. Homogenize with the culture medium which has been sterilized and cooled to 50°C. The use of natural milk is not recommended due its high variability.

The medium must be quickly poured into Petri dishes because if it remains hot for too long flocculation and abnormal precipitates may appear. If the sample is not diluted or the volume in the plate is more than 2 mL, it is not necessary to add the skimmed milk (Art. No. 06-019) because it is assumed that the sample provides the required growth factors.

References

- APHA-AWWA-WEF (1998) Standard Metods for the Examinationof Water and Wastewater. 20th ed. APHA. Washington.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- FDA (Food and Drug Adminstrations). (1998) Bacteriological Analytical Manual 8th ed. Revision A. AOAC International,Gaitherburg. MD.
- HORWITZ, W. (2000) Official Methods of Analysis. AOAC International. Gaitherburg. MD.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MARSHALL, R.T. (Ed.) (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptone Glucose Extract Agar (TGE Agar)

Art. No. 01-082

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Bacillus subtilis ATCC 6633	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Yersinia enterocolitica ATCC 9610	Productivity > 0.70	-

Bacillus subtilis ATCC 6633

Staphylococcus aureus ATCC 6538

Escherichia coli ATCC 25922

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Tryptone Sulfite Neomycin Agar (TSN Agar)

Art. No. 01-195

Specification

Solid selective medium for *Clostridium perfringens* isolation.

Formula* in g/L

Casein peptone	15,00
Sodium sulfite	1,00
Neomycin sulfate	0,05
Polymyxin B	0,02
Yeast extract	10,00
Ferric citrate	0,50
Agar	13,50

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 40 g of powder in 1 L of distilled water and bring to the boil. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

For improved results, add 20 mL/L of a solution containing 1 g/L di-potassium phosphate, 0,5 g/L; sodium carbonate and 1 g/L sodium thioglycollate just before use.

Description

This culture medium was formulated taking advantage of the tolerance of *C. perfringens* to high concentrations of sulfite, which apart from being an inhibitor agent, provides a strong reducing environment.

Selection of *C. perfringens* is almost complete when it is incubated at 46°C, since neomycin and polymyxin included in the medium restrain the development of *C. bifermentans* and all the accompanying Gram negative bacteria.

The medium is especially suitable for the investigation of food products, and it may be used in tubes as well as in plates. If the incubation is not performed in an anaerobic jar, buffered thioglycollate solution must be added or the inoculated surface must be covered with a sterile layer of medium.

Colonies of *C. perfringens* form very characteristic black colonies that, if exposed to air, become decolourised by oxidation.

TSN has a very short storage period once prepared, so it is advisable to re-hydrate or reconstitute it in small amounts and use it on the day of its preparation.

References

- ATLAS, R.M., & L.C. PARK (1993) Handbook of Microbiological Media, CRC Press Inc., London.
- MacFADDIN, J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Williams & Wilkins. Baltimore, USA.
- MARSHALL, R.S., STEENBERGEN, J.F., MacCLUNG, L.S. (1955) Rapid Technique for the enumeration of *Clostridium perfringens*. Appl. Microbiol. 13:559-563.
- MOSSEL, D.A.A. (1959) Enumeration of sulfite reducing clostridia occurring in foods. J. sci. Food Agr. 10:662-669.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptone Sulfite Neomycin Agar (TSN Agar)

Art. No. 01-195

Quality control

Incubation temperature: 46°C ± 1,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). In anaerobic conditions

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Pseudomonas aeruginosa ATCC 27853	Inhibited	-
Clostridium perfringens ATCC 13124	Good - very good	Black colonies
Clostridium perfringens ATCC 10543	Good - very good	Black colonies

Clostridium perfringens ATCC 13124
on membrane filter

Clostridium perfringens ATCC 13124
in double layer

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Tryptone Water (Peptone Water)

Art. No. 03-156

Specification

Substrate with low nutrient capacity, for the detection of indol production in coliform microorganisms according to ISO 7251 standard.

Formula* in g/L

Casein peptone.....10,00
Sodium chloride.....5,00
Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 15 g of powder in 1 L of distilled water and dispense into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

The standard protocol requires that one loop from each suspected tube is inoculated into 10 mL of Tryptone Water.
Incubate for 48 hours at 44°C before investigating the indol production with Kovacs' Reagent for Indol (Art. No. 06-018).
As an alternative method, Ehrlich's Reagent can also show indol production. After 48 hours of incubation at 37°C, take 0,5 mL of growth and mix it with 0,5 mL of Ehrlich's Reagent. Let them settle a few minutes. A pink colour indicates a positive test. Colour appearance is accelerated if a few drops of a saturated solution of potassium per-sulfate is added.
Other authors prefer extraction and concentration of indol with 1 mL of Ether prior to addition of reagent.

References

- APHA-AWWA-WEF (1998) Standard Methods for the examination of water and wastewater. 20th ed. APHA. Washington. DC.
- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Food. 4th ed. APHA. Washington.
- ISO 7251 Standard (2005) Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of presumptive *Escherichia coli* - Most Probable Number Technique.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptone Water (Peptone Water)

Art. No. 03-156

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Good	-
Salmonella typhimurium ATCC 14028	Good	Indol (-)
Escherichia coli ATCC 8739	Good	Indol (+)
Escherichia coli ATCC 25922	Inhibited	Indol (+)

Left: Uninoculated tube (Control)
Centre: *S. typhimurium* ATCC 14028
Right: *Escherichia coli* ATCC 25922

Indol Test
Left: Uninoculated tube (Control)
Centre: *S.typhimurium* ATCC 14028
Right: *Escherichia coli* ATCC 25922

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Tryptone Water pH 7,5

Art. No. 03-577

Specification

Liquid medium used for the detection of indol in coliform bacteria.

Formula* in g/L

Tryptone.....10,00
Sodium chloride.....5,00
Final pH 7,5 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 15 g of powder in 1 L of distilled water and dispense into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

The standard protocol requires one loop from each suspected tube or colony to be inoculated in 10 mL of Tryptone Water. Incubate for 48 hours at 44°C before investigating the indol production with Kovacs' Reagent for Indol (Art. No. 06 018).
As an alternative method, Ehrlich's Reagent can also show indol production. After 48 hours of incubation at 37°C, take 0,5 mL of growth and mix it with 0,5 mL of Ehrlich's Reagent. Let them settle a few minutes. A pink colour indicates a positive result. Colour appearance is accelerated if a few drops of a saturated solution of potassium per-sulfate is added. Other authors prefer extraction and concentration of indol with 1 mL of ether prior to addition of reagent.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. London.
- HAVELAAR, A.H & M. DURING (1988) Evaluation of the Anderson Baird-Parker direct plating method for enumerating *Escherichia coli* in water. J. Appl. Bact. 64:89-98.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Membrane Filter Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Poor to good	-
Salmonella typhimurium ATCC 14028	Good	Indol (-)
Escherichia coli ATCC 25922	Good	Indol (+)
Escherichia coli ATCC 8739	Good	Indol (+)

Right: Uninoculated tube (Control)
Centre: *Escherichia coli* ATCC 25922
Left: *Staphylococcus aureus* ATCC 25923

Tryptone Yeast Extract Agar

Art. No. 01-590

Also known as

Water Plate Count Agar

Specification

Solid medium used for the enumeration of water microorganisms according to ISO standards.

Formula* in g/L

Tryptone	6,00
Yeast extract	3,00
Agar	15,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 24 g of powder in 1 L of distilled water and bring to the boil. Distribute into containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This medium, formulated according to ISO Standard 6222 and others is for the enumeration of heterotrophic microorganisms from water.

Technique

Using a water sample obtained according to the ISO Standard 5667-2 and 5667-3, make a decimal dilution series (see ISO Standard 6887) using 1/4 Ringer Solution (Art. No. 06-073) and take aliquots to 2 parallel series of plates. Pour the sterilized Tryptone Yeast Extract Agar cooled to 45°C, and homogenize with the sample (see ISO Standard 8199). Once solidified, incubate one of the series at 36 ± 2°C for 48 ± 2 hours and the other one at 22°C for 3 days (72 ± 4 hours).

In order to achieve a good count, select plates with 30-300 colonies. Express the results as number of colony forming units per millilitre (CFU/mL) of sample for each temperature of incubation. If there are no colonies with the undiluted sample express the results as "none detected in one mL". If there are more than 300 colonies in the highest dilution express the results as ">300 CFU/mL".

References

- ISO Standard 6222 Water Quality - Enumeration of cultivable microorganisms. Colony count by inoculation in a nutrient agar culture.
- ISO Standard 5667-2 (1991) Water Quality - Sampling - Guidance on sampling techniques.
- ISO Standard 5667-3 (1996) Water Quality - Sampling - Guidance on the preservation and handling of samples.
- ISO Standard 6887 (1999) Microbiology - General - Guidance for the preparation of dilutions for microbiological examination.
- ISO Standard 8199 (1988) Water Quality - General guide to the enumeration of microorganisms by culture.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptone Yeast Extract Agar

Art. No. 01-590

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method / or Membrane Filter Method according to ISO/TS 11133-1/2

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Productivity > 0.70	-
Enterococcus faecalis ATCC 19433	Productivity > 0.70	-
Bacillus cereus var. mycoides ATCC 11778	Productivity > 0.70	-
Escherichia coli ATCC 25922	Productivity > 0.70	-
Salmonella typhimurium ATCC 14028	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC 27853	Productivity > 0.70	-

Bacillus cereus var. mycoides ATCC 11778

Staphylococcus aureus ATCC 25923

Salmonella typhimurium ATCC 14028

Tryptophan Broth

Art. No. 02-418

Specification

Liquid medium used for the detection of indol production according to ISO standards.

Formula* in g/L

Meat peptone	10,00
DL-Tryptophan	1,00
Sodium chloride	5,00

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 16 g of powder in 1 L of distilled water. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This broth allows indol to be produced from tryptophan, and therefore it is suitable for the differentiation and identification of coliforms from water and food. Its formulation is according to the German standards for waters and foods, and complies with the specifications of several ISO standards related to the presence and identification of *Escherichia*, *Salmonella* and *Shigella* in different products.

Technique

Medium is inoculated with a previously isolated culture, and then incubated at 30-32°C for 24-48 hours.

Indol production is observed by adding a few drops of Kovacs' Reagent (Art. No. RE0007) to the broth (with or without previous extraction) and shaking gently. Formation of a red ring indicates indol production.

References

- BUNDESGESMELHEITSAMT: Amtliche Samnulung von Untersuchungs Verfahren nach #35LMBG. Beuth Verlag. Berlin-Köln.
- ISO Standard 6785:2001. Milk and Milk Products. Detection of *Salmonella spp*.
- ISO Standard 9308-1:2000. Water Quality. Detection and enumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method.
- ISO Standard 21567:2004. Microbiology of Foods and animal feeding stuffs - Horizontal method fort he detection of *Shigella spp* .
- VERORDNUNG über Trinkwasser und über Wasser für Lebensmittelbetriebe vom 12-12-1990. Bundesgesatbl. I. 2613-2619.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10.000-100.000 CFU

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	Indol (+)
Escherichia coli ATCC 25922	Good	Indol (+)
Salmonella typhimurium ATCC 14028	Good	Indol (-)
Shigella sonnei ATCC 9290	Good	Indol (-)

Left: Uninoculate tube (control)
Centre: *Salmonella typhimurium* ATCC 14028
Right: *Escherichia coli* ATCC 25922

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Tryptose Lauryl sulfate Broth

Art. No. 02-108

Also known as

LST

Specification

Liquid medium used for the detection and enumeration of coliform bacteria according to IDF-FIL 73B and ISO standards.

Formula* in g/L

Tryptose	20,00
Sodium Lauryl sulfate	0,10
Lactose	5,00
Dipotassium phosphate	2,75
Monopotassium phosphate	2,75
Sodium chloride	5,00

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 35,6 g of powder in 1 L of distilled water. Distribute into tubes or containers with inverted Durham tubes (for gas detection). Sterilize at 121°C for 15 minutes. For double concentration medium, dissolve 71,2 g/L and proceed as indicated above.

Preferably store the broth at room temperature, and use screw-capped bottles to prevent evaporation of water. refrigerated broth generally becomes cloudy or forms precipitates but clears at room temperature. Clarity is not important as gas production is the most significant criterion.

Description

Lauryl sulfate broth is used for the MPN Presumptive Test of coliforms in water and sewage, confirmatory test of lactose fermentation with gas production for milk and detection of coliforms in food.

The high nutrient quality and the presence of phosphate buffer in this medium ensure rapid growth and increased gas production, even by slow lactose-fermenting coliforms.

Indol production is observed by adding a few drops of Kovacs' Reagent (Art. No. RE0007) to the broth (with or without previous extraction) and shaking gently. Formation of a red ring indicates indol production.

This medium can be used as a presumptive broth for *E. coli* (by fluorescent reaction) if, before sterilization, MUG (Art. No. 06-102CASE or 06-102-LYO) is added.

Technique

If the volume of sample is substantial, then reconstitute the medium such that the final concentration remains normal.

Incubate at 37°C for 24-48 hours. Lactose fermentation is shown by the appearance of gas in the Durham tubes, indicating the presence of coliform bacteria.

Verification can be done by the isolation and identification of the coliforms on an appropriate medium.

References

- APHA AWWA WPCF (1995) Standard Methods for the examination of water and wastewater. APHA. Washington.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Food. 4th ed. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8 th ed. Revision A. AOAC International Gaithersburg. MD.
- FIL IDF Standard 73B (1998) Milk and milk products. Enumeration of coliforms. IDF. Brussels.
- HORWITZ, W. (2000) Official methods ofAnalysis. 17th ed. AOAC International. Gaithersburg. MD.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 4831 Standard (1991) General guidance for the enumeration of coliforms - MPN technique.
- ISO 7251 Standard (1993) General guidance for enumeration of *E.coli* by MPN technique.
- MARSHALL R.T. (1992) Standard Methods for the examination of dairy products. 16th ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Tryptose Lauryl sulfate Broth

Art. No. 02-108

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited to poor	-
Staphylococcus aureus ATCC 6538	Inhibited to poor	-
Escherichia coli ATCC 25922	Good	Gas (+)
Escherichia coli ATCC 8739	Good	Gas (+)
Salmonella typhimurium ATCC 14028	Good	Gas (-)
Citrobacter freundii ATCC 43864	Good	Gas (+)

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Tryptose Lauryl sulfate Mannitol Tryptophan Broth

Art. No. 02-460

Specification

Liquid medium used for the production of indol and gas in a single tube, according to ISO 9308-1/2 standards.

Formula* in g/L

Tryptose	20,00
Mannitol	5,00
Sodium chloride	5,00
Monopotassium phosphate	2,75
Dipotassium phosphate	2,75
Sodium Lauryl sulfate	0,10
L-Tryptophan	0,20

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 35,8 g of powder in 1 L of distilled water, heating if necessary. Distribute in tubes containing Durham´s tubes and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat.**

Description

This broth is described in the ISO 9308-1 Standard as an alternative medium for the production of indol and gas in a single tube and to confirm the presence of thermotolerant coliforms and the presumptive presence of *E. coli* in water samples.

Technique

Tubes are inoculated from suspicious colonies from a filter membrane and then incubated at 44°C for 24 hours. Gas production, which appears in the Durham tubes, confirms the presence of thermotolerant coliforms. If after the addition of 0,2-0,3 mL of Kovacs' Reagent (Art. No. RE0007) a cherry red colour appears on the surface of the medium (Indol +), a presumptive presence of *E. coli* is considered and should be confirmed.

References

- ISO Standard 9308:1990. Water quality detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *E.coli*. Part 1. Membrane filtration method. Part 2. MPN method.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 44°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	Indol (+) Gas (+)
Escherichia coli ATCC 25922	Good	Indol (+) Gas (+)
Salmonella typhimurium ATCC 14028	Good	Indol (-) Gas (-)

Left (K): Uninoculated tube (Control)
Centre: *Salmonella typhimurium* ATCC 14028
Right: *Escherichia coli* ATCC 25922

Indol test

Tryptose Sulfite Cycloserine Agar (TSC Agar)

Art. No. 01-278

Specification

Solid selective and differential medium for isolation and presumptive identification of *Clostridium perfringens*, according to ISO standards.

Formula* in g/L

Tryptose	15,00
Soya peptone	5,00
Yeast extract	5,00
Sodium metabisulfite	1,00
Ferric ammonium citrate	1,00
Agar	18,00

Final pH 7,6 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 45 g of powder in 1 L of distilled water and soak. Heat to boiling and distribute into suitable containers, do not add more than 250 mL to each one. Sterilize in the autoclave at 121°C for 10 minutes. Let it cool to 60°C and add 1 flask of Cycloserine Selective Supplement (Art. No. 06-116CASE or 06-116-LYO) to every 250 mL of medium. Mix well and pour into plates. If it is desired to include egg yolk, add Egg Yolk Sterile Emulsion (Art. No. 06-016) in a concentration of 80 mL/L.

Description

The medium is a modification of the classical TSN Agar (Art. No. 01-195) in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine. Cycloserine has been found more selective for *Clostridium perfringens*, and reduces the production of diffuse blackening. *Clostridium perfringens* is more resistant to cycloserine than to sulfadiazine, polymyxin and neomycin, hence reducing the dosage. The presence of sodium meta-bisulfite and ferric ammonium citrate allow three differential characteristics of this anaerobic species to be verified with just one assay. These characteristics are sulfite reduction, growth at 46°C and cycloserine resistance.

Cycloserine does not tolerate temperatures above 100°C and its stability in a solution is variable. Therefore, it is advisable to prepare the exact number of plates that are going to be used.

A solution of cycloserine in phosphate buffer at pH 8,0 may be prepared (Di potassium phosphate 16,73 g/L and mono-potassium phosphate 0,52 g/L) and if it is maintained refrigerated, can be used for approx. 5 days.

Technique

The standard procedure recommends surface inoculation of the samples or their dilutions, and once absorbed, to pour a second layer as a seal for anaerobiosis. After incubation at 46°C for 18-20 hours, proceed to enumerate the black colonies that appear in the plate.

Necessary supplements

D-Cycloserine Selective Supplement (Art. No. 06-116CASE/06-116-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

D-Cycloserine	100,00 mg

Distilled water (Solvent)

References

- ATLAS, R.M., LC. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DIN Standard 10165. Referenz Verfahren fur Bestimmung von *Clostridium perfringens*. Fleisch und Fleischerzeugnissen.
- DOWNES, F.P. y K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. American Public Health Association. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International Inc. Gaithersburg. MD.
- ISO Norma 7973 (2004) Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for Enumeration of *C. perfringens*. Colony-count technique.
- ISO Norma 6461-2 (1986) Water Quality.- Detection and enumeration of the spores of sulfite-reducing anaerobes (Clostridia).- Part 2: Method by Membrane Filtration.
- SMITH, L.D. (1981) Clostridial Anaerobic Infections, in Diagnostic Procedures for Bacterial Mycotic and Parasitic Infections. 6th ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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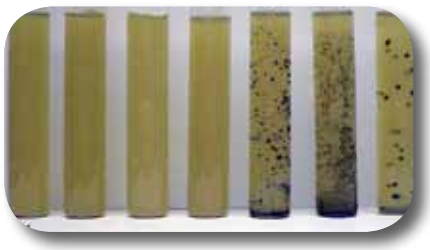
Tryptose Sulfite Cycloserine Agar (TSC Agar)

Art. No. 01-278

Quality control

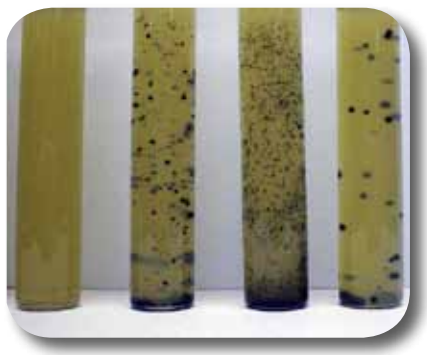
Incubation temperature: 35°C ± 2,0
Incubation time: 20 - 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited	Anaerobiosis
Escherichia coli ATCC 25922	Inhibited	Anaerobiosis
Pseudomonas aeruginosa ATCC 27853	Inhibited	Anaerobiosis
Clostridium perfringens ATCC 10543	Productivity > 0.70	Black colonies (Anaerobiosis)
Clostridium perfringens ATCC 13124	Productivity > 0.70	Black colonies (Anaerobiosis)



Growth

Clostridium perfringens ATCC 13124



First: Uninoculated Control
Second: *Clostridium perfringens* ATCC 10543
Third: *Clostridium perfringens* ATCC 13124
Fourth: *Clostridium sporogenes* ATCC 11437

Urea Agar Base

Art. No. 01-261

Also known as

CHRISTENSEN Agar

Specification

Solid medium for the detection of urease, according to ISO standards and DIN standard.

Formula* in g/L

Gelatin peptone	1,000
Dextrose	1,000
Sodium chloride	5,000
Monopotassium phosphate	2,000
Phenol red	0,012
Agar	15,000

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 24 g of powder in 950 mL of distilled water and bring to the boil. Sterilize in the autoclave at 121°C for 15 minutes. Let it cool to 50-55°C. Add 50 mL of Urea Sterile Solution 40% (Art. No. 06-083) and mix well. Distribute aseptically in tubes and let them solidify in a slanted position.

Description

Urea Agar complies with Christensen's specifications, and is recommended for the detection of ureolytic or urea degrading microorganisms, especially Enterobacteriaceae, although it can be used with Gram positive bacteria.

Technique

A pure culture is inoculated by surface streaking, and then incubated at 37°C. Generally, organisms with strong urease activity can be read after 3-5 hours.

Reaction is evident as the medium changes colour form orange to pink-fuchsia, due to a strong alkalinization produced by ammonia release.

References

- ATLAS, R.M. & L.C. PARK (1993) Handbook of Microbiological Media. CRC Press Inc. London.
- CHRISTENSEN W.B. (1946) Urea decomposition as means of differentiating *Proteus* aqnd *Paracolon* cultures from each other and from *Salmonella* and *Shigella* types. J. Bact. 52:461.
- DIN Standard 10160. Untersuchung von Fleisch und Fleischerzeugnissen. Nachweis von Salmonellen. Referenzverfahren.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington DC. USA.
- EDWARDS & EWING (1962) Identificacion of Enterobacteriaceae Burgess Pub. Co.
- FIL-IDF 93 Standard (2001) Milk and Milk products. Detection of *Salmonella*.
- ISO 6340 Standard (1995) Water Quality - Detection of *Salmonella spp*.
- ISO 6579 Standard (2002) Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella spp*.
- ISO 6785 Standard (2001) Milk and Milk products - Detection of *Salmonella spp*.
- ISO 21567 Standard (2004) Microbiology of food and animal feeding stuffs.- Horizontal method for the detection of *Shigella spp* .
- MARSHALL, R.T. (1992) Standard methods for the examination of dairy products. 16th ed. APHA. Washington DC. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Urea Agar Base

Art. No. 01-261

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 5 - 18 h
Inoculum: Pure culture is inoculated by surface streaking

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good to very good	Urease (-)
Enterobacter aerogenes ATCC 13048	Good to very good	Urease (-)
Proteus mirabilis ATCC 12453	Good to very good	Urease (+)
Proteus vulgaris ATCC 6380	Good to very good	Urease (+)
Proteus mirabilis ATCC 29906	Good to very good	Urease (+)
Salmonella typhimurium ATCC 14028	Good to very good	Urease (-)
Proteus mirabilis ATCC 43071	Good to very good	Urease (+)

Escherichia coli ATCC 25922

Proteus mirabilis ATCC 43071

Urea Broth Base

Art. No. 02-202

Specification

Liquid medium for urease detection according to the Rustigian and Stuart formulation.

Formula* in g/L

Monopotassium phosphate	9,10
Disodium phosphate	9,50
Yeast extract	0,10
Phenol red	0,01

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 19 g of powder into 950 mL of distilled water and sterilize in the autoclave at 121°C for 15 minutes. Let it cool to 50-55°C and then add 50 mL of Urea Sterile Solution 40% (Art. No. 06-083). Mix well and dispense in haemolysis tubes (3,0 mL/tube).

Description

According to Rustigian and Stuart, this Urea Broth is excellent for identifying enterobacteria, since within this family, only *Proteus* may alkalinize the medium over pH 8,1. Despite the fact that some authors prefer a buffering potency 10 to 100 times lower to obtain faster results this does not compensate for the instability of the medium.

Urease production is shown by the indicator turning to dark pink, produced by strong alkalinization by ammonium. With plenty of inoculum (2-3 loops in 3-5 mL of medium), *Proteus* produces the colour change after 6-8 hours, other positive enterobacteria need up to 24-48 hours.

References

- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Rev. A. AOAC International. Gaithersburg. MD. USA.
- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos. S.A. Madrid.
- RUSTIGIAN, R. & C.A. STUART (1941) Decomposition of urea by *Proteus*. Proc. Soc. Exp. Biol. Med. 47:108.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 6 - 8 h
Inoculum: 10.000 - 100.000 CFU

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	Urease (-)
Enterobacter aerogenes ATCC 13048	Good	Urease (-)
Salmonella typhimurium ATCC 14028	Good	Urease (-)
Proteus mirabilis ATCC 43071	Good	Urease (+)
Proteus mirabilis ATCC 29906	Good	Urease (+)
Proteus vulgaris ATCC 6380	Good	Urease (+)

Left: *Proteus mirabilis* ATCC 43071
Right: Uninoculated tube (Control)

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Violet Red Bile Agar (VRB Agar)

Art. No. 01-164

Also known as

VRB Agar; VRBA; VRBL

Specification

Medium for the detection and enumeration of coliforms in milk and other dairy products, according to APHA and ICMSF, FIL-IDF and ISO standards.

Formula* in g/L

Yeast extract	3,000
Peptone	7,000
Bile salts No. 3	1,500
Lactose	10,000
Sodium chloride	5,000
Neutral red	0,030
Crystal violet	0,002
Agar	13,000

Final pH 7,4 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 39,5 g in 1 L of distilled water. Heat and boil for 1 minute and pour into plates. The medium must to be used preferably on the same day of preparation. **Do not autoclave.**

Description

The Violet Red Bile Agar corresponds to the classic formulation of standardized media for the screening of coliforms in milk and other dairy products. This medium has been adopted for the enumeration of coliforms as well as for differentiating between lactose-fermenting and non-lactose fermenting organisms, due to its contents of crystal violet and bile salts, whose inhibiting or selective properties have been widely confirmed.

Technique

The recommended procedure is inoculation directly into Petri dishes, with the molten agar cooled to 45-47°C. Plates can be read after 24 hours of incubation at 37°C.

The size of the colonies ranges from 2 to 5 mm, depending on the amount per plate. If enterococci develop they will appear small in size and pink coloured. Lactose fermenting enterobacteria acquire a dark red colour with a clearing zone around them, while lactose non-fermenting ones form colourless colonies.

References

- DOWNES, F.P. & K. ITO (2001). Compendium of Methods for the Microbiological Examination of Food. 4th ed. APHA, Washington. DC.
- FIL-IDF. (1998) Standard 73B. Enumeration of coliform bacteria. ICMSF (1978). Microorganisms in Food, University of Toronto Press.
- ISO (1986) Standard 5541-1 Milk and Milk Products. enumeration of coliforms. Colony count technique at 30ºC.
- ISO (2006) Standard 4832:2006 (E) -Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coliformes- Colony - count- technique.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA, Washington. DC.
- PASCUAL ANDERSON, Mª R. (1992) Microbiología Alimentaria. Díaz de Santos, S.A.,Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Violet Red Bile Agar (VRB Agar)

Art. No. 01-164

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Selectivity
Pseudomonas aeruginosa ATCC 27853	Poor to good	Colourless colonies w/o precipitate
Escherichia coli ATCC 8739	Productivity > 0.50	Dark violet colonies w. precipitate zone
Escherichia coli ATCC 25922	Productivity > 0.50	Dark violet colonies w. precipitate zone
Salmonella abony NCTC 6017	Productivity > 0.50	Colourless colonies w/o precipitate
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Colourless colonies w/o precipitate

Escherichia coli ATCC 25922

Escherichia coli ATCC 25922
"Detail"

Salmonella typhimurium ATCC 14028

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Violet Red Bile Dextrose Agar (VRBD Agar) (Eur. Pharm.)

Art. No. 01-295

Also known as

MacConkey Dextrose Agar; VRBG

Specification

Selective solid medium for the enumeration of enterobacteria, acccording to ISO standard 21528 and Pharmacopeial Harmonised Methods.

Formula* in g/L

Yeast extract	3,000
Peptone	7,000
Bile salts	1,500
D(+)-Glucose	10,000
Sodium chloride	5,000
Neutral red	0,030
Crystal violet	0,002
Agar	13,000

Final pH 7,4 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 39,5 g in 1 L of distilled water and let it soak. Bring to the boil with constant stirring do not overheat. Distribute in suitable containers. Prolonged heating in a water bath could cause slight precipitates. Do not autoclave.

Description

This medium is a modification of the Violet Red Bile Agar (Art. No. 01-164) and the MacConkey Agar (Art. No. 01-118) as described by Mossel *et al.* The addition of glucose to the Violet Red Bile Agar enhances both the growth of the most fastidious enterobacteria and the recovery of those having suffered from adverse conditions. Mossel himself realized that by removing the lactose and keeping the glucose, the medium's efficiency remained stable.

This medium can be used as a presumptive medium for *E. coli* (by fluorescent reaction) if before sterilization MUG (Art. No. 06-102CASE) is added.

Technique

Violet Red Bile Dextrose Agar is widely used in the analysis of food, medicines and cosmetics. It is particularly indicated for the recovery of bacteria which have been damaged during preparation. In such cases, a progressive enrichment is recommended in TSB (Art. No. 02-200) and subsequently in EE Broth (Art. No. 02-064). The enriched culture can be inoculated in tubes or on Violet Red Bile Dextrose Agar plates. For a count of enterobacteria, follow the technique described for Violet Red Bile Agar.

Results can be read after 24 hours of incubation at 35°C ± 2,0. Enterobacterial colonies are an intense purple colour surrounded by a clearer zone. If enterococci colonies eventually develop, they will be small and pink coloured.

References

- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- ISO Norma 21528-1:2004. Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 1: Detection and enumeration by MPN technique with pre-enrichment.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- PASCUAL ANDERSON, Mª R. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Violet Red Bile Dextrose Agar (VRBD Agar) (Eur. Pharm.)

Art. No. 01-295

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 6538	Total inhibition	-
Pseudomonas aeruginosa ATCC 9027	Productivity > 0.50	Dark violet colonies w. precipitate zone
Escherichia coli ATCC 8739	Productivity > 0.50	Dark violet colonies w. precipitate zone
Escherichia coli ATCC 25922	Productivity > 0.50	Dark violet colonies w. precipitate zone
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Dark violet colonies w. precipitate zone
Salmonella abony NCTC 6017	Productivity > 0.50	Dark violet colonies w. precipitate zone

Escherichia coli ATCC 25922

Salmonella typhimurium ATCC 14028
"Detail"

Salmonella typhimurium ATCC 14028

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Violet Red Bile Lactose Dextrose Agar

Art. No. 01-220

Also known as

VRBLD Agar; VRBLDA Medium; Eur Pharm. Agar Medium F

Specification

Solid selective medium for the detection of Enterobacteriaceae according to the European Pharmacopoeia.

Formula* in g/L

Yeast extract	3,000
Peptone	7,000
Sodium chloride	5,000
Bile salts No. 3	1,500
Lactose monohydrate	10,000
Dextrose monohydrate	10,000
Neutral red	0,030
Crystal violet	0,002
Agar	15,000

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 51,5 g of powder in 1 L of distilled water and heat to the boil. Pour into Petri dishes immediately. **Do not sterilize in the autoclave nor overheat**.

Description

This medium developed in 1962 by Mossel et al. is more effective than MacConkey Agar for the detection of Enterobacteriaceae in foods. The formulation has been officially adopted by the European Pharmacopoeia 5.0 for the microbiological examination of non-sterile products. The medium is especially used in the recovery of process stressed bacteria using a progressive enrichment technique.

This medium can be used as presumptive medium for *E. coli* (by fluorescent reaction) if before sterilization MUG (Art. No. 06-102CASE or 06-102-LYO) is added.

Technique

The product sample is diluted 1:10 in Lactose Broth (Art. No. 02-105) and incubated for 2-5 hours at 35-37°C. The pre-enrichment is then diluted ten fold in EE Broth (Art. No. 02-064) and incubated at 35-37°C for 18-24 hours. From this enrichment the surface of several plates of VRBDL Agar are inoculated. The product passes the test if after 18-24 hours of incubation at 35-37°C there is no growth of Gram negative bacteria.

The Enterobacteriaceae colonies are deep purple in colour surrounded by a clearing zone. Sometimes colonies of *Pseudmonas* or Aeromonas are present, these can be easy differentiated using the oxidase test.

References

- EUROPEAN PHARMACOPOEIA (2005) § 2.6.13 Microbiological examination of non-sterile products. Tests for specified organisms. EDQM. Council of Europe. Strasbourg.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- MOSSEL, D.A.A., M. VISER y A.M.R. CORNELISSEN (1963) The examination of foods for Enterobacteriaceae using a test of the type generally adopted for the detection of salmonellae J. Appl. Bact. 26:444-452.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Violet Red Bile Lactose Dextrose Agar

Art. No. 01-220

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Selectivity
Pseudomonas aeruginosa ATCC 27853	Good	-
Escherichia coli ATCC 25922	Productivity > 0.50	Dark violet colonies, with a precipitate
Escherichia coli ATCC 8739	Productivity > 0.50	Dark violet colonies, with a precipitate
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Dark violet colonies, with a precipitate
Salmonella abony NCTC 6017	Productivity > 0.50	Dark violet colonies, with a precipitate

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Vogel Johnson Agar (VJ Agar)

Art. No. 01-206

Also known as

Tellurite-Glycine-Phenol Red Agar Base

Specification

Solid and selective medium for isolation and identification of staphylococci according to ISO standard 22718.

Formula* in g/L

Casein peptone	10,000
Yeast extract	5,000
Mannitol	10,000
Dipotassium phosphate	5,000
Lithium chloride	5,000
Glycine	10,000
Phenol red	0,025
Agar	15,000

Final pH 7,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 60 g of powder in 1 L of distilled water and bring to the boil. Dispense in suitable containers and sterilize at 121°C for 15 minutes. Cool it to 50°C approx. and add aseptically 20 mL of Potassium Tellurite Solution 1% (Art. No. 06-089) or 6,0 mL of Potassium Tellurite Solution 3.5% (Art. No. 06-011). **Do not reheat** after tellurite addition.

Description

VJ Agar is a selective medium for detection and enumeration of pathogenic staphylococci.The medium's strong selective action is due to lithium chloride, glycine and potassium tellurite. They inhibit almost all accompanying organisms, while staphylococci are not affected. Staphylococci reduce tellurite to tellurium, producing black colonies. For pathogenic Staphylococci a high correlation between tellurite reduction and mannitol fermentation has been proven, and this is shown in the medium by the indicator turning to yellow due to the amount of acid produced.

The medium's selectivity avoids, in the first 24 hours, the development of any other bacteria, so heavy inoculation is permitted. After this period, it is possible that other bacteria may appear like micrococci, which produce tiny colonies, and staphylococci can ferment mannitol, therefore it is recommended to verify their identification separately.

Due to reduced tellurite, staphylococci generally appear as black colonies over red medium (if they do not ferment mannitol) or yellow medium (if they do, and these are a presumptive pathogen). Saprophytic staphylococci (*S. epidermidis, S. saprophiticus* and *S. intermedius*) have a grey-black colour and are mannitol negative. Complete medium may be stored up to 1 week in the refrigerator. Do not re-melt it after tellurite is added.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. Md.
- ISO Norma 22718 (2006) Cosmetics. Detection of *Staphylococcus aureus.*
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- USP (2002) 25th ed. <61> Microbial Limit Tests. US Pharmacopeial Convention Inc. Rockville. Md.
- VOGEL, R.A. y M. JOHNSON (1960) A modification of the tellurite-glycine medium for the use in the identification of *Staphylococcus aureus*. Pub. Health. Lab. 18:131-133.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Vogel Johnson Agar (VJ Agar)

Art. No. 01-206

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Inhibited	Selectivity
Bacillus subtilis ATCC 6633	Inhibited	Selectivity
Staphylococcus epidermidis ATCC 12228	Fair to good	Brownish. Puntiform colonies
Staphylococcus aureus ATCC 6538	Productivity 0.50	Black colonies; Yellow medium
Staphylococcus aureus ATCC 25923	Productivity > 0.50	Black colonies; Yellow medium

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WL Nutrient Agar

Art. No. 01-210

Specification

Solid medium for the culture and enumeration of yeast and bacteria for microbiological control in brewing and other fermentation industries.

Formula* in g/L

Yeast extract	4,0000
Tryptone	5,0000
Dextrose	50,0000
Monopotassium phosphate	0,5500
Magnesium sulfate	0,1250
Calcium chloride	0,1250
Potassium chloride	0,4250
Iron (III) chloride	0,0025
Manganese sulfate	0,0025
Bromocresol green	0,0220
Agar	20,0000

Final pH 5,5 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 80 g of the powder in 1 L of distilled water. Mix thoroughly. Heat with frequent agitation and boil. If a final pH of 6,5 is desired, the pH may be adjusted with one percent aqueous sodium carbonate, using approx. 30 mL per litre of medium.

Dispense and sterilize the medium in the autoclave at 121°C for 15 minutes.

Note: The WL Differential Agar has the same formula as the WL Nutrient Agar with the addition of 2 vials/L of Cycloheximide Selective Supplement (Art. No. 06-022CASE or 06-022-LYO).

Description

WL Nutrient Agar was formulated by Green and Gray in the Wallerstein Laboratory for use in the control of industrial fermentations, particularly the processing of beer. It is recommended for examination of worts, beers, liquids containing yeast and other materials.

WL Nutrient Agar has a pH of 5,5 which is optimal for the enumeration of brewers yeast . If bakers or distillers yeast is to be examined, the pH should be adjusted to 6,5. When cultivating the microorganisms from an alcoholic mash, tomato juice should be added to the medium.

WL Differential Agar contains cycloheximide to suppress yeast and any other moulds, which may be present; this medium allows reliable counting of all bacteria which may be encountered in brewery laboratories.

Technique

Dilute the sample material and spread 0,1 mL onto a WL Nutrient Agar plate and 2 WL Differential Agar plates.

The WL Nutrient Agar plate is incubated aerobically to obtain a total count, mainly of yeast colonies. One WL Differential Agar plate is incubated aerobically for growth of acetic acid bacteria, *Flavobacterium*, *Proteus*, and other organisms; the second plate is incubated anaerobically for detection of such organisms as lactic acid bacilli and *Pediococcus* species.

Plates prepared with both the media are generally incubated at 25°C, if brewing materials are being studied, and at 30°C for bakers yeast and alcohol mash samples. Incubation may be continued for a week, or ten days to two weeks, depending upon the microbiota present. Counts can be made at intervals during the incubation period.

Necessary supplements

Cycloheximide Selective Supplement (Art. No. 06-022CASE/06-022-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Cycloheximide	2,00 mg
Sodium chloride (excipient)	100,00 mg

Distilled water (Solvent)

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
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WL Nutrient Agar

Art. No. 01-210

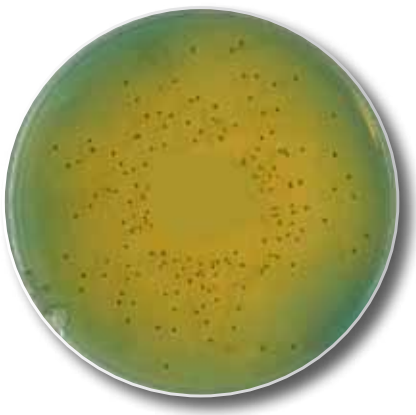
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Quality control

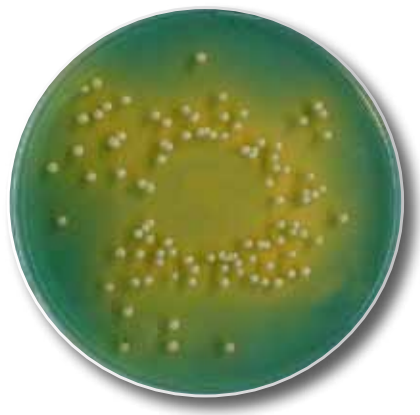
Incubation temperature: 30°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	Green colonies. Yellowish medium
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	White colonies. Yellowish medium
Lactobacillus fermentum ATCC 9338	Productivity > 0.70	Green colonies. Yellowish medium
Candida albicans ATCC 2091	Productivity > 0.70	White colonies. Yellowish medium
Candida albicans ATCC 10231	Productivity > 0.70	White colonies. Yellowish medium



Lactobacillus fermentum ATCC 9338

Uninoculated plate (Control)



Candida albicans ATCC 10231

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Wort Agar

Art. No. 01-132

Specification

General solid medium for the cultivation of fungi.

Formula* in g/L

Malt extract	15,00
Casein peptone	0,75
Maltose	12,75
Dextrine	2,75
Dipotassium hydrogen phosphate	1,00
Ammonium chloride	1,00
Agar	17,00

Final pH 4,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 50,25 g of powder in 1 L of distilled water and add 2-3 mL of glycerol and bring to the boil to dissolve completely. Distribute into final containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat.** Prolonged heating will diminish the gelling strength of the medium.

Description

Wort Agar is used for the cultivation, isolation and enumeration of yeast and moulds.It is particularly well adapted for counting osmophilic yeast in butter, sugar and syrups, in lemonade and more generally in sweet or soft drinks.

For a more selective utilization it is possible to adjust the pH to 4,5 or 3,5 but this acidification can inhibit the agar solidification. In order to disminish this effect it is advisable to supplement the medium with 10 g/L Bacteriological Agar (Art. No. 07-004). Never heat the medium after adding acid, in order to prevent the loss of solidifying properties of the agar. The acid pH inhibits the growth of bacteria and favours that of yeast.

Technique

A decimal dilution series is performed from the original simple. Aliquots of 1 mL pf each dilution are deposited in sterile Petri dishes. The medium melted and cooled to 45-50°C is poured into the dishes and the mixture is homogenized and allowed to set. Read the plates after incubation for 5 days at 25°C.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
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- PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- RAPP, M. (1974) Indikator-zusätze zur Keimdifferenzierung auf Würze und Malzextrakt-Agar. Milchwiss. 29:341-344.
- SCARR (1959) Selective media used in the microbiological examination of sugar products. J. Sci. Food Agric. 10:678-681.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Wort Agar

Art. No. 01-132

Quality control

Incubation temperature: 25°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Candida albicans ATCC 10231	Productivity > 0.70	-
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Penicillium aurantiogriseum ATCC 16025	Productivity > 0.70	-
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	-

Aspergillus brasiliensis ATCC 16404

Saccharomyces cerevisiae ATCC 9763

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Wort Broth

Art. No. 02-132

Specification

Liquid medium for the production of yeasts suspensions.

Formula* in g/L

Malt extract	15,00
Casein peptone	1,00
Maltose	12,50
Dextrine	2,50
Dipotassium phosphate	1,00
Ammonium chloride	1,00

Final pH 4,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 33 g of powder in 1 L of distilled water and add 2-3 mL of glycerol and bring to the boil to dissolve completely. Distribute into final containers and sterilize in the autoclave at 121°C for 15 minutes. **Do not overheat.**

Description

This Broth is the liquid version of the classical Wort Agar (Art. No. 01-132). It is especially designed to propagate yeasts, and often has been employed as a semi-selective or enrichment medium, due to its high acidity, which makes it inhibitory for bacteria. This effect may be enhanced by adding, before sterilization, 10 mL/L of a 10% solution of lactic or tartaric acid. To avoid precipitate it is recommended to sterilize by filtration.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- SCARR (1959) Selective media used in the microbiological examination of sugar products. J. Sci. Food Agric. 10:678-681.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 25 - 30°C
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (Productivity) (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Candida albicans ATCC 10231	Good	-
Saccharomyces cerevisiae ATCC 9763	Good	-
Aspergillus brasiliensis ATCC 16404	Good	-
Penicillium aurantiogriseum ATCC 16025	Good	-

Xylose Lysine Deoxycholate Agar (Eur. Pharm.)

Art. No. 01-211

Also known as

XLD Agar

Specification

Solid medium for the isolation of enteropathogenic species, especially *Salmonella* according to Pharmacopeial Harmonised Method and ISO Standard 6340.

Formula* in g/L

Xylose	3,50
L-Lysine	5,00
Lactose	7,50
Sucrose	7,50
Sodium chloride	5,00
Yeast extract	3,00
Phenol red	0,08
Sodium deoxycholate	2,50
Sodium thiosulfate	6,80
Ammonium ferric citrate	0,80
Agar	15,00

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 56,68 g of powder in 1 L of distilled water. Heat with constant stirring until boiling. Pour immediately into plates. **Do not autoclave and avoid remelting**.

Description

Xylose Lysine Deoxycholate Agar is a selective differential medium, suitable for the detection of pathogenic enterobacteria, especially *Shigella*. Gram negative microbiota are inhibited by the low amount of deoxycholate, whilst *Shigella* grows.

Xylose, lactose or sucrose fermentation produces the acidification of the medium, and this is seen by the indicator turning yellow, surrounding the colonies. This colour disappears after 24 hours, so observations must be carried out between 18 and 24 hours.

Hydrogen sulfide production from thiosulfate is easily detected because colonies become darker, due to the ferric sulfide precipitate. Lysine decarboxylation to cadaverine may also be observed in the medium, since it produces alkalinization and consequently the indicator turns to red.

All these reactions allow a good differentiation of *Shigella*. *Edwardsiella* and *Proteus inconstans* are the only enterobacteria other than *Shigella* which do not ferment xylose and therefore show negative fermentation reaction. *Salmonella* ferment xylose, but it is consumed quickly and alkalinization of the medium due to lysine decarboxylation, may mask the reaction. *Salmonella* colonies become darker due to ferrous sulfide precipitates, which is also a common property with *Edwardsiella*.

Other types of enterobacteria do not suffer this phenomenon, since acid accumulation due to lactose and sucrose fermentation is so high that it avoids pH reversion by decarboxylation and even ferrous sulfide precipitate in the first 24 hours.

In the table on the next page, typical colonial appearances on XLD medium after 24-36 hours of incubation at 37°C are described.

References

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- TAYLOR, W.J. (1965) Isolation of *Shigella*. I. Xylose Lysine Agars: New media for isolation of enteric pathogens. Am. J. Clin. Path 44:471-475.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Xylose Lysine Deoxycholate Agar (Eur. Pharm.)

Art. No. 01-211

COLONIAL APPEARANCE	MICROORGANISM
Transparent red colonies	*Shigella sp., Proteus incontans, Salmonella paratyphi A.*, sometimes *S.cholerasuis* and *S. pullorum*
Transparent red colonies with black nucleus	*Edwarsiella* and most species of *Salmonella*
Orange and slightly opaque colonies	*Salmonella typhi*
Colonies red, translucent without zone	Pseudomonas, *Proteus rettgeri*
Yellow opaque colonies	*Escherichia* (when growth) *Enterobacter,* Aeromonas, *Citrobacter*.
Yellow, mucous, opaque and black-nucleated colonies.	*Klebsiella, Citrobacter intermedius* (when growth)
Yellow, transparent colonies with black nucleus	Most strains of *Proteus mirabilis, P. vulgaris*.
Yellow opaque colonies without zone	*Serratia, Hafnia*.

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Inhibited	Selectivity
Escherichia coli ATCC 8739	Partial Inhibition	Selectivity
Proteus mirabilis ATCC 43071	Productivity > 0.50	Colourless colonies w. black centre (H_2S +)
Salmonella abony NCTC6017	Productivity > 0.50	Colourless colonies w. black centre (H_2S +)
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Colourless colonies w. black centre (H_2S +)
Salmonella enteritidis ATCC 13076	Productivity > 0.50	Colourless colonies w. black centre (H_2S +)
Shigella flexneri ATCC 12022	Productivity > 0.50	Colourless colonies w. transparent centre (H_2S -)

Salmonella typhimurium ATCC 14028

Uninoculate plate (Control)

Shigella flexneri ATCC 12022

Xylose Lysine Deoxycholate Modified Agar

Art. No. 01-552

Specification

Medium for isolation of enteropathogenic species, especially *Shigella* and *Salmonella* in food and animal feeding stuffs, according to ISO standards.

Formula* in g/L

Xylose	3,75
L-Lysine	5,00
Lactose	7,50
Sucrose	7,50
Sodium chloride	5,00
Yeast extract	3,00
Phenol red	0,08
Sodium deoxycholate	1,00
Sodium thiosulfate	6,80
Ammonium ferric citrate	0,80
Agar	15,00

Final pH 7,4 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 55,43 g of powder in 1 L of distilled water. Heat with constant stirring until boiling. Pour immediately into plates. **Do not sterilize and avoid remelting.**

Description

Xylose Lysine Deoxycholate Agar is a selective differential medium, suitable for the detection of pathogenic enterobacteria in food, especially *Shigella*. A modification in the original formulation of Taylor allows the medium to perform to the specifications of the ISO standards. Gram negative microbiota are inhibited by the low amount of deoxycholate, whilst *Shigella* grows. Xylose, lactose or sucrose fermentation produce acidification of the medium which is shown by the indicator surrounding the colonies turning yellow. This colour disappears after 24 hours, so readings must be carried out between 18 and 24 hours.

Sulfide production from thiosulfate is easily detected because colonies become darker, due to the ferric sulfide precipitate. Lysine decarboxylation to cadaverine may also be observed in the medium, since it produces alkalinization and consequently the indicator turns red.

All these reactions allow a good differentiation of *Shigella*, which other than *Edwarsiella* and *Proteus inconstans* are the only enterobacteria that do not ferment xylose and therefore show a negative fermentation reaction. *Salmonella* does ferment xylose, but it is consumed quickly and the medium becomes alkaline due to lysine decarboxylation, which may hide the reaction. The difference between *Shigella* and *Salmonella* is that the latter colonies become darker due to ferrous sulfide precipitates, which is also a common characteristic of *Edwarsiella*. Other types of enterobacteria do not suffer this phenomenon, since acid accumulation due to lactose and sucrose fermentation is so great that it avoids pH reversion by decarboxylation and even ferrous sulfide precipitate in the first 24 hours.

In the table on the next page, typical colonial appearances on XLD Agar after 24-36 hours of incubation at 37°C are described.

References

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- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
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- ISO 6579:2002 Standard. Microbiology of Foods and animal feeding stuffs. Horizontal method for the detection of *Salmonella spp*.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ISO 21567 Standard (2004) Microbiology of food and animal feeding stuffs.- Horizontal method for the detection of *Shigella spp* .
- PASCUAL ANDERSON, MªR. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- TAYLOR, W.J. (1965) Isolation of *Shigella*. I. Xylose Lysine Agars: New media for isolation of enteric pathogens. Am. J. Clin. Path 44:471-475.
- US FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual 8th ed. AOAC Internacional. Gaithersburg. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

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Xylose Lysine Deoxycholate Modified Agar

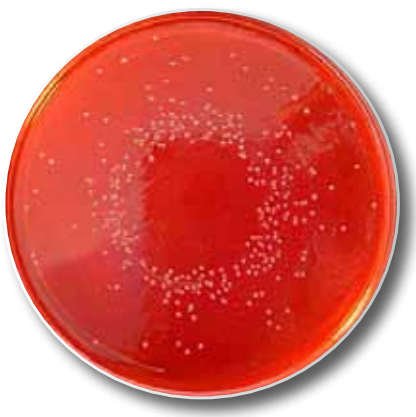
Art. No. 01-552

COLONIAL APPEARANCE	MICROORGANISM
Transparent red colonies	*Shigella sp., Proteus incontans, Salmonella paratyphi A.*, sometimes *S.cholerasuis* and *S. pullorum*
Transparent red colonies with black nucleus	*Edwarsiella* and most species of *Salmonella*
Orange and slightly opaque colonies	*Salmonella typhi*
Colonies red, translucent without zone	Pseudomonas, *Proteus rettgeri*
Yellow opaque colonies	*Escherichia* (when growth) *Enterobacter,* Aeromonas, *Citrobacter*.
Yellow, mucous, opaque and black-nucleated colonies	*Klebsiella, Citrobacter intermedius* (when growth)
Yellow, transparent colonies with black nucleus	Most strains of *Proteus mirabilis, P. vulgaris*.
Yellow opaque colonies without zone	*Serratia, Hafnia*.

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Total inhibition	-
Escherichia coli ATCC 25922	Partial inhibition	-
Salmonella abony NCTC 6017	Productivity > 0.50	Colourless colonies / Black centre (H_2S+)
Salmonella typhimurium ATCC 14028	Productivity > 0.50	Colourless colonies / Black centre (H_2S+)
Salmonella enteritidis ATCC 13076	Productivity > 0.50	Colourless colonies / Black centre (H_2S+)
Shigella flexneri ATCC 12022	Productivity > 0.50	Colourless colonies



Shigella flexneri ATCC 12022

Enterococcus faecalis ATCC 29212

Salmonella typhimurium ATCC 14028
24 h-48 h

Yeasts Extract Peptone Dextrose Broth (YEPD)

Art. No. 02-473

Also known as

YEPD Broth

Specification

Liquid medium for the cultivation of yeast in molecular biology procedures.

Formula* in g/L

Peptone	20,00
Yeast extract	10,00
Dextrose	20,00

Final pH 6,8 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 50 g of powder in 1 L of distilled water, heating if necessary. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This media supports the growth of most heterotrophic microorganisms,. Its simple composition has been adopted as the basal media for the routine cultivation of yeasts for molecular biology studies.

References

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	24 h
Saccharomyces cerevisiae ATCC 9763	Good	-
Candida albicans ATCC 10231	Good	-
Aspergillus brasiliensis ATCC 16404	Good	Black sporulation at 5 days

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Yeast Malt Agar

Art. No. 01-219

Also known as

YMA; YM Agar

Specification

Solid medium for the cultivation of fungi and actinomycetes.

Formula* in g/L

Dextrose	10,00
Peptone	5,00
Malt extract	3,00
Yeast extract	3,00
Agar	20,00

Final pH 6,2 ± 0,2 at 25ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 41 g of powder in 1 L of distilled water and let it soak. Bring to the boil and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

This is a classical culture medium for the cultivation of moulds, yeasts and acidophilic actinomycetes. The medium may be made selective to one or other group of microorganisms by adding appropriate antibiotics when the medium is at 50°C.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. Londres.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SAMSON, R.A., E.S. HOEKSTRA, J.C. FRISVAD y O. FILTENBORG (2002) Introduction to food and airborne fungi. 6th ed. CBS. Utrech. Holanda.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Good	48 h
Escherichia coli ATCC 8739	Good	48 h
Aspergillus brasiliensis ATCC 16404	Productivity > 0.70	Black sporulation (5 days)
Saccharomyces cerevisiae ATCC 9763	Productivity > 0.70	-
Candida albicans ATCC 10231	Productivity > 0.70	-
Penicillium aurantiogriseum ATCC 16025	Good	-

Yeast Malt Broth

Art. No. 02-219

Also known as

YM Broth

Specification

Liquid medium for the cultivation of fungi and actinomycetes.

Formula* in g/L

Dextrose	10,00
Peptone	5,00
Malt extract	3,00
Yeast extract	3,00

Final pH 6,2 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 21 g of powder in 1 L of distilled water. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

This is a classical culture medium for the cultivation of moulds, yeasts and acidophilic actinomycetes. The medium may be made selective to one or other group of microorganisms by adding appropriate antibiotics when the medium is at 50°C.

References

- ATLAS, R.M. & L.C. PARK (1993) Handbook of Microbiological Media for the Examination of Food. CRC Press Inc. .London.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 20-25°C ± 2,0
Incubation time: 48 h - 5 days
Inoculum: 10-100 CFU (according to standard ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Penicillium aurantiogriseum ATCC 16025	Good	5 days, yellow-green sporulation
Trichophyton rubrum ATCC 28188	Good	5 days, white-pink sporulation
Aspergillus brasiliensis ATCC 16404	Good	5 days, black sporulation
Candida albicans ATCC 10231	Good	-
Saccharomyces cerevisiae ATCC 9763	Good	-

Yeast Starch Glucose Agar

Art. No. 01-673

Also known as

YSG Agar

Specification

Solid medium for the detection and isolation of *Alicyclobacillus*, in fruit juices and other acidic food, according to IFU standard Method No. 12.

Formula* in g/L

Yeast extract	2,00
Dextrose	1,00
Soluble starch	2,00
Agar	20,00

Final pH 3,7 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 25 g of the powder in 1 L of distilled water and bring to the boil to dissolve. Distribute it in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C and adjust the pH to 3,7 ± 0,2 by adding 1N HCl. Mix well to homogenize and pour into sterile Petri dishes. **Avoid heating or remelting the medium after the pH adjustment.**

Description

Alicyclobacillus have emerged as food spoilage organisms of major significance to the fruit juice industry (Baumgart & Menje, 2000). Spoilage is generally manifested as the formation of off flavours and odours from compounds such as guaiacol and the halogenated phenols. The economic impact of such incidents can be very high, to date, no human risk are known to be associated with the consumption of juices and other food products containing *Alicyclobacillus* bacteria.

An acidified environment is required to grow alicyclobacilli and YSG media supports the growth of all currently known species of *Alicyclobacillus* (*A. acidocaldarius, A. acidoterrestris, A. cycloheptanicus* and *A. hesperidium*). The media complies the Standard IFU Method for the detection of taint producing organisms in fruit juices.

The low pH-value of the media, in combination with the high incubation temperature inhibits the contaminating microbiota. K Agar (Art. No. 01-674) when incubated at 45°C supports the growth of predominantly *A. acidoterrestris* and limited growth of other species of the genus. Therefore, K Agar (Art. No. 01-674) is used to detect predominantly *A. acidoterrestris* strains.

Technique

The IFU Standard provides three methods of detection depending on the sample composition and the time since processing:

1. Raw materials (including process water): A heat shock treatment is prescribed followed by direct plating (optional), filtration or enrichment in liquid medium.
2. Final products sampled directly after (heat) processing where an additional heat shock is unnecessary: Pre-incubation of the sample in liquid medium is prescribed.
3. Final products taken from the market: Pre-incubation of the sample, as per the heat shock treatment. If spoilage is suspected and no alicyclobacilli detected after direct plating, a heath shock and enrichment is recommended.

In all methodology incubation for 3-5 days at 45 ± 1°C is prescribed. Count all colonies growing on the YSG Agar as presumptive alicyclobacilli. Confirm the suspicious colonies by further testing.

References

- BAUMGART, J. (2003) Media for detection and enumeration of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* in foods. In Handbook of Culture Media for Food Microbiology. J.E.L. Corry *et al.* (Eds.) Elsevier Sci B.V. Amsterdam.
- BAUMGART, J. & S. MENJE (2000) The impact of *Alicyclobacillus acidoterrestris* on the quality of juices and soft drinks. Fruit Processing 7:251-254.
- CERNY, G., W. HENNLICH & K. PORALLA (1984) Fruchsaftverdeb durch Bazillen: Isolierung und Charakterisierung des Verdebserregers. Z. Lebens. Unter Forsch. 179:224-227.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
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- IFU STANDARDS (2004) Method No. 12 on the detection of taint producing *Alicyclobacillus* in fruit juices. Revision march 2007.
- WITTHUHN, R.C., W. DUVENAGE & P.A. GOUWS (2007) Evaluation of different growth media for the recovery of the species of *Alicyclobacillus*. Letters Appl. Microbiol. 45:224-229.

Storage

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Yeast Starch Glucose Agar

Art. No. 01-673

Quality control

Incubation temperature: 45°C ± 2,0
Incubation time: 72 h - 5 days
Inoculum: 10-100 CFU. Spiral Plate Method (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Alicyclobacillus acidoterrestris ATCC 49025	Productivity > 0.70	-
Alicyclobacillus acidocalcarius ATCC 27009	Productivity > 0.70	-
Escherichia coli ATCC 25922	Inhibited	-

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Yeast Starch Glucose Broth

Art. No. 02-673

Also known as

YSG Broth

Specification

Liquid medium used for the detection and enrichment of *Alicyclobacillus*, in fruit juices and other acidic foods, according to IFU standard Method No 12.

Formula* in g/L

Yeast extract	2,00
Dextrose	1,00
Soluble starch	2,00

Final pH 3,7 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 5 g in 1 L of distilled water. Gently heat it to dissolve. Adjust the pH to 3,7 ± 0,2 by adding 1N HCl Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Alicyclobacillus has emerged as a food spoiling microorganism of major significance in the fruit juice industry (Baumgart & Menje, 2000). Spoilage is generally manifested as the formation of off flavours and odours from compounds such as guaiacol and the halogenated phenols. The economic impact of spoilage can be very high, to date, no human risk has been associated with the consumption of such juices and other food products containing *Alicyclobacillus* bacteria.

An acidic environment is required to grow alicyclobacilli and YSG media supports the growth of all currently known species of *Alicyclobacillus* (*A. acidocaldarius, A. acidoterrestris, A. cycloheptanicus* and *A. hesperidium*). The media complies the Standard IFU Method for the detection of taint producers in fruit juices.

The low pH-value of the media, in combination with the high incubation temperature inhibits the growth of contaminating microbiota. K Agar (Art. No. 01-674) when incubated at 45°C predominantly supports the growth of *A. acidoterrestris* and limited growth of other species of the same genus. Therefore, K Agar (Art. No. 01-674) is used to detect predominantly *A. acidoterrestris* strains.

Technique

The IFU Standard provides three methods of detection depending on the sample composition and the elapsed time since processing:

1. Raw materials (including processed water): A heat shock treatment is prescribed followed by direct plating (optional), filtration or enrichment in liquid medium.
2. Final products sampled directly after (heat) processing where an additional heat shock is unnecessary: Pre-incubation of the sample in liquid medium is prescribed.
3. Final products taken from the market: Pre-incubation of the sample, as per the heat shock treatment. If spoilage is suspected and no alicyclobacilli detected after direct plating, a heath shock and enrichment is recommended.

In all methods, incubation for 2-4 days at 45 ± 1°C is prescribed for the enrichment step.

References

- BAUMGART, J. (2003) Media for detection and enumeration of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* in foods. In "Handbook of Culture media for food Microbiology". J.E.L. Corry et al. (Eds.) Elsevier Sci B.V. Amsterdam.
- BAUMGART, J. & S. MENJE (2000) The impact of *Alicyclobacillus acidoterrestris* on the quality of juices and soft drinks. Fruit Processing 7:251-254.
- CERNY, G., W. HENNLICH & K. PORALLA (1984) Fruchtsaftverderb durch Bazillen: Isolierung und Charakterisierung des Verderberregers. Z. Lebens. Unter Forsch. 179:224-227.
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- IFU Standard (2004) Method No. 12 on the detection of taint producing *Alicyclobacillus* in fruit juices. Revision march 2007.
- WITTHUHN, R.C., W. DUVENAGE & P.A. GOUWS (2007) Evaluation of different growth media for the recovery of the species of *Alicyclobacillus*. Letters Appl. Microbiol. 45:224-229.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Yeast Starch Glucose Broth

Art. No. 02-673

Quality control

Incubation temperature: 45°C ± 2,0
Incubation time: 72 h - 5 days
Inoculum: 10-100 CFU (according to standard ISO/TR 11133-1:2000 and ISO/TS 11133-2:2003)

Microorganism	Growth	Remarks
Alicyclobacillus acidoterrestris ATCC 49025	Good	-
Alicyclobacillus acidocalcarius ATCC 27009	Good	-
Escherichia coli ATCC 25922	Inhibited	-

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Yersinia CIN Agar Base

Art. No. 01-444

Also known as

CIN Agar; *Yersinia* Selective Agar, Cefsulodin-Irgasan®-Novobiocin Agar Base

Specification

Solid differential medium used for the selective isolation of *Yersinia spp.* from highly polluted samples, according to ISO 10273 standard.

Formula* in g/L

Special peptone	20,000
Yeast extract	2,000
Mannitol	20,000
Sodium pyruvate	2,000
Sodium chloride	1,000
Sodium deoxycholate	0,500
Magnesium sulfate	0,010
Neutral red	0,030
Crystal violet	0,001
Agar	15,000

Final pH 7,4 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 30,25 g in 500 mL of distilled water and bring to the boil. Sterilize in the autoclave at 121°C for 15 minutes. Let it cool to 50-55°C and, aseptically, add the content of a vial of *Yersinia* Selective Supplement, (Art. No. 06-143-LYO). Homogenize and pour into plates.

Description

Cefsuloidin-Irgasan™-Novobiocin Agar CIN Agar was originally formulated by Schiemann (1979) for detection of *Yersinia enterocolitica*. He subsequently (1982) revised it by substituting sodium deoxycholate for bile salts and reducing the novobiocin content. It relies on the use of selective inhibitory components sodium deoxycholate, crystal violet, cefsulodin, Irgasan® and novobiocin. The basic principle involved is fermentation of mannitol with localised pH reduction which forms a red colony due to the neutral red and a zone of precipitation due to the deoxycholate.

The characteristic appearance of *Yersinia spp*. colonies after an incubation of 18-24 hours at 30°C or 48 hours at 22°C on CIN Agar in air, are round, pink, about 2 mm in diameter with a dark pink centre and surrounded with a precipitation zone. Confirmatory tests are required.

Typical colonies of *Yersinia enterocolitica* will develop as a red bull's-eye surrounded by a transparent border, but will vary considerably among serotypes in colony size, smoothness and the ratio of the border to centre diameter. Most other organisms that are capable of growing on this medium produce larger colonies (> 2 mm in diameter) with diffuse pinkish centres and opaque outer zones. Some strains of *Serratia*, *Citrobacter* and *Enterobacter* on CIN Agar may give a colonial morphology resembling *Yersinia enterocolitica*.

These organisms can be differentiated by simple biochemical tests.

Technique

At present no single isolation procedure is available for the recovery of all pathogenic strains of *Yersinia enterocolitica*. The isolation procedure used will depend on the bio/serogroups of *Yersinia spp.* sought and on the type of sample to be examined. The ISO method for the detection of presumptive pathogenic *Yersinia enterocolitica* includes the parallel use of two isolation procedures:

1. Enrichment in Peptone, Sorbitol and Bile Salts (PSB) Broth for 2-3 days at 22-25°C with agitation or 5 days without agitation; plating on CIN Agar directly and after alkaline treatment and incubation for 24 hours at 30°C.

2. Enrichment in ITC (Irgasan®-Ticarcillin-Chlorate) Broth for 2 days at 24°C; plating on SSDC (*Salmonella-Shigella*-Deoxycholate-Calcium Chloride) Agar and incubation for 2 days at 30°C.

Necessary supplements

Yersinia Selective Supplement (Art. No. 06-143-LYO)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Cefsulodin	7,50 mg
Irgasan®	2,00 mg
Novobiocin	1,25 mg

Distilled water (Solvent)

References

- ATLAS, R.M. & J.W. SNYDER (1995) Handbook of Media for Clinical Microbiology. CRC Press. Boca Raton. Fla. USA.
- BAYLIS, C.L. (Ed.) (2007) Manual of Microbiological Methods for the Food and Drinks Industry. 5th ed. Guideline No. 43, Campden & Chorleywood Food Research Association. (CCFRA). U.K.
- CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD (2003) Handbook of Culture Media for Food Microbiology. Progress in Industrial Microbiology, vol. 37. Elsevier Science Amsterdam.
- De BOER, E. (2003) Isolation of *Yersinia enterocolitica* from foods in "Handbook of Culture Media for Food Microbiology". J.E.L. Corry *et al.* (Eds.) Elsevier Sci. B.V.
- FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed, revision A. AOAC International. Gaithersburg. MD. USA.
- ISENBERG, H.D. (ed.) (1992) Clinical Microbiology Procedures Handbook. ASM. Washington. DC. USA.

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Yersinia CIN Agar Base

Art. No. 01-444

- ISO Standard 10273 (2003) Microbiology of food and animal feeding stuffs - Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica.*
- ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- SCHIEMAN, D.A. (1979) Synthesis of a selective medium for *Yersinia enterocolitica*. Can. J. Microbiol. 25:1298-1304.
- SCHIEMAN, D.A. (1980) *Yersinia enterocolitica*: Observations on some growth characteristics and response to selective agents. Can. J. Microbiol. 26:1232-1240.
- SCHIEMAN, D.A. (1982) Development of a two step enrichment procedure for recovery of *Yersinia enterocolitica* from food. Appl. Environm. Microbiol. 43:14-27.
- WEAGANT, S.D. & P. FENG (2001) *Yersinia*, in "Compendium of Methods for the Microbiological Examination of Foods". 4th ed. Downes & Ito (Eds.) APHA. Washington. DC. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 22 - 25°C
Incubation time: 3 days
Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Bacillus subtilis ATCC 6633	Inhibited	-
P. aeruginosa ATCC 27853	Poor	3 days
Yersinia enterocolitica ATCC 9610	Good	-
E. coli ATCC 8739	Good	48 h

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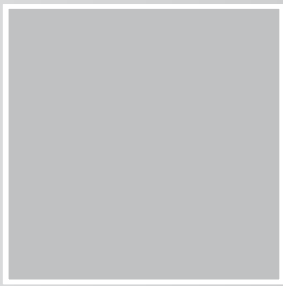







Additives

Polysorbate 80

Art. No. TW0080

Also known as

Polyoxy-ethylene sorbitan mono-oleate; Tween®80

Specification

Culture Media Additive.

Description

Under the name of Polysorbate 80 or poly-oxy-ethylene-sorbitan-mono-oleate are included many derivates of polyose-1,2-ethano-diol sorbitan-mono-9-octodecenoate.

The product supplied by Scharlau is can be used as a nutrient in some cases and an emulsifier in others, but is always compatible with the rest of the components in the culture medium. It is a viscous liquid, amber in colour, and has a density of approx. 1,08. It is very soluble in water, but has average solubility in organic diluents, but is not soluble in mineral lipids.

The incorporation of Polysorbate 80 to culture media may have a slight affect on the final pH, if the original medium formulation does not incorporate polysorbate.

Although it may bear sterilization in the autoclave, when it is present in a concentration greater than 1%, it is usual to homogenize the medium after sterilization, since with autoclaving, the polysorbate sometimes separates out of the medium.

Polysorbate is a surfactant agent that decreases the surface tension of the cell, modifying at the same time the cellular exchange speed. Resulting usually in faster growth or an increase in some bacterial activities.

Physical data

Density a 25ºC	1,07 g/cm^3
Solubility in water at 25ºC	miscible
Boiling point	> 100 ºC
Flash point	> 149 ºC
Ignition point	> 180 ºC
Vapour pressure at 20ºC	< 1,33 hPa
Viscosity a 25ºC	375-480 mPa
pH 5% aq. soln at 20ºC	5 - 7

Chemical data

Arsenic (As)	max. 0,0001 %
Heavy metals (as Pb)	max. 0,0010 %
Sulphated ash	max. 0,5000 %
Acidity index	3
Hydroxyl index	65 - 80
Iodine index	18 - 24
Saponification index	45 - 55

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Potassium Tellurite Sterile Solution 1%

Art. No. 06-089

Specification

Aqueous solution of potassium tellurite at 1%, sterilized by filtration and suitable for use as an inhibitor additive in culture media.

Formula

Potassium tellurite.....10 g
Distilled water.....1 L
Filter- sterilized aqueous solution.

Description

Potassium Tellurite Solution is added to culture media as an inhibitor. Its purpose is to prevent the growth of most Gram negative bacteria and of those Gram positive bacteria unable to reduce it.

It is used in media such as Giolitti-Cantoni Broth (Art. No. 02-230), Vogel-Johnson Agar (Art. No. 01-206) and other selective media for staphylococci. This solution is also contained in selective media for corynebacteria, streptococci and vibrios.

There is a high correlation between the ability to reduce potassium tellurite to tellure and the pathogenicity of staphylococci. Therefore, the presence of potassium tellurite in a medium together with other tests helps to determine staphylococci of clinical interest.

Potassium Tellurite Solution should be stored at room temperature, since low temperatures will cause crystallization and later precipitation of the product. Should this occur, intense agitation will help redissolve the sediment. Due it its thermolability, potassium tellurite is supplied, sterile filtered.

Technique

The technique is described in each of the culture media where the additive is required.

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- FDA (1998) Bacteriological Analytical Manual. 8th ed. Reviskion A. AOAC International. Gaithersburg. MD.
- ISO Standard 22718 (2006) Cosmetics. Detection of *Staphylococcus aureus*.
- USP (2002) 25th ed. <61> Microbial Limit Tests. US Pharmacopeial Convention Inc. Rockville. MD.
- VOGEL, R.A. & M. JOHNSON (1960) A modification of the tellurite-glycine medium for the use in the identification of *Staphylococcus aureus*. Pub. Health. Lab. 18:131-133.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C).

Quality control

Culture medium: 02-230
Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h.
Inoculum: 10-100 cfu (Productivity) // 1.000-10.000 cfu (Selectivity). (ISO 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	48h (black precipitate)
Staphylococcus aureus ATCC 6538	Good	48h (black precipitate)
E. coli ATCC 8739	Inhibited	-

First: Control
Second: *Staphylococcus aureus* ATCC 25923 (Black precipitate)
Third: *Staphylococcus aureus* ATCC 6538 (Black precipitate)
Fourth: *Escherichia coli* ATCC 8739

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Potassium Tellurite Sterile Solution 3,5%

Art. No. 06-011

WARNING

H: 3.1.O/4: H302

P: P264-P270-P301+P312-P330-P501a

Specification

Aqueous solution of potassium tellurite at 3,5%, sterilized by filtration and suitable for use as an inhibitor additive in culture media.

Formula

Potassium tellurite ..... 35 g
Distilled water..... 1 L
Filter- sterilized aqueous solution.

Description

Potassium Tellurite Solution is added to culture media as an inhibitor. Its purpose is to prevent the growth of most Gram negative bacteria and of those Gram positive bacteria unable to reduce it.

It is used in media such as Giolitti-Cantoni Broth (Art. No. 02-230), Vogel-Johnson Agar (Art. No. 01-206) and other selective media for staphylococci. This solution is also contained in selective media for corynebacteria, streptococci and vibrios.

There is a high correlation between the ability to reduce potassium tellurite to tellurium and the pathogenicity of staphylococci, therefore, the presence of potassium tellurite in a medium, together with other tests, helps to determine staphylococci of clinical interest. Potassium Tellurite Solution should be stored at room temperature, since low temperatures will cause crystallization and later precipitation of the product. Should this occur, intense agitation will help redissolve the sediment. Due to its thermolability, the potassium tellurite is supplied, sterile filtered.

Technique

The technique is described in each of the culture media where the additive is requiered.

References

- ATLAS R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- BAIRD-PARKER, A.C. (1962) An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bact. 25:12.
- COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4rd ed. APHA. Washington. USA.
- EUROPEAN PHARMACOPOEIA (2007) 5ª ed. Suppl. 5.6 § 2.6.13. Microbiological examination of non-sterile products. EDQM. Council of Europe. Strasbourg.
- ISO 5944:2001 Standard. Milk and milk based products - Detection of coagulase positive staphylococci - MPN Technique. Geneva.
- ISO 6888-1:1999 Standard. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci - Part 1: Technique using Baird-Parker Agar medium. Geneva.
- ISO 22718:2006 Standard. Cosmetics - Detection of *Staphylococcus aureus.*
- FIL-IDF 60:2001 Standard. Lait et produits à base de lait - Détection des staphylocoques à coagulase positive - Technique du nombre le plus probable. Brussels.
- USP 31 - NF 26 (2008) <61> Microbial Limit Tests. US Phamacopeial Conv. Inc. Rockville. MD. USA.
- ZANGERL, P. & H. ASPERGER (2003) Media used in the detection and enumeration of *Staphylococcus aureus.* In "Handbook of Culture Media for Food Microbiology". Corry *et al.* Eds. Elsevier Sci. BV Ámsterdam.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C).

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Potassium Tellurite Sterile Solution 3,5%

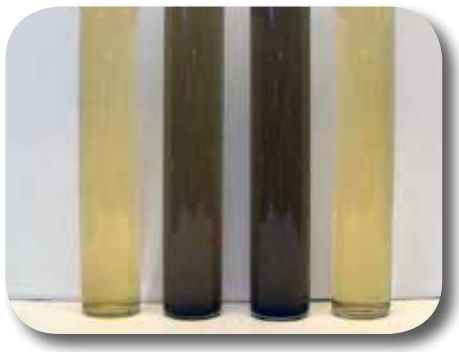
Art. No. 06-011

WARNING
H: 3.1.O/4; H302
P: P264-P270-P301+P312-P330-P501a

Quality control

Culture medium: 02-230
Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 cfu (Productivity) // 1.000-10.000 cfu (Selectivity). (ISO 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	48h (black precipitate)
Staphylococcus aureus ATCC 6538	Good	48h (black precipitate)
E. coli ATCC 8739	Inhibited	-



First: Control
Second: *Staphylococcus aureus* ATCC 25923 (Black precipitate)
Third: *Staphylococcus aureus* ATCC 6538 (Black precipitate)
Fourth: *E. coli* ATCC 8739

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Skimmed Milk Powder

Art. No. 06-019

Specification

Culture Media Additive.

Directions

100 g of powder produces 1 L of skimmed milk. Addition of water must be gradual, until the mixture is a homogeneous paste. Then, complete the process by adding more water until the desired volume is obtained. Sterilization may be under fluent steam for 30 minutes and three consecutive days or in the autoclave at 121ºC for 15 minutes or at 114ºC for 15-20 minutes (this last way is the best). Care should be taken not to overheat the prepared milk since natural sugars may become burnt and produce toxic compounds.

Description

Skimmed milk powder for bacteriology is obtained after a careful spray drying process that keeps it free from thermophilic organisms that would otherwise interfere with its use.

Skimmed milk may be used alone or as an additive to other culture media. It is a suitable medium for the culture of lactic acid bacteria and for identification in general, where an organism's capacity to coagulate or peptonize milk results in visible changes to the medium. With the addition of suitable indicators such as bromocresol purple at 0,004% pH changes due to bacterial activity may be detected. It also accepts redox indicators like methylene blue, resazurin or TTC (Art. No. 06-023) to verify microbial development.

Skimmed milk may be added to media such as Tryptone Soy Agar (Art. No. 01-200) or Nutrient Agar (Art. No. 01-144 and 01-140) to detect caseolytic activity.

Chemical data

Fat	0,5 %
Protein	33,0 %
Ash	8,0 %
Moisture	< 5,0 %
Acid lactic	1,5 %
Phosphatase Test	Negative
Inhibitor Test (Antibiotics)	Negative

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Sodium Biselenite

Art. No. SO0160

DANGER

H: 3.1.O/3; H301-3.1.I/3; H331-3.9/2; H373-4.1.C/1; H410

P: P260-P261-P301+P310-P321-P405-P501a

Also known as

Sodium Hydrogen Selenite

Specification

Chemical compound to be added to selenite based culture media.

Description

The toxicity and potential teratogenicity of this product recommend its exclusion from the dehydrated powder mixture of culture media in order to minimize the hazard of accidental inhalation or contact. The supply of this product in a separate form from the medium base reduces potential risk to the user.

The intended use of this product is to complete the following culture media by adding the specified amounts:

- Art. No. 02-602 Selenite Cystine Broth Base.....4 g/L
- Art. No. 02-598 Selenite Broth Base.....4 g/L

The use of this product is restricted to technically qualified personnel. It should not be handled by untrained personnel.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Sterile Egg Yolk Emulsion

Art. No. 06-016

Specification

Egg yolk emulsion, for use in bacteriology.

Formula

Egg Yolk	200,00 mL
Destilled water	800,00 mL

Description

Sterile Egg yolk emulsion is a common additive in culture media. Scharlau Microbiology's Egg Yolk is formulated according to the ISO standards for food and cosmetics, and is used with *Bacillus cereus* Agar Base (Art. No. 01-262). *Bacillus cereus* Selective Agar (Art. No. 01-487) and Tryptose Sulfite and Cycloserine Agar (Art. No. 01-278).

It is usually used for the detection of lecithinase in the genera Bacillus, *Clostridium*, and *Staphylococcus,* and can also be used to demonstrate the enzyme in the psychrotrophic and lactic group of microorganisms.

Lecithinase test

Prepare the medium by aseptically adding 0.5-1.0 ml of the egg yolk emulsion to 10 mL of sterile molten agar cooled to about 55-60°C. Tryptic Soy Agar (Art. No. 01-200), Nutrient Agar (Art. No. 01-140) and Nutrient Agar according to APHA (Art. No. 01-144), are all suitable. To perform the test in liquid medium the corresponding broths may be used. If the turbidity is considered excessive considerable clarification is achieved by adding 1% sodium chloride prior to sterilization.

The solid or liquid medium is inoculated with the strain to be tested and incubated for 5 days at 35-37°C. If lecithinase activity is present broths become opalescent and an opaque halo surrounds the colonies on solid media.

Bacillus cereus, has potent lecithinase activity, producing visible results within a few hours coagulating the broth within a day and sometimes sooner. In the case of staphylococci, the results are more apparent when the culture medium base includes 1% glucose.

Egg yolk emulsion has been used extensively as a neutralizer of bactericides in liquid samples. This effect is greatly enhanced if some Tween 20 is included in the formulation. 1% (v / v) is usually adequate.

References

- IDF 60 Standard (2001) Milk and Milk based products - Detection of coagulase-positive staphylococci- Most probable technique.
- ISO 5944 Standard (2001) Milk and Milk based products - Detection of coagulase-positive staphylococci- Most probable technique.
- ISO 6888-1 Standard (1999) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker Agar Medium.
- ISO 22718 Standard (2006) Cosmetics- Microbiology- Detection of *Staphylococcus aureus*.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+8 °C to 12 °C and <60 % RH).

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Sterile Egg Yolk Emulsion

Art. No. 06-016

Quality control

Incubation temperature: 30°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 cfu (Productivity)

Microorganism	Growth	Remarks
Bacillus cereus ATCC 11778	Good	Red colonies irregulars borders

Bacillus cereus var. mycoides ATCC

Sterile Egg Yolk Tellurite Emulsion

Art. No. 064-BA1018

Specification

Formulated for addition to Baird-Parker Agar Base (Art. No. 01-030) according to the ISO standard 6888-1.

Formula

Egg Yolk	200,00 mL
Potassium tellurite	2,00 g
Sterile water	800,00 mL

Recommended techniques

Add, aseptically, 50 mL of egg yolk tellurite emulsion, to 1 litre of sterile Baird-Parker Agar Base (Art No. 01-030), melted and cooled to around 55-60°C. Mix thoroughly avoiding bubble and foam formation and pour into Petri dishes.

The presumptive colonies of *Staphylococcus aureus* express their lecithinase activity with a clear zone (digestion) around the colonies, whilst simultaneously blackening in the centre due to the reduction of tellurite.

References

- ISO 6888-1 Standard (1999) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker Agar Medium.

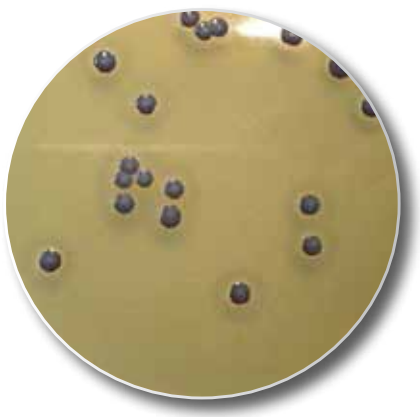
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+ 8 °C to 12 °C and <60 % RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 cfu (Productivity)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	Black colonies; Lecithinase (+)



Staphylococcus aureus ATCC 25923

Sterile Egg Yolk Tellurite Emulsion

Art. No. 06-026

Specification

Specially formulated for addition to Baird-Parker Agar Base (Art. No. 01-030)

Formula

Egg Yolk	200,00 mL
Potassium tellurite	2,10 g
Sodium Chloride	4,25 g
Sterile water	800,00 mL

Recommended techniques

Add, aseptically, 50 mL of egg yolk tellurite emulsion, to 1 litre of sterile Baird-Parker Agar Base (Art. No. 01-030), melted and cooled to around 55-60°C. Mix thoroughly avoiding bubble and foam formation and pour into Petri dishes.

The presumptive colonies of *Staphylococcus aureus* express their lecithinase activity with a clear zone (digestion) around the colonies, whilst simultaneously blackening in the centre due to the reduction of tellurite.

References

- IDF 60 Standard (2001) Milk and Milk based products - Detection of coagulase-positive staphylococci- Most probable technique.
- ISO 5944 Standard (2001) Milk and Milk based products - Detection of coagulase-positive staphylococci- Most probable technique.
- ISO 6888-1 Standard (1999) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker Agar Medium.
- ISO 22718 Standard (2006) Cosmetics Microbiology Detection of *Staphylococcus aureus*.

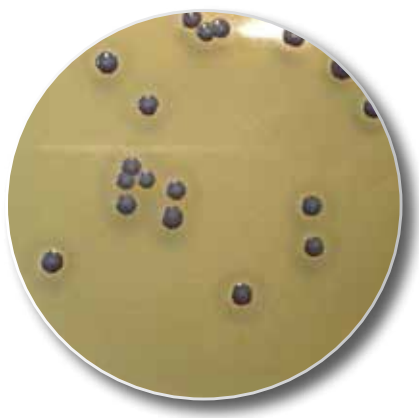
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+8 °C to 12 °C and <60 % RH).

Quality control

Incubation temperature: 35°C ± 2,0
Incubation time: 24 - 48 h
Inoculum: 10-100 cfu (Productivity)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	Black colonies; Lecithinase (+)



Staphylococcus aureus ATCC 25923

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TTC Sterile Solution 1%

Art. No. 06-023

Specification

Reagent to be added, as an aerobic indicator, to culture media.

Formula

2,3,5-Triphenyltetrazolium chloride.....10 g
Distilled water.....1 L

Description

1% 2-3-5-triphenyl-2H-tetrazolium chloride sterile solution. Is used as an additive for culture media to indicate biological activity. The colourless form is hydrogenated or reduced to a red insoluble pigment: triphenyl-formazan, which may be easily observed.

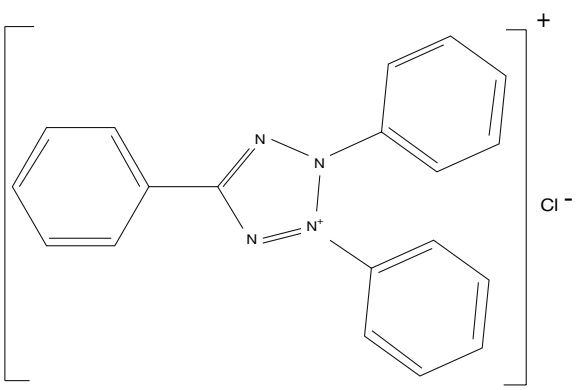
It is not advisable to incorporate TTC into culture media prior to sterilization, because it can lose it's efficacy. The best results achieved when the addition is carried out aseptically with medium cooled to 60ºC maximum. TTC is photolabile and can become yellow through the effect of light, therefore it must be kept in the refrigerator and direct light avoided.

The concentration used varies depending on the medium, but generally it is between 0,3 and 1% (v/v).

This product is especially produced to be added to the following media:

- Chapman TTC Agar Base (Art. No. 01-053)
- Slanetz Bartley Agar Base (Art. No. 01-579)

The general structure is the following:



Technique

The technique is described in each of the culture media where the additive is required.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
- ISO 7899-2:2000 Standard. Water Quality. Detection and enumeration of enterococci by membrane filtration method.
- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- CHAPMAN G.H. (1951) A culture medium for detecting and confirming *E. coli* in ten hours. Am. J. Publ. Hlth 41:1381-1386.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. APHA.Washington.
- GUINEA, SANCHO, PARES (1979) Análisis Microbiológico de Aguas. Ed. Omega. Barcelona.
- ISO 9308-1:2000 Standard. Water Quality. Detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method.
- LACHICA, LV.F. & P.A. HARTMAN (1968) Two improved media for isolating and enumerating enterococci in certain frozen foods. J. appl. Bact. 31:151-156.
- SLANETZ, L.W. & BARTLEY, C.H. (1957) Numbers of enterococci in water, sewage and faeces determined by the membrane filter technique with an improved medium. J. Bact. 74:591-596.
- SPECK, M (Ed.) (1982) Compendium of Methods for the Microbiological Examination of Foods. 2nd. ed. APHA.Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 12ºC).

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TTC Sterile Solution 1%

Art. No. 06-023

Quality control

Culture medium: Art. No. 01-053
Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 10-100 cfu.

Microorganism	Growth	Remarks
Escherichia coli ATCC 8739	Good	Yellow- orange Colonies
Salmonella typhimurium ATCC 14028	Good	Violet or dark red Colonies

Escherichia coli ATCC 25922

Urea Sterile Solution 40%

Art. No. 06-083

Specification

Additive for the Urease test.

Formula

Urea	400,00 g
Distilled water	1000,00 mL

Description

Aqueous urea solution 40%, sterilized by filtration is suitable for use as an additive in culture media.
It is supplied for use with dehydrated Urea Agar according to Christensen (Art. No. 01-261) and Urea Broth (Art. No. 02-202). It must be added to these media after sterilization and with the media cooled below 55°C.
Once it is added, do not reheat the media because urea is thermolabile and heating causes it to break down and liberate ammonia.

Technique

The technique is described in each of the culture media where the additive is required.

References

- ATLAS, R.M. & L.C. PARK (1993) Handbook of Microbiological Media. CRC Press Inc. London.
- CHRISTENSEN W.B. (1946) Urea decomposition as means of differentiating *Proteus* aqnd Paracolon cultures from each other and from *Salmonella* and *Shigella* types. J. Bact. 52:461.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 12°C).

(continues on the next page)

Urea Sterile Solution 40%

Art. No. 06-083

Quality control

Culture medium: Art. No. 01-261 and Art. No. 02-202
Incubation temperature: 35°C ± 2,0
Incubation time: 6 - 12 h
Inoculum: Pure culture

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	Urease (-)
Proteus mirabilis ATCC 43071	Good	Urease (+)

Escherichia coli ATCC 25922
(Colourless: Urease -)

Left: *Proteus mirabilis* ATCC 43071
(Pink colour: Urease +)
Right: Control

Proteus mirabilis ATCC 43071
(Pink colour: Urease +)

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Scharlau

Supplements

Selective Supplements for Culture Media

Supplements in unique press-shake-use vials

What's different?

This packaging system consists in vials and caps that further simplify the handling of supplements. There is no need to apply hard pressure and no need to remove the tamper-proof cover. This allows for quick opening and easy manipulation: press, shake, open and dispense. Offered in boxes of 10 vials; each vial contains sufficient supplement for 500mL of reconstituted medium.

Screw cap → **No need to remove tamper-proof cover**
Screw cap → **Less pressure required to break the seal and mix the components**
Larger vial → **Wider variety of supplements now available**
Larger vial → **Larger volumes of supplement are now available**

Features & Benefits

Our Tamper-proof vials make it easy to add supplements to media. The sterile powdered supplement is contained inside the plastic cap and the diluent is contained inside the vial. Simply prepare by depressing the cap; the supplement base is released into the solvent ready to be mixed.

Shelf life and storage

Products should be stored in the fridge at temperatures between 2 and 8°C and the shelf life is between 3 and 4 years, depending on the product.*

1. Depress the cap to break the seal and release the powdered supplement.
2. The powder then falls into the diluent. Shake vigorously for total dissolution.
3. The supplement is now ready. Open the vial aseptically, close to a flame or in a safety cabinet. Pour into the medium base, which has been cooled down to 45-50°C.
4. Mix to evenly distribute the supplement in the medium and now it is ready to dispensed into the desired containers: flasks, tubes or plates.

Standard reference for press-shake-use supplements: 06-XXXCASE (XXX 3 numbers depending on the supplement)

Freeze dried supplements in vials with safe screw caps

Supplements - freeze-dried

By means of the freeze-drying technique we can assure a standardised quality that will yield reliable results without any loss in performance of the selective medium. The freeze-dried solids dissolve easily and quickly.

Screw cap for easy and safe handling

The standard closure offered by other manufactures, which consists of a rubber septum with an outer aluminium cap may lead to undesirable accidents. Its removal requires a tool, takes some time and may cause injuries.

Therefore we offer our vials with a screw cap a much safer, faster and easier to open alternative.
You may directly remove the septum from the freeze-dried supplement vial and the solvent vial and pour the solvent on to the supplement. Alternatively you may use a syringe, perforate our septum with a needle and that way transfer the solvent to the supplement vial.

Method 1

Method 2

Standard reference for freeze dried supplements: 06-XXX-LYO (XXX 3 numbers depending on the supplement)

Sterilised solvents included

Each box contains 5 vials of the supplements and 5 vials of the appropriate diluent. This enables the user to start using our supplements right away and saves the tedious work of preparing and sterilising the solvent.

Supplements

Our standard vials contain sufficient 500mL of prepared medium.

Shelf life and storage

Products should be stored in the fridge at temperatures between 2 and 8°C and the shelf life is between 3 and 4 years, depending on the product.*

*Some supplements have lower shelf life depending on composition.

Selective Supplements for Culture Media

Microorganisms	Supplement	Art. No.
Aeromonas	Ampicillin Selective Supplement	06-126-LYO
Bacillus cereus	Polymyxin B sulfate Selective Supplement	06-021CASE 06-021-LYO
Campylobacter	*Campylobacter* Bolton Selective Supplement	06-131-LYO
Campylobacter	*Campylobacter* CCDA Selective Supplement	06-133-LYO
Campylobacter	*Campylobacter* Growth Supplement	06-128-008
Campylobacter	*Campylobacter* Preston Selective Supplement	06-130-LYO
Campylobacter	*Campylobacter* Preston Modified Selective Supplement	06-135-LYO
Campylobacter	*Campylobacter* Skirrow Selective Supplement	06-132-LYO
Coliforms	Basic Fuchsin 250 Selective Supplement	06-607-LYO
Coliforms	MUG Fluorescent Supplement	06-102CASE 06-102-LYO
Coliform / *E. coli*	Coliform CV Selective Supplement	06-140 LYO
Clostridium	D-Cycloserine Selective Supplement (250 mL of medium)	06-116CASE 06-116-LYO
Clostridium	m-CP Selective Supplement	06-125-LYO
Clostridium	Ferric Ammonium Citrate Supplement (312 mg)	06-113CASE 06-113-LYO
Clostridium	Sodium disulfite (Sodium Meta-Bisulfite) for Bacteriology	06-114CASE 06-114-LYO
***E. coli* 0157:H7**	CT SMAC Selective Supplement	06-146-LYO
E. coli* 0157:H7** ***Salmonella	Novobiocin Selective Supplement	06-139-LYO
Fermenting microorganisms	Cycloheximide Selective Supplement	06-022CASE 06-022-LYO

[1] Needs addition of 06-016-100 [6] Needs addition of 06-128-008 [7] Needs addition of 06-130-LYO or 06-135-LYO

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	Base	Composition per vial	Directive, Standard
	Blood Agar Base (Art. No. 01-352) Blood Agar Base (Columbia) (Art. No. 01-034)	2,5 mg of Ampicillin	
	Bacillus cereus Agar Base (MYPA) (Art. No. 01-262) [1] *Bacillus cereus* Agar Base (PEMBA) (Art. No. 01-487) [1]	50.000 IU Polymyxin B sulfate	BAM, COMPF, IDF, ISO, NMLK
	Bolton Enrichment Broth Base (Art. No. 02-688)	10 mg of Vancomycin 10 mg of Cefoperazone 10 mg of Trimethoprim 5 mg of Amphotericin B sulfate	ISO
	Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base (Art. No. 01-685)	16 mg of Cefoperazone 5 mg of Amphotericin B sulfate	ISO
	Preston *Campylobacter* Agar Base (Art. No. 01-451) [7] Preston *Campylobacter* Broth Base (Art. No. 02-561) [7] Skirrow Selective Medium: with Blood Agar Base (Art. No. 01-352) or Blood Agar Base (Columbia) (Art. No. 01-034)	0,125 g of Sodium pyruvate 0,125 g of Sodium metabisulfite 0,125 g of Ferrous sulfate	
	Preston *Campylobacter* Agar Base (Art. No. 01-451) [6] Preston *Campylobacter* Broth Base (Art. No. 02-561) [6]	2500 IU Polymyxin B sulfate 5 mg Rifampicin 5 mg of Trimethoprim 50 mg of Cycloheximide	
	Preston *Campylobacter* Agar Base (Art. No. 01-451) [6] Preston *Campylobacter* Broth Base (Art. No. 02-561) [6]	2500 IU Polymyxin B sulfate 5 mg Rifampicin 5 mg of Trimethoprim 50 mg of Cycloheximide	
	Blood Agar Base (Columbia) (Art. No. 01-034) Blood Agar Base no. 2 (Art. No. 01-505)	5 mg of Vancomycin 2,5 mg of Trimethoprim 1250 IU Polymyxin B sulfate	
	Endo Agar (Art. No. 01-589)	250 mg of Basic Fuchsin	AOAC, COMPF, SMD
	Brilliant Green Bile 2% Broth (Art. No. 02-041) *E. coli* Broth (Art. No. 02-060) Lactose Broth (Art. No. 02-105) MacConkey Agar (Art. No. 01-118) MacConkey Broth (Art. No. 02-118) MacConkey Broth (Eur. Pharm.) (Art. No. 02-611) Tryptose lauryl sulfate Broth (Art. No. 02-108) Violet Red Bile Agar (VRB Agar) (Art. No. 01-164) Violet Red Bile Lactose Dextrose Agar (Eur. Pharm. Medium F) (Art. No. 01-220)	50 mg of MUG (4-methilumbeliferil-β-D glucuronide)	
	Colinstant Agar (Art. No. 01-618) Chromogenic Coliform Agar (CCA Agar) (Art. No. 01-695)	2,5 mg of Cefsulodin 2,5 mg of Vancomycin	Spanish Ministry of Health
	Tryptose Sulfite Cycloserine Agar (TSC Agar) (Art. No. 01-278)	100 mg of D-Cycloserine	BAM, COMPF, DIN, ISO
	m-CP Agar Base (Art. No. 01-513)	200 mg of D-Cycloserine 12,5 mg of Polymyxin B sulfate 30 mg of 3-indoxyl-β-D-glucopyranoside 50 mg of Phenolphthalein diphosphate 45 mg of Iron III Chloride	Directive 12767/97
	Lactose Sulfite Broth Base (Art. No. 02-519)	312 mg of Ferric ammonium citrate	EP, ISO
	Lactose Sulfite Broth Base (Art. No. 02-519)	375 mg of Di-sodium sulfite	EP, ISO
	MacConkey Sorbitol Agar (Art. No. 01-541)	1,25 mg Potassium tellurite 0,025 mg Cefixime	ISO
	Rappapport Vassiliadis semi-solid Medium (MSRV) (Art. No. 03-376) TSB Modified (Art. No. 02-691)	10 mg of Novobiocin	ISO BAM, ISO
	WL Nutrient Agar (Art. No. 01-210)	2 mg of Cycloheximide	

Selective Supplements for Culture Media

Microorganisms	Supplement	Art. No.
Legionella	*Legionella* BCYE Growth Supplement	06-137-LYO
Legionella	*Legionella* BCYE without Cysteine NO Growth Supplement	06-134-LYO
Legionella	*Legionella* GVPC Selective Supplement	06-138-LYO
Listeria	Ferric Ammonium Citrate Supplement (250 mg)	06-112-LYO
Listeria	*Listeria* Selective Supplement for Primary Enrichment (UVM I)	06-106CASE 06-106-LYO
Listeria	*Listeria* Selective Supplement for Primary Enrichment Half Fraser (Each vial supplements 225mL of medium)	06-136-LYO
Listeria	*Listeria* Selective Supplement for Primary Enrichment Half Fraser (Each vial supplements 500 mL of medium)	06-145-LYO
Listeria	*Listeria* Selective Supplement for Secondary Enrichment (UVM II / Fraser)	06-111CASE 06-111-LYO
Listeria	*Listeria* Selective Supplement for Enrichment (FDA and IDF/FIL)	06-107CASE 06-107-LYO
Listeria	Oxford Agar Selective Supplement	06-127-LYO
Listeria	Palcam Agar Selective Supplement	06-110 CASE 06-110 LYO
Neisseria * Glucose is included in the diluent vial	GPS - Growth Promotion Supplement	06-144-LYO
Neisseria	VCAT Selective Supplement	06-141-LYO
Neisseria	VCNT Selective Supplement	06-142-LYO
Pseudomona	Nalidixic Acid Selective Supplement	06-124CASE 06-124-LYO

[2] Needs addition of 06-137-LYO [3] Needs addition of 06-0111CASE or 06-111-LYO [4] Needs addition of 06-0112CASE or 06-112-LYO [8] Can be added 06-144-LYO

* 10 tubes of RPF freeze dried supplement without solvent. Reconstitute by adding 10 mL of pre-warmed sterile water at 37°C to 90 mL of medium base.

	Medium to be added	Composition per vial	Directive, Standard
	BCYE Legionella Agar Base (Art. No. 01-687)	3,6 g of ACES Buffer 1,4 g of Potassium hydroxide 0,125 g of Ferric pyrophosphate 0,2 mg of L-Cysteine HCl 0,5 g Potassium α–Ketoglutarate	ISO
	BCYE *Legionella* Agar Base (Art. No. 01-687)	3,6 g of ACES Buffer 1,4 g of Potassium hydroxide 0,125 g of Ferric pyrophosphate 0,5 g Potassium α–Ketoglutarate	ISO
	BCYE *Legionella* Agar Base (Art. No. 01-687)[2]	0,5 mg of Vancomycin 40.000 IU of Polymyxin B sulfate 40 mg of Cycloheximide 1,5 g of Glycine (ammonia free)	ISO
	Listeria Enrichment Broth Base (Fraser) (Art. No. 02-496) [3]	250 mg of Ammonium ferric citrate	ISO
	Listeria Enrichment Broth Base (UVM) (Art. No. 02-472) [3]	10 mg of Nalidixic acid, sodium salt 6 mg of Acriflavine	AOAC
	Listeria Enrichment Broth Base (Fraser) (Art. No. 02-496)	2,25 mg of Nalidixic acid, sodium salt 2,80 mg of Acriflavine 112,5 mg of Ammonium ferric citrate	ISO
	Listeria Enrichment Broth Base (Fraser) (Art. No. 02-496)	5 mg of Nalidixic acid, sodium salt 6,2 mg of Acriflavine 250 mg of Ammonium ferric citrate	ISO
	Listeria Enrichment Broth Base (Fraser) (Art. No. 02-496) [4] *Listeria* Enrichment Broth Base (UVM) (Art. No. 02-472)	10 mg of Nalidixic acid, sodium salt 12,5 mg of Acriflavine	ISO AOAC
	Listeria Enrichment Broth Base (Lovett) (Art. No. 02-498)	20 mg of Nalidixic acid, sodium salt 25 mg of Cycloheximide 7,5 mg of Acriflavine	BAM, IDF
	Oxford Agar Base (Art. No. 01-471)	5 mg of Fosfomycin 1 mg of Sodium cefotetan 10 mg of Colistin sulfate 200 mg of Cycloheximide 2,5 mg of Acriflavine	ISO
	Palcam Agar Base (01-470)	5 mg of Polymyxin B sulfate 10 mg of Sodium ceftazidime 2,5 mg of Acriflavine	ISO
	GC Agar Base (Art. No. 01-310)	0,1 mg of Vitamin B12 100 mg of L-glutamine 10 mg of Adenine SO_4 0,3 mg of Guanine HCl 0,13 mg of p-Aminobenzoic acid 11 mg of L-cystine 2,5 mg of NAD 1 mg of Cocarboxylase 0,2 mg of Ferric nitrate 0,03 mg of Vitamin B1 (Thiamnine HCl) 259 mg of Cysteine HCl 1 g of Glucose *	
	GC Agar Base (Art. No. 01-310) [8]	1 mg of Vancomycin 3,75 mg of Colistin sulfate 1,5 mg of Trimethoprim 0,5 mg of Anphotericine B	
	GC Agar Base (Art. No. 01-310) [8]	1,5 mg of Vancomycin 3,75 mg of Colistin sulfate 2,5 mg of Trimethoprim 6.250 IU of Nystatin	
	CN Selective Agar Base (Art. No. 01-609)	7,5 mg of Nalidixic acid, sodium salt	EN, ISO

Selective Supplements for Culture Media

Microorganisms	Supplement	Art. No.
Salmonella	Brilliant Green + Novobiocin Selective Supplement	06-017CASE 06-017-LYO
Salmonella	Novobiocin Selective Supplement (20 mg)	06-147-LYO
Salmonella ***E. coli* 0157:H7**	Novobiocin Selective Supplement (10 mg)	06-139-LYO
Staphylococci / Streptococci **(Gram-positive cocci)**	CP Selective Supplement for Gram-positive Cocci (CNA)	06-013CASE 06-013-LYO
Staphylococci (Coagulase positive)	RPF Supplement * (Each vial supplement 100 mL of medium)	064-TA0155
Yeasts and Moulds	Chloramphenicol Selective Supplement (25 mg)	06-118CASE 06-118-LYO
Yeasts and Moulds	Oxytetracycline Selective Supplement	06-115CASE 06-115-LYO
Yersinia	*Yersinia* Selective Supplement	06-143-LYO

[5] Needs addition of 064-V11108

* 10 tubes of RPF freeze dried supplement without solvent. Reconstitute by adding 10mL of pre-warmed sterile water at 37°C to 90mL of medium base.

	Medium to be added	Composition per vial	Directive, Standard
	Muller-Kauffmann Tetrathionate Broth (Art. No. 02-335) [5]	5 mg of Brilliant green 20 mg of Novobiocin	DIN, ISO
	Tetrathionate Bile Brilliant Green Broth Base (Eur. Pharm.) (Art. No. 02-629)	20 mg of Novobiocin, sodium salt	EP
	Rappaport Vassiliadis Modified Semi-solid Medium Base (Art. No. 03-376) Tryptic Soy Broth Modified (Art. No. 02-691)	10 mg of Novobiocin, sodium salt	BAM, ISO
	Blood Agar Base (Art. No. 01-352) Blood Agar Base (Columbia) (Art. No. 01-034)	5 mg of Colistin sulfate 7,5 mg of Nalidixic acid	
	Baird Parker Agar (Art. No. 01-030)	2,5 mL of Rabbit Plasma - EDTA 0,5 g of Bovine Fibrinogen 2,5 mg of Trypsin Inhibitor 2,5 mg of Potassium Tellurite	ISO
	Sabouraud Dextrose Agar (Art. No. 01-165)	25 mg of Chloramphenicol	EP, ISO, USP
	Sabouraud Oxytetracycline Agar Base (OGYEA) (Art. No. 01-275)	50 mg of Oxytetracycline HCl	EP, ISO, USP
	Yersinia CIN Agar Base (Art. No. 01-444)	7,5 mg of Cefsulodin 2 mg of Irgasan® 1,25 mg of Novobiocin	ISO

Ampicillin Selective Supplement

Art. No. 06-126-LYO

DANGER

H: 3.4.R/1; H334-3.8/3; H335-3.2/2; H315-3.3/2; H319-3.4.S/1; H317

P: P285-P261-P305+P351+P338-P321-P405-P501a

Specification

Selective supplement for Aeromonas *hydrophila* isolation.

Contents

The box contains 5 vials with antibiotic and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Blood Agar, Art. No. 01-352, or 500 mL of Columbia Blood Agar (Art. No. 01-034).

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Ampicillin.....2,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Does not use beyond stated expiry date.

Applicable media

Art. No. 01-352 Blood Agar

Art. No. 01-034 Columbia Blood Agar

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light, at 2°C to 8°C.

Basic Fuchsin (250) Selective Supplement

Art. No. 06-607-LYO

Specification

Selective and differential supplement for the detection of coliform microorganisms.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL). Each vial contains sufficient inhibitor to add to 500mL of Endo Agar Base Art. No. 01-589.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Basic fuchsin..... 250,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Endo Agar Base Art. No. 01-589

Sterility control

No growth within 7 days at 32,5 ± 2,5ºC and 22,5 ± 2,5ºC in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light, at 2ºC to 8ºC.

Brilliant Green + Novobiocin Selective Supplement

Art. No. 06-017CASE

Specification

Selective supplement for the isolation of *Salmonella* in Müeller-Kauffman Medium.

Contents

The box contains 10 vials. Each vial contains sufficient inhibitors to add to 500 mL of Müller-Kauffmann Tetrathionate Broth Base Art. No. 02-335.

Vial contents

Amount required for 500 mL of complete medium.

Brilliant green.....5,00 mg
Novobiocin, sodium salt.....20,00 mg
Solvent (1:20 Ethanol : distilled water).....5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the vial contents to 500 mL of boiled broth base, cooled to 50°C. Homogenize and use as per the mediums specification.

Note: Do not heat the medium once the supplements have been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-335 Müller-Kauffmann Tetrathionate Broth Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light, at 2°C to 8°C.

Brilliant Green + Novobiocin Selective Supplement

Art. No. 06-017-LYO

Specification

Selective supplement for the isolation of *Salmonella* in Müeller-Kauffman Medium.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL). Each vial contains sufficient inhibitors to add to 500 mL of Tetrathionate Broth Base Art. No. 02-033 or Muller Kauffmann Broth Art. No. 02-335.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Brilliant green.....5,00 mg
Novobiocin, sodium salt.....20,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-335 Müller-Kauffmann Tetrathionate Broth Base

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light, at 2°C to 8°C.

Campylobacter Bolton Selective Supplement

Art. No. 06-131-LYO

DANGER

H: 3.4.R/1; H334-3.2/2; H315-3.3/2; H319-3.4.S/1; H317

P: P285-P261-P280-P305+P351+P338-P321-P501a

Specification

Selective supplement for *Campylobacter* isolation.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Campylobacter* Bolton Broth Base, Art. No. 02-688, in order to prepare 500 mL of *Campylobacter* Bolton Broth, according to the ISO Standard 10272.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Vancomycin	10,00 mg
Cefoperazone	10,00 mg
Trimethoprim	10,00 mg
Amphotericin B sulfate	5,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-688 *Campylobacter* Bolton Broth

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Campylobacter CCDA Selective Supplement

Art. No. 06-133-LYO

Specification

Selective supplement for *Campylobacter* isolation.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Charcoal Cefoperazone Deoxycholate Modified Agar Art. No. 01-685 in order to prepare 500 mL of *Campylobacter* Blood-free Selective Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Amphotericin B.....5,00 mg
Cefoperazone.....16,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-685 Charcoal Cefoperazone Deoxycholate Modified Agar (CCDA).

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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Campylobacter Growth Supplement

Art. No. 06-128-008

Specification

Supplement for the enhanced growth of *Campylobacter*.

Contents

The box contains 10 vials. Each vial content 8 mL of the solution sufficient to supplement 500 mL of *Campylobacter* Preston Agar Base (Art. No. 01-451 (or Art. No. 02-561)), in order to prepare 500 mL of *Campylobacter* Preston Agar.

Supplement vial contents

Amount required for 500 mL of complete medium.

Sodium pyruvate.....0,125 g
Sodium metabisulfite.....0,125 g
Ferrous sulfate.....0,125 g

Directions

Shake the liquid and add the solution to the prepare media.
Alternatively you may use a syringe, and transfer the solution to the prepare media.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-451 Nutrient Agar No. 2 (*Campylobacter* Preston Agar)
Art. No. 02-561 Nutrient Broth No. 2

Sterility control

No growth within 7 days at 32,5 ± 2,5ºC and 22,5 ± 2,5ºC in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2ºC to 8ºC.

Campylobacter Preston Modified Selective Supplement

Art. No. 06-135-LYO

Specification

Selective supplement for *Campylobacter* isolation.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Campylobacter* Preston Agar Base (Art. No. 01-451) in order to prepare 500 mL of *Campylobacter* Preston Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Polymyxin B sulfate	2500,00 IU
Rifampicin	5,00 mg
Trimethoprim	5,00 mg
Amphotericin B sulfate	5,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-451 Nutrient Agar No. 2 (*Campylobacter* Preston Agar)
Art. No. 02-561 Nutrient Broth No. 2

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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Campylobacter Preston Selective Supplement

Art. No. 06-130-LYO

Specification

Selective supplement for *Campylobacter* isolation.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Campylobacter* Preston Agar Base (Art. No. 01-451) in order to prepare 500 mL of *Campylobacter* Preston Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Polymyxin B sulfate	2500,00 IU
Rifampicin	5,00 mg
Trimethoprim	5,00 mg
Cycloheximide	50,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-451 Nutrient Agar No. 2 (*Campylobacter* Preston Agar)
Art. No. 02-561 Nutrient Broth No. 2

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Campylobacter Skirrow Selective Supplement

Art. No. 06-132-LYO

WARNING

H: 3.4.S/1; H317

P: P261-P280-P321-P363-P333+P313-P501a

Specification

Selective supplement for *Campylobacter* isolation.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Blood Agar Base No. 2 Art. No. 01-505 or Columbia Blood Agar Base Art. No. 01-034 in order to prepare 500 mL of Skirrow Selective Medium.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Vancomycin	5,00 mg
Trimethoprim	2,50 mg
Polymyxin B sulfate	1250,00 IU

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-034 Columbia Blood Agar
Art. No. 01-505 Blood Agar Base No. 2

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Chloramphenicol Selective Supplement

Art. No. 06-118CASE

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

Specification

Selective supplement for the isolation of fungi.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of Sabouraud Dextrose Agar Art. No. 01-165.

Vial contents

Amount required for 500 mL of complete medium.

Chloramphenicol..... 25,00 mg

Distilled water..... 5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile agar base cooled to 50°C. Homogenize and distribute the complete medium into suitable containers.

Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-165 Sabouraud Dextrose Agar.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Chloramphenicol Selective Supplement

Art. No. 06-118-LYO

DANGER

H: 3.6/1A; H350

P: P281-P201-P202-P308+P313-P405-P501a

Specification

Selective supplement for the isolation of fungi.

Contents

The box contains 5 vials with antibiotic and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Sabouraud Dextrose Agar Art. No. 01-165.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Chloramphenicol..... 25,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-165 Sabouraud Dextrose Agar.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Coliform CV Selective Supplement

Art. No. 06-140-LYO

Specification

Selective supplement for the detection of enterobacteria.

Contents

The box contains 5 vials with antibiotics and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Colinstant Agar, Art. No. 01-618 in order to prepare 500 mL of Colinstant Selective Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Cefsulodin.....2,50 mg
Vancomycin.....2,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-618 Colinstant Agar
Art. No. 01-695 Chromogenic Coliform Agar

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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m-CP Selective Supplement

Art. No. 06-125-LYO

Specification

Selective and differential supplement for the isolation of *Clostridium perfringens* according to the European Directive 12767/97.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL). Each vial is sufficient to supplement 500 mL of m-CP Agar Base, Art. No. 01-513, in order to prepare 500 mL of m-CP (*Clostridium perfringens*) Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

D-Cycloserine	200,00 mg
Polymyxin B sulfate	12,50 mg
3-Indoxyl-β-D-glucopyranoside	30,00 mg
Phenolphthalein bi-phosphate	50,00 mg
Iron III chloride	45,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-513 m-CP Agar Base

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

CP Selective Supplement for Gram Positive Cocci (CNA)

Art. No. 06-013CASE

DANGER

H: 3.4.R/1; H334-3.1.O/4; H302-3.4.S/1; H317
P: P285-P261-P280-P321-P342+P311-P501a

Specification

Selective supplement for Gram positive cocci in Blood Agar based media.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of Blood Columbia Agar Base Art. No. 01-034 or Blood Agar Base Art. No. 01-352 in order to prepare 500 mL of *Staphylococcus* and *Streptococcus* selective blood agar.

Vial contents

Amount required for 500 mL of complete medium.

Colistin sulfate	5,00 mg
Nalidixic acid, sodium salt	7,50 mg
Distilled water	5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile agar base cooled to 50°C. Homogenize and use as per each mediums specification.
Note: Do not heat the media once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-034 Blood Columbia Agar Base.
Art. No. 01-352 Blood Agar Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

CP Selective Supplement for Gram Positive Cocci (CNA)

Art. No. 06-013-LYO

DANGER

H: 3.4.R/1; H334-3.1.O/4; H302-3.4.S/1; H317
P: P285-P261-P280-P321-P342+P311-P501a

Specification

Selective supplement for the Gram positive cocci in the Blood Agar based media.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Blood Agar Art. No. 01-352 or to supplement 500 mL of Columbia Blood Agar Art. No. 01-034.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Colistin sulfate	5,00 mg
Nalidixic acid, sodium salt	7,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-034 Blood Columbia Agar Base.
Art. No. 01-352 Blood Agar Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

CT SMAC Selective Supplement

Art. No. 06-146-LYO

Specification

Selective supplement for the isolation of *E. coli* O157: H7.

Contents

The box contains 5 vials with the selective mixture and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of MacConkey Sorbitol Agar Art. No. 01-541.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Cefixime.....0,025 mg
Potassium tellurite.....1,250 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-541 MacConkey Sorbitol Agar

Sterility control

No growth within 7 days at 32,5 ± 2,5ºC and 22,5 ± 2,5ºC in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2ºC to 8ºC.

Cycloheximide Selective Supplement

Art. No. 06-022CASE

Specification

Inhibitor supplement for yeasts and moulds in culture media used to detect microorganisms in brewery samples.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of WL Nutrient Agar or Broth Art. No. 01-210 or 2-210 in order to prepare 500 mL of WL Differential Agar or Broth.

Vial contents

Amount required for 500 mL of complete medium.

Cycloheximide	2,00 mg
Sodium chloride (excipient)	100,00 mg
Distilled water	5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile agar base or sterile broth cooled to 50°C.

Note: Do not heat the media once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-210 WL Nutrient Agar
Art. No. 02-210 WL Nutrient Broth

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Cycloheximide Selective Supplement

Art. No. 06-022-LYO

Specification

Inhibitor supplement for yeasts and moulds in culture media used to detect microorganisms in brewery samples.

Contents

The box contains 5 vials with antibiotic and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of WL Nutrient Agar or Broth Art. No. 01-210 or 02-210 in order to prepare 500 mL of WL Differential Agar or Broth.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Cycloheximide	2,00 mg
Sodium chloride	100,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-210 WL Nutrient Agar
Art. No. 02-210 WL Nutrient Broth

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

D-Cycloserine Selective Supplement

Art. No. 06-116CASE

Specification

Selective supplement for the isolation of *Clostridium perfringens*.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 250 mL of Tryptose Sulfite Cycloserine Agar Base (TSC Agar) Art. No. 01-278.

Vial contents

Amount required for 250 mL of complete medium.

D-Cycloserine.....100,00 mg
Distilled water.....5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 250 mL of sterile agar base cooled to 50°C. If desired, add 20 mL of Sterile Egg Yolk Emulsion (Art. No. 06-016). Homogenize and distribute the complete medium into plates.
Note: Do not heat the medium once the supplements have been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-278 Tryptose Sulfite Cycloserine Agar (TSC Agar)

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

D-Cycloserine Selective Supplement

Art. No. 06-116-LYO

Specification

Selective supplement for the isolation of *Clostridium perfringens*.

Contents

The box contains 5 vials with antibiotic and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 250 mL of Tryptose Sulfite Cycloserine Agar Base (TSC Agar) Art. No. 01-278.

Inhibitor vial contents

Amount required for 250 mL of complete medium.

D-Cycloserine.....100,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-278 Tryptose Sulfite Cycloserine Agar (TSC Agar)

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Ferric Ammonium Citrate Supplement (250 mg)

Art. No. 06-112-LYO

Specification

Supplement to demostrate the production of esculetin form esculin hydrolysis.

Contents

The box contains 5 vials with reagent and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth according to Fraser Art. No. 02-496 in order to prepare 500 mL of Fraser Broth.

Supplement vial contents

Amount required for 500 mL of complete medium.
Ferric ammonium citrate.....250,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-496 *Listeria* Enrichment Broth Base according to Fraser

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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Ferric Ammonium Citrate Supplement (312 mg)

Art. No. 06-113CASE

Specification

Indicator for sulfide production as a result of sulfite reduction.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of Lactose Sulfite Broth Base Art. No. 02-519 in order to prepare 500 mL of Lactose Sulfite Broth.

Vial contents

Amount required for 500 mL of complete medium.
Ferric ammonium citrate.....312,00 mg
Distilled water.....5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and add aseptically to 500 mL of sterile broth base cooled to 50°C.
Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-519 Lactose Sulfite Broth Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Ferric Ammonium Citrate Supplement (312 mg)

Art. No. 06-113-LYO

Specification

Indicator for sulfide production as a result of sulfite reduction.

Contents

The box contains 5 vials with reagent and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Lactose Sulfite Broth Base Art. No. 02-519 in order to prepare 500 mL of Lactose Sulfite Broth.

Supplement vial contents

Amount required for 500 mL of complete medium.
Ferric ammonium citrate.....312,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-519 Lactose Sulfite Broth Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Legionella BCYE w/o Cysteine NO Growth Supplement

Art. No. 06-134-LYO

Specification

Buffering supplement with growth factors to complete the Medium *Legionella* BCYE w/o Cysteine Agar.

Contents

The box contains 2 vials with the inhibitor and 2 vials with the solvent (40 ml Sterile solvent).
Each vial is sufficient to supplement 500 mL of Legionella CYE Agar Base Art. No. 01-687 in order to prepare 500 mL of Legionella BCYE Selective Agar.

Supplement vial contents

Amount required for 500 mL of complete medium.

ACES Buffer	3,600 g
Potassium hydroxide	1,400 g
Ferric pyrophosphate	0,125 g
Potassium α–ketoglutarate	0,500 g

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-687 *Legionella* CYE Agar Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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Legionella BCYE Growth Supplement

Art. No. 06-137-LYO

Specification

Buffering supplement with growth factors to complete the Medium *Legionella* BCYE Agar.

Contents

The box contains 2 vials with the inhibitor and 2 vials with the solvent (40 ml Sterile solvent).
Each vial is sufficient to supplement 500 mL of Legionella CYE Agar Base Art. No. 01-687 in order to prepare 500 mL of Legionella BCYE Selective Agar.

Supplement vial contents

Amount required for 500 mL of complete medium.

ACES Buffer	3,600 g
Potassium hydroxide	1,400 g
Ferric pyrophosphate	0,125 g
L-Cysteine HCl	0,200 g
Potassium α–ketoglutarate	0,500 g

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-687 *Legionella* CYE Agar Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Legionella GVPC Selective Supplement

Art. No. 06-138-LYO

Specification

Selective supplement for *Legionella* isolation.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Legionella* CYE Agar Base Art. No. 01-687 in order to prepare 500 mL of *Legionella* BCYE Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Vancomycin	0,50 mg
Polymyxin B sulfate	40000,00 IU
Cycloheximide	40,00 mg
Glycine (ammonia free)	1,50 g

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-687 *Legionella* CYE Agar Base

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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Listeria Selective Supplement for Enrichment (FDA and IDF/ FIL)

Art. No. 06-107CASE

Specification

Selective supplement for *Listeria* enrichment according to FDA and FIL-IDF methods.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth Base according to Lovett Art. No. 02-498 in order to prepare 500 mL of *Listeria* enrichment broth according to the FDA and IDF/FIL.

Vial contents

Amount required for 500 mL of complete medium.

Nalidixic acid, sodium salt	20,00 mg
Cycloheximide	25,00 mg
Acriflavine	7,50 mg
Distilled water	5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile broth base cooled to 50°C.

Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-498 *Listeria* Enrichment Broth Base according to Lovett.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Listeria Selective Supplement for Enrichment (FDA and IDF/FIL)

Art. No. 06-107-LYO

Specification

Selective supplement for *Listeria* enrichment according to FDA and FIL-IDF methods.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth Base according to Lovett Art. No. 02-498 in order to prepare 500 mL of *Listeria* enrichment broth according to FDA and IDF/ FIL.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Nalidixic acid, sodium salt	20,00 mg
Cycloheximide	25,00 mg
Acriflavine	7,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-498 *Listeria* Enrichment Broth Base according to Lovett.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Listeria Selective Supplement for Primary Enrichment (Half Fraser) 225 mL

Art. No. 06-136-LYO

Specification

Selective supplement for the primary enrichment of *Listeria.*

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 225 mL of *Listeria* Enrichment Broth Base according to Fraser Art. No. 02-496 in order to prepare 225 mL of Half Fraser *Listeria* Primary Enrichment Medium.

Inhibitor vial contents

Amount required for 225 mL of complete medium.

Nalidixic acid, sodium salt	2,25 mg
Acriflavine	2,80 mg
Ammonium ferric citrate	112,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-496 *Listeria* Enrichment Broth Base according to Fraser

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Listeria Selective Supplement for Primary Enrichment (Half Fraser) 500 mL

Art. No. 06-145-LYO

Specification

Selective supplement for the primary enrichment of *Listeria.*

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth Base according to Fraser Art. No. 02-496 in order to prepare 500 mL of Half Fraser *Listeria* Primary Enrichment Medium.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Nalidixic acid, sodium salt	5,00 mg
Acriflavine	6,20 mg
Ammonium ferric citrate	250,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-496 *Listeria* Enrichment Broth Fraser

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Listeria Selective Supplement for Primary Enrichment (UVM I)

Art. No. 06-106CASE



DANGER

H: 3.4.R/1; H334-3.3/1; H318-3.4.S/1; H317-4.1.C/2; H411

P: P285-P261-P305+P351+P338-P310-P321-P501a

Specification

Selective supplement for the primary enrichment of *Listeria*.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth Base (UVM) Art. No. 02-472 in order to prepare 500 mL of *Listeria* primary enrichment medium.

Vial contents

Amount required for 500 mL of complete medium.

Nalidixic acid, sodium salt.....10,00 mg
Acriflavine.....6,00 mg
Distilled water.....5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile broth base cooled to 50°C.

Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-472 *Listeria* Enrichment Broth Base (UVM).

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Listeria Selective Supplement for Primary Enrichment (UVM I)

Art. No. 06-106-LYO



DANGER

H: 3.4.R/1; H334-3.3/1; H318-3.4.S/1; H317-4.1.C/2; H411

P: P285-P261-P305+P351+P338-P310-P321-P501a

Specification

Selective supplement for the primary enrichment of *Listeria*.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth Base (UVM) Art. No. 02-472 in order to prepare 500 mL of *Listeria* primary enrichment medium.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Nalidixic acid, sodium salt.....10,00 mg
Acriflavine.....6,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-472 *Listeria* Enrichment Broth Base (UVM).

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Listeria Selective Supplement for Secondary Enrichment (UVM II / Fraser)

Art. No. 06-111CASE

Specification

Selective supplement for the secondary enrichment of *Listeria*.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth Base (UVM) Art. No. 02-472 in order to prepare 500 mL of *Listeria* Secondary Enrichment Medium (UVM II formulation); or to supplement 500 mL of *Listeria* Enrichment Broth according to Fraser Art. No. 02-496 in order to prepare 500 mL of Fraser Broth.

Vial contents

Amount required for 500 mL of complete medium.

Nalidixic acid, sodium salt.....10,00 mg
Acriflavine.....12,50 mg
Distilled water.....5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile broth base cooled to 50°C.

Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-472 *Listeria* Enrichment Broth Base (UVM)
Art. No. 02-496 *Listeria* Enrichment Broth Base according to Fraser

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

DANGER

H: 3.4.R/1; H334-3.3/1; H318-3.2/2; H315-3.4.S/1; H317-4.1.C/2; H411
P: P285-P261-P305+P351+P338-P310-P321-P501a

Listeria Selective Supplement for Secondary Enrichment (UVM II / Fraser)

Art. No. 06-111-LYO

Specification

Selective supplement for the secondary enrichment of *Listeria*.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6mL Sterile distilled water). Each vial is sufficient to supplement 500 mL of *Listeria* Enrichment Broth Base (UVM) Art. No. 02-472 in order to prepare 500 mL of *Listeria* Secondary Enrichment Medium (UVM II formulation); or to supplement 500 mL of *Listeria* Enrichment Broth according Fraser Art. No. 02-496 in order to prepare 500 mL of Fraser Broth.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Nalidixic acid, sodium salt.....10,00 mg
Acriflavine.....12,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-472 *Listeria* Enrichment Broth Base (UVM)
Art. No. 02-496 *Listeria* Enrichment Broth Base according to Fraser

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

DANGER

H: 3.4.R/1; H334-3.3/1; H318-3.2/2; H315-3.4.S/1; H317-4.1.C/2; H411
P: P285-P261-P305+P351+P338-P310-P321-P501a

MUG Fluorescent Supplement

Art. No. 06-102CASE

Specification

Differential supplement for coliform detection.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of culture medium.

Vial contents

Amount required for 500 mL of complete medium.
MUG (4-Methil-Umbeliferil-β-D-Glucuronide).....50,00 mg
Distilled water.....5,00 mL

Directions

MUG Supplement may be added to most of the media that allow the growth of *Escherichia coli* for its identification. However, results will be more reliable in media that are selective for coliforms. In the presence of MUG and UV light, the *E. coli* growth fluorescence blue-green.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-047 CLED Agar
Art. No. 01-118 McConkey Agar
Art. No. 01-164 Violet Red Bile Agar
Art. No. 01-220 VRB Dextrose-Lactose
Art. No. 02-041 Brilliant Green Bile 2%
Art. No. 02-060 EC Broth
Art. No. 02-105 Lactose Broth
Art. No. 02-108 Triptose Lauryl sulfate
Art. No. 02-118 McConkey Broth
Art. No. 02-611 McConkey G, Broth

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

MUG Fluorescent Supplement

Art. No. 06-102-LYO

Specification

Differential supplement for coliform detection.

Contents

The box contains 5 vials with fluorochrome and 5 vials with solvent (6mL Sterile distilled water). Each vial is sufficient to supplement 500 mL medium.

Supplement vial contents

Amount required for 500 mL of complete medium.
MUG (4-Methil-Umbeliferil-β-D-Glucuronide).....50,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-047 CLED Agar
Art. No. 01-118 McConkey Agar
Art. No. 01-164 Violet Red Bile Agar
Art. No. 01-220 VRB Dextrose-Lactose
Art. No. 02-041 Brilliant Green Bile 2%
Art. No. 02-060 EC Broth
Art. No. 02-105 Lactose Broth
Art. No. 02-108 Triptose Lauryl sulfate
Art. No. 02-118 McConkey Broth
Art. No. 02-611 McConkey G, Broth

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Nalidixic Acid Selective Supplement

Art. No. 06-124CASE

Specification

Selective supplement for the isolation of *Pseudomonas aeruginosa* according to ISO 16266:2006 and EN 12780:2002 Standards.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of CN Selective Agar for Pseudomonas Art. No. 01-609.

Vial contents

Amount required for 500 mL of complete medium.
Nalidixic acid, sodium salt.....7,50 mg
Distilled water.....5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile agar base cooled to 50°C.
Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-609 CN Selective Agar Base for Pseudomonas.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Nalidixic Acid Selective Supplement

Art. No. 06-124-LYO

Specification

Selective supplement for the isolation of *Pseudomonas aeruginosa* according to ISO 16266:2006 and EN 12780:2002 Standards.

Contents

The box contains 5 vials with antibiotic and 5 vials with solvent (6mL sterile distilled water). Each vial is sufficient to supplement 500 mL of CN Selective Agar for Pseudomonas Art. No. 01-609.

Inhibitor vial contents

Amount required for 500 mL of complete medium.
Nalidixic acid, sodium salt.....7,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-609 CN Selective Agar Base for Pseudomonas.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Novobiocin Selective Supplement (10 mg)

Art. No. 06-139-LYO

Specification

Selective supplement for the isolation of *Salmonella* and/or *E. coli* 0157:H7.

Contents

The box contains 5 vials with the antibiotic and 5 vials with solvent (6 mL sterile distilled water). After its reconstitution each vial is sufficient to supplement 500 mL of Medium Base and obtain 500 mL of complete medium.

Inhibitor vial contents

Amount required for 500 mL of complete medium.
Novobiocin, sodium salt.....10,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 03-376 Rappaport Vassiliadis Modified Semisolid Medium Base
Art. No. 02-691 Tryptic Soy Broth Modified

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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Novobiocin Selective Supplement (20 mg)

Art. No. 06-147-LYO

Specification

Selective supplement for the isolation of *Salmonella* in Müeller-Kauffman Medium.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial contains sufficient inhibitor for 500 mL of Tetrathionate Broth Base Art. No. 02-629.

Inhibitor vial contents

Amount required for 500 mL of complete medium.
Novobiocin, sodium salt.....20,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-629 Tetrathionate Bile Brilliant Green Broth base.

Sterility control

No growth within 7 days at 32,5 ± 2,5ºC and 22,5 ± 2,5ºC in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2ºC to 8ºC.

Oxford Agar Selective Supplement

Art. No. 06-127-LYO



DANGER

H: 3.1.O/2; H300-3.7/1B; H360-3.5/2; H341-3.3/2; H319-3.4.
S/1; H317-4.1.C/2; H411
P: P261-P301+P310-P305+P351+P338-P321-P405-P501a

Specification

Selective supplement for the isolation of *Listeria* in using Oxford Agar.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Oxford Agar Base Art. No. 01-471 in order to prepare 500 mL of *Listeria* Selective Agar (Oxford formulation).

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Acriflavine	2,50 mg
Phosphomycin	5,00 mg
Sodium cefotetan	1,00 mg
Colistin	10,00 mg
Cycloheximide	200,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-471 Oxford Agar Base

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

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Oxytetracycline Selective Supplement

Art. No. 06-115CASE

Specification

Selective Supplement for the isolation of fungi.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of Sabouraud with Oxytetracycline Agar Base also known as Oxytetracycline Glucose Yeast Extract Agar (OGYEA) Art. No. 01-275.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Oxytetracycline HCl..... 50,00 mg
Distilled water..... 5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile agar base cooled to 50°C. Homogenize and distribute the complete medium into plates.

Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-275 Sabouraud with Oxytetracycline Agar (OGYEA)

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Oxytetracycline Selective Supplement

Art. No. 06-115-LYO

Specification

Selective Supplement for the isolation of fungi.

Contents

The box contains 5 vials with antibiotic and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Sabouraud with Oxytetracycline Agar Base Art. No. 01-275.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Oxytetracycline HCl..... 50,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-275 Sabouraud with Oxytetracycline Agar (OGYEA)

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Palcam Agar Selective Supplement

Art. No. 06-110CASE

Specification

Selective supplement for *Listeria* isolation using Palcam Agar.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of Palcam Agar Base Art. No. 01-470 in order to prepare 500 mL of *Listeria* Selective Agar (Palcam formulation).

Vial contents

Amount required for 500 mL of complete medium.

Acriflavine	2,50 mg
Polymyxin B sulfate	5,00 mg
Sodium ceftazidime	10,00 mg
Distilled water	5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile agar base cooled to 50°C.

Note: Do not heat the media once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-470 Palcam Agar Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Palcam Agar Selective Supplement

Art. No. 06-110-LYO

Specification

Selective supplement for *Listeria* isolation using Palcam Agar.

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Palcam Agar Base Art. No. 01-470 in order to prepare 500 mL of *Listeria* Selective Agar (Palcam formulation).

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Acriflavine	2,50 mg
Polymyxin B sulfate	5,00 mg
Sodium ceftazidime	10,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-470 Palcam Agar Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Polymyxin B Sulfate Selective Supplement

Art. No. 06-021CASE

Specification

Selective supplement for the isolation of *Bacillus cereus*.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of *Bacillus cereus* Agar Base Art. No. 01-262 or *Bacillus cereus* Selective Agar Art. No. 01-487 (Polymixin pyruvate egg yolk mannitol bromothymol blue agar) (PEMBA).

Vial contents

Amount required for 500 mL of complete medium.

Polymyxin B sulfate	50000,00 IU
Mannitol (excipient)	100,00 mg
Distilled water	5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 450 mL of sterile agar base cooled to 50°C. Also add 50 mL of sterile Egg Yolk Emulsion (Art. No. 06-016). Homogenize and pour the complete medium into the plates.
Note: Do not heat the media once the supplements have been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-262 *Bacillus cereus* Agar Base
Art. No. 01-487 *Bacillus cereus* Selective Agar (PEMBA)

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Polymyxin B Sulfate Selective Supplement

Art. No. 06-021-LYO

Specification

Selective supplement for the isolation of *Bacillus cereus*.

Contents

The box contains 5 vials with antibiotic and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Bacillus cereus* Agar Base Art. No. 01-262 or *Bacillus cereus* Selective Agar Art. No. 01-487.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Polymyxin B sulfate	50000,00 IU

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-262 *Bacillus cereus* Agar Base
Art. No. 01-487 *Bacillus cereus* Selective Agar (PEMBA)

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Sodium Disulfite (Sodium Meta-Bisulfite) for Bacteriology

Art. No. 06-114CASE

DANGER

H: 3.3/1; H318-3.1.O/4; H302+EUH031

P: P280-P264-P305+P351+P338-P310-P301+P312-P501a

Specification

Indicator for sulfide production as a result of sulfite reduction.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 mL of Lactose Sulfite Broth Base Art. No. 02-519 in order to prepare 500 mL of Lactose Sulfite Broth.

Vial contents

Amount required for 500 mL of complete medium.

Di-sodium sulfite..... 375,00 mg

Distilled water..... 5,00 mL

Directions

Mix the liquid with the powder by pressing down on the cap. Shake to dissolve and aseptically add the solution to 500 mL of sterile broth base cooled to 50°C.

Note: Do not heat the medium once the supplement has been added.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-519 Supplement for Lactose Sulfite Broth.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Sodium Disulfite (Sodium Meta-Bisulfite) for Bacteriology

Art. No. 06-114-LYO

DANGER

H: 3.3/1; H318-3.1.O/4; H302+EUH031

P: P280-P264-P305+P351+P338-P310-P301+P312-P501a

Specification

Indicator for sulfide production as a result of sulfite reduction.

Contents

The box contains 5 vials with reagent and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of Lactose Sulfite Broth Base Art. No. 02-519 in order to prepare 500 mL of Lactose Sulfite Broth.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Di-sodium sulfite..... 375,00 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 02-519 Supplement for Lactose Sulfite Broth.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

VCAT Selective Supplement

Art. No. 06-141-LYO

WARNING

H: 3.1.0/4; H302

P: P264-P270-P301+P312-P330-P501a

Specification

Selective supplement for the isolation of pathogenic *Neisseria*.

Contents

The box contains 5 vials with antibiotics and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of GC Agar Base Art. No. 01-310 in order to prepare 500 mL of GC Selective Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Vancomycin	1,00 mg
Colistin sulfate	3,75 mg
Amphotericin B	0,50 mg
Trimethoprim	1,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-310 GC Agar Base.

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

VCNT Selective Supplement

Art. No. 06-142-LYO

WARNING

H: 3.1.O/4: H302-3.4.S/1: H317

P: P261-P280-P321-P301+P312-P363-P501a

Specification

Selective supplement for the isolation of pathogenic *Neisseria*.

Contents

The box contains 5 vials with antibiotics (Vancomycin, Colistin, Nystatin and Trimethprim (VCNT)) and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of GC Agar Base in order to prepare 500 mL of Thayer-Martin Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Vancomycin	1,50 mg
Colistin sulfate	3,75 mg
Nystatin	6250,00 IU
Trimethoprim	2,50 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-310 GC Agar Base

Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Yersinia Selective Supplement

Art. No. 06-143-LYO

WARNING

H: 3.4.R/1; H334-4.1.A/1; H400-3.4.S/1; H317

P: P285-P261-P280-P321-P342+P311-P501a

Specification

Selective supplement for the isolation of *Yersinia enterocolitica.*

Contents

The box contains 5 vials with inhibitor and 5 vials with solvent (6 mL sterile distilled water). Each vial is sufficient to supplement 500 mL of *Yersinia* Cefsulodin, Irgasan, Novobiocin (CIN) Agar Base Art. No. 01-444 in order to prepare 500 mL of *Yersinia* CIN Agar.

Inhibitor vial contents

Amount required for 500 mL of complete medium.

Cefsulodin.....7,50 mg
Irgasan®.....2,00 mg
Novobiocin.....1,25 mg

Directions

Dissolve the pellet using the total volume of diluent contained in the provided vial, according to instructions.

Precautions

- This product is for laboratory use only.
- Do not use beyond stated expiry date.

Applicable media

Art. No. 01-444 *Yersinia* CIN Agar Base

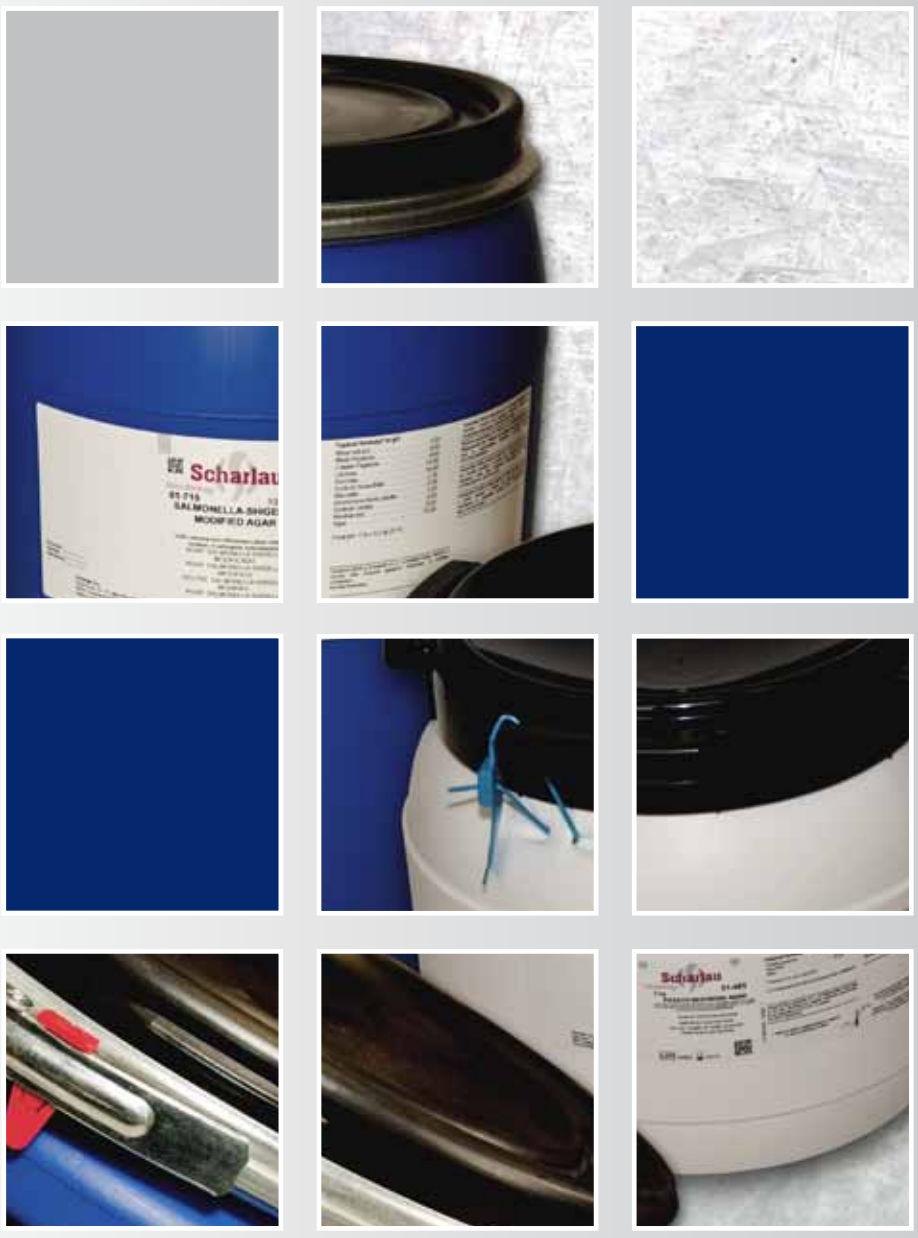
Sterility control

No growth within 7 days at 32,5 ± 2,5°C and 22,5 ± 2,5°C in Thioglycollate Fluid Medium USP and in Tryptone Soy Broth (TSB).

Storage

Keep tightly closed, away from bright light at 2°C to 8°C.

Scharlau



Culture Media Ingredients

Culture Media Ingredients

Definitions

EXTRACTS are concentrated preparations that are liquid, solid or semisolid in nature usually obtained from dried vegetal or animal matter. For some preparations, the matter extracted may undergo a preliminary treatment, for example, inactivation of enzymes, grinding or defatting. Extracts are prepared by maceration, percolation or other, validated methods, using water, ethanol or another suitable solvent. After extraction unwanted matter is removed if necessary.

PEPTONES are complex water-soluble mixes of free amino acids, peptides, sugars, mineral salts and other components obtained by acid, alkaline or enzymatic hydrolysis of protein substrates. Their very variable characteristics depend on:

- The nature of the substrate(s).
- The nature of the hydrolysis: enzyme(s), alkali(s), acid(s), and the duration of the hydrolysis.
- The technique of purification (filtration, ultrafiltration, …)
- Other operating conditions used in the production process.

The term peptone is more commonly applied to the hydrolysates obtained by enzymatic digestion. The enzymes most frequently used are:

Papain which acts on the links adjacent to arginine, lysine, phenylalanine and glycine. *Bromelain* and *Ficin* are also used because they act in a similar manner to papain but act a different temperature and pH and are sourced from other plants.

Pepsin, acts on the links adjacent to phenylalanine or leucine.

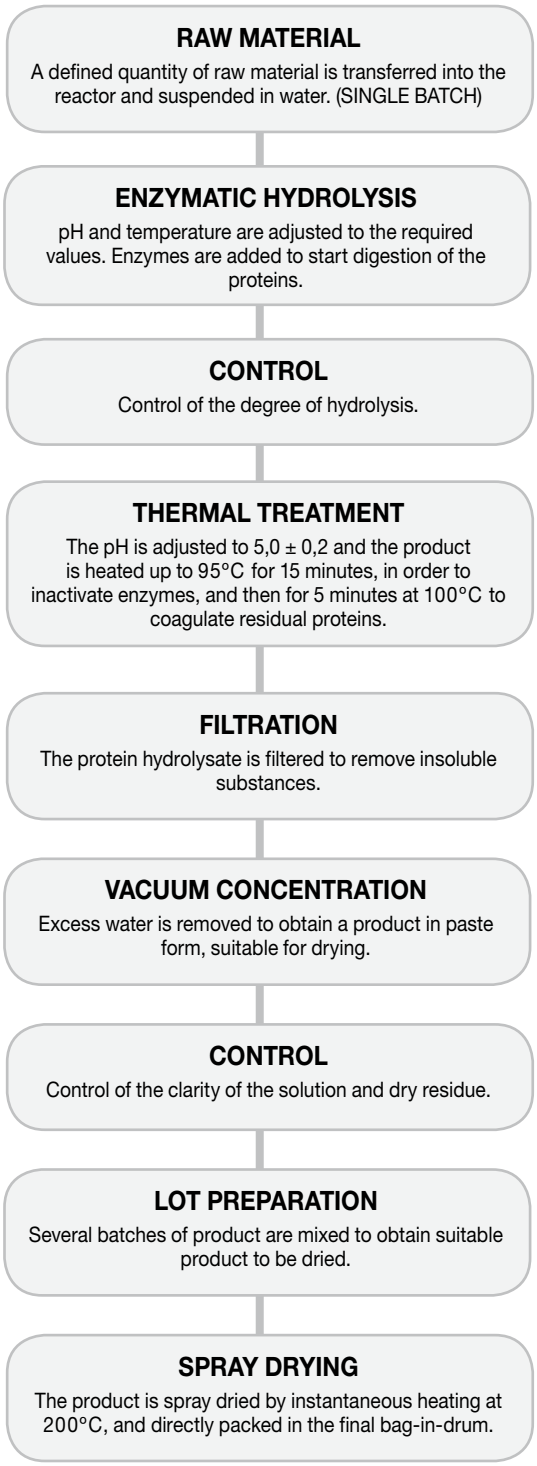
Pancreatin (a variable mixture of *trypsin* and *chymotrypsin*) acts on the links adjacent to arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine.

Microbial Proteases are obtained from microbial cultures as extracellular enzymes and are also used in the production of peptones. Acid and Neutral microbial proteases works in a similar way to papain while alkaline microbial work in a similar way to pancreatin.

Papain is obtained from plant material while pepsin and pancreatin, are animal enzymes, of porcine origin.

Production process

The general production process is schematized in the following flow-chart.



Culture Media Ingredients

Warranty of health and origin

All the animal tissue raw materials used in the manufacture of Scharlau's Peptones come from approved slaughterhouses and are covered by certificates obtained from the veterinary authorities.

The country of origin of bovine animal tissues and casein used in the manufacture of each batch of peptone is specified in the health certificate. These documents certify that animals from which tissues have been removed were in good health and fit for human consumption. They are used by the Scharlau Microbiology Quality Control Department to issue a health certificate for each batch of product manufactured: a copy of this certificate is submitted to our customers upon each delivery.

Analytical data and control methods

All data figuring in the following documentation results from the analysis of a significant number of batches of each product. These data may be:

Typical data, which are in fact average values (comparative tables and technical data sheets).

Norms of acceptability (Technical data sheets).

Bacteriological controls as well as most general physical-chemical controls are carried out on each batch systematically. The other characteristics are verified according to routine testing.

For the control methodology, a norm is defined specifically for each characteristic. When a pharmacopeial monograph (Eur. Pharm. 4th ed. 2002; USP 25th Ed. 2002) is available, it is adopted as a routine method. If not another documented methodology is accepted.

Typical data are shown in the following Tables (tables 1 to 6) and in the specific description of each product. The information contained in this publication is based on our own research and development work and is to the best of our knowledge true and accurate. Users should however conduct their own tests to determine the suitability of our products for their own purpouses. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for the infringement of any patents.

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media CRC Press London.
- BRYDSON, E.Y. (1978) Natural and Synthetic Culture Media for Bacteria. In M. Rechcigl Jr. (ed) Handbook Series in Nutrition and Food. Section G. Vol III. CRC Press. Cleveland
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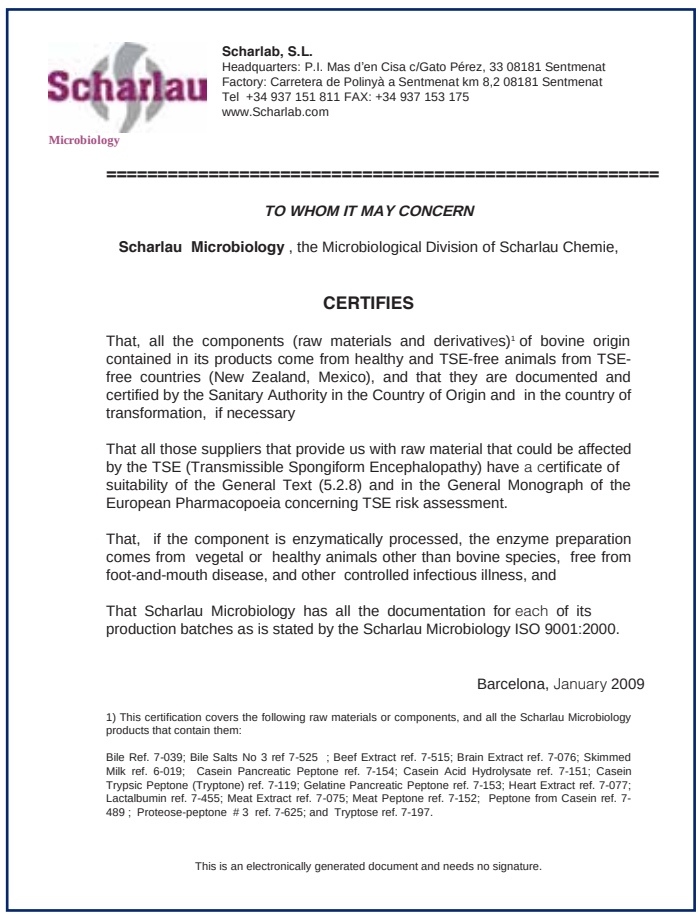

Scharlau
Microbiology

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TO WHOM IT MAY CONCERN

Scharlau Microbiology , the Microbiological Division of Scharlau Chemie,

CERTIFIES

That, all the components (raw materials and derivatives)[1] of bovine origin contained in its products come from healthy and TSE-free animals from TSE-free countries (New Zealand, Mexico), and that they are documented and certified by the Sanitary Authority in the Country of Origin and in the country of transformation, if necessary

That all those suppliers that provide us with raw material that could be affected by the TSE (Transmissible Spongiform Encephalopathy) have a certificate of suitability of the General Text (5.2.8) and in the General Monograph of the European Pharmacopoeia concerning TSE risk assessment.

That, if the component is enzymatically processed, the enzyme preparation comes from vegetal or healthy animals other than bovine species, free from foot-and-mouth disease, and other controlled infectious illness, and

That Scharlau Microbiology has all the documentation for each of its production batches as is stated by the Scharlau Microbiology ISO 9001:2000.

Barcelona, January 2009

1) This certification covers the following raw materials or components, and all the Scharlau Microbiology products that contain them:

Bile Ref. 7-039; Bile Salts No 3 ref 7-525 ; Beef Extract ref. 7-515; Brain Extract ref. 7-076; Skimmed Milk ref. 6-019; Casein Pancreatic Peptone ref. 7-154; Casein Acid Hydrolysate ref. 7-151; Casein Trypsic Peptone (Tryptone) ref. 7-119; Gelatine Pancreatic Peptone ref. 7-153; Heart Extract ref. 7-077; Lactalbumin ref. 7-455; Meat Extract ref. 7-075; Meat Peptone ref. 7-152; Peptone from Casein ref. 7-489 ; Proteose-peptone # 3 ref. 7-625; and Tryptose ref. 7-197.

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Table 1. PHYSICAL AND CHEMICAL CHARACTERISTICS (1) Typical Data

Art. No.	Product	Maximum Solubility (g/100 ml)	Turbidity (NTU) 1 % Soln	Filtrability (mg/cm²)	pH	Stability after autoclave	Osmolality (mmol / kg)	Loss on drying (%)
	EXTRACTS							
07-515	Beef Extract	18,0	1,8	500	6.8	no precipitate	128	3,3
07-080	Malt Extract	60	3,3	ND	5,3	no precipitate	97	2,6
07-075	Meat Extract	7,0	1,7	700	6,7	no precipitate	135	3,5
07-079	Yeast Extract	11,0	1,4	2500	7,1	no precipitate	115	4,7
	CASEIN PEPTONES							
07-151	Casein Acid Hydrolysate	41.0	0,4	2800	6,7	no precipitate	302	3,0
07-154	Casein Pancreatic Peptone	41,0	0,6	2600	6.9	no precipitate	110	3,4
07-119	Casein Trypsic Peptone (Tryptone)	40,5	0,5	2000	7,0	no precipitate	95	4,9
07-489	Peptone from casein	40,0	0,4	2400	7,0	no precipitate	93	3,1
	VEGETAL PEPTONES							
07-155	Soy Peptone	17,0	1,0	1300	7,1	no precipitate	115	3,7
07-620	Potato Peptone	0,4	0,8	1700	7,1	no precipitate	120	3,5
	ANIMAL PEPTONES							
07-153	Gelatin Peptone	ND	1,3	800	7,1	no precipitate	75	3,0
07-455	Lactalbumin hydrolysate	20	0,7	5200	6,5	no precipitate	120	4,3
07-152	Meat Peptone	15,0	0,6	1100	6,9	no precipitate	160	2,4
07-625	Proteose Peptone n. 3	25	2	750	6,7	no precipitate	132	3,6
07-197	Tryptose	30	0,9	2100	7,2	no precipitate	105	3,5

Table 2. PHYSICAL AND CHEMICAL CHARACTERISTICS (2) Typical Data

Art. No.	Product	Obtained by	α-A.N. (%)	T.N. (%)	AN/TN	Molecular Weight Distribution (Dalton) in %					Average mw (daltons)
						< 300	300-500	500-1000	1000-10000	>10000	
	EXTRACTS										
07-515	Beef Extract	Enzymatic hydrolysis	4,8	12,5	0,38	29,0	19,2	31,7	32,0	0	1040
07-080	Malt Extract	Purification	ND	ND	ND	ND	ND	ND	ND	ND	ND
07-075	Meat Extract	Enzymatic hydrolysis	4,0	12,1	0,33	28,1	21,6	22,3	28,0	0	1350
07-079	Yeast Extract	Autolysis	5,1	10,9	0,46	59,1	17,2	13,0	10,7	0	438
	CASEIN PEPTONES										
07-151	Casein Acid Hydrolysate	Acid hydrolysis	5,7	7,8	0,73	57,7	38,8	2,5	1	0	379
07-154	Casein Pancreatic Peptone	Pancreatic hydrolysis	4,7	13,1	0,35	20,1	27,9	32,8	19,2	0	695
07-119	Casein Trypsic Peptone	Pancreatic hydrolysis	4,3	12,6	0,34	31,7	37,1	23,0	8,2	0	490
07-489	Peptone from casein	Pancreatic hydrolysis	6,1	12,1	0,26	19,9	30,2	32,1	17,8	0	668
	VEGETAL PEPTONES										
07-620	Potato Peptone	Enzymatic hydrolysis	5,5	10,1	0,54	55,3	18,7	17,7	8,0	0,3	263
07-155	Soy Peptone	Papaic hydrolysis	3,1	9,8	0,31	37,7	31,0	21,8	9,5	0	476
	ANIMAL PEPTONES										
07-153	Gelatin Pancreatic Peptone	Pancreatic hydrolysis	2,7	15,8	0,17	8,9	18,9	38,8	33,5	0	1111
07-455	Lactalbumin hydrolysate	Pancreatic hydrolysis	5	11,7	0,42	23,8	68,6	7,3	0,3	0	461
07-152	Meat Peptone	Enzymatic hydrolysis	3,4	12,7	0,26	14,3	20,3	33,7	31,7	0	985
07-625	Proteose Peptone n. 3	Enzymatic hydrolysis	4,1	11,9	0,34	18,4	24,3	32,3	25,1	0	890
07-197	Tryptose	Enzymatic hydrolysis	4,4	12,1	0,36	43,1	26,4	21,3	9,1	0	473

Table 3. PHYSICAL AND CHEMICAL CHARACTERISTICS (3) Mineral Components

Art. No.	Product	Residue on ignition	Chloride (as NaCl)	Phosphate (as P_2O_5)	Sulfate (%)	Calcium (ppm)	Magnesium (ppm)	Potassium (%)	Sodium (%)
	EXTRACTS								
07-515	Beef Extract	25,7	20,4	2,2	ND	0,16	0,03	1,90	2,10
07-080	Malt Extract	2,7	0,6	ND	ND	ND	ND	ND	ND
07-075	Meat Extract	15,4	5,0	2,3	ND	0,13	0,04	2,5	2,4
07-079	Yeast Extract	14,9	0,2	3,5	0	0,02	0,03	4,8	0,11
	CASEIN PEPTONES								
07-151	Casein Acid Hydrolysate	33,8	2,4	77	ND	0,02	ND	ND	15,7
07-154	Casein Pancreatic Peptone	10,1	4,8	1,7	0,18	0,08	0,01	0,02	4,0
07-119	Casein Trypsic Peptone (Tryptone)	12,7	0,2	1,8	0,2	0,09	0,01	0,03	3,8
07-489	Peptone from casein	13,8	4,4	1,8	0,22	0,10	0,01	0,50	3,6
	VEGETAL PEPTONES								
07-155	Soy Peptone	18,0	0,5	0,86	0,37	0,11	0,13	3,2	3,0
07-620	Potato Peptone	20,5	9,5	ND	ND	0,60	ND	0,0	4,80
	ANIMAL PEPTONES								
07-153	Gelatin Pancreatic Peptone	6,9	3,7	0,25	2,4	0,01	< 0,01	0,13	2,0
07-455	Lactalbumin hydrolysate	7,5	0,6	1,20	0,10	0,67	0,03	0,9	0,8
07-152	Meat Peptone	6,0	1,8	0,45	1,4	0,40	0,02	0,70	0,70
07-625	Proteose Peptone n. 3	15,2	3,4	2,31	0,1	0,09	0,03	2,05	2,5
07-197	Tryptose	12,6	2,3	1,50	0,17	0,07	0,02	0,89	1,9

Table 4. PHYSICAL AND CHEMICAL CHARACTERISTICS (4) Mineral Components

Art. No.	Product	Arsenic (ppm)	Cadmium (ppm)	Copper (ppm)	Iron (ppm)	Manganese (ppm)	Mercury (ppm)	Lead (ppm)	Zinc (ppm)
	EXTRACTS								
07-515	Beef Extract	1,4	24,00	0,6	36	1,1	0,31	5,00	37
07-080	Malt Extract	< 0,1	< 0,10	0,3	6	1,0	0,10	< 0,20	8
07-075	Meat Extract	1,5	0,29	0,8	48	1,3	0,36	6,00	43
07-079	Yeast Extract	< 0,5	< 0,10	1,25	62	0,7	< 0,05	< 0,20	60
	CASEIN PEPTONES								
07-151	Casein Acid Hydrolysate	ND	ND	ND	ND	ND	ND	ND	ND
07-154	Casein Pancreatic Peptone	1,4	0,27	< 0,2	17	0,7	0,78	2,50	36
07-119	Casein Trypsic Peptone (Tryptone)	1,3	0,25	<0,2	15	0,6	0,63	2,43	36
07-489	Peptone from casein	1,2	0,24	< 0,2	14	0,5	0,53	2,38	37
	VEGETAL PEPTONES								
07-155	Soy Peptone	1,1	0,26	0,2	23	2,5	0,38	2,51	19
07-620	Potato Peptone	ND	ND	ND	ND	ND	ND	ND	ND
	ANIMAL PEPTONES								
07-153	Gelatin Pancreatic Peptone	1,3	0,15	< 0,1	10	0,4	0,71	0,73	2
07-455	Lactalbumin hydrolysate	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
07-152	Meat Peptone	1,5	0,34	< 0,2	16	0,5	0,36	1,77	11
07-625	Proteose Peptone n. 3	1,3	0,24	0,67	40	1,0	0,42	3,85	44
07-197	Tryptose	1,1	0,20	0,9	77	0,6	0,54	2,05	41

Table 5. TOTAL AMINO ACIDS (g / 100 g) Typical Values

Art. No.	Product	ALA	ARG	ASP	CYS	GLU	GLY	HIS	ILE	LEU	LYS	MET	PHE	PRO	SER	THR	TRP	TYR	VAL	TOTAL
	EXTRACTS																			
07-515	Beef Extract	4,97	4,42	6,14	0,70	12,28	7,09	2,90	2,63	4,86	4,49	1,25	2,59	7,31	2,38	2,04	0,73	1,27	3,45	71,50
07-080	Malt Extract	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
07-075	Meat Extract	3,99	3,84	6,17	0,73	12,38	3,70	1,73	3,03	4,81	5,25	1,99	2,91	4,48	3,08	3,24	0,70	1,67	3,91	67,61
07-079	Yeast Extract	4,06	2,77	5,95	0,60	10,95	2,53	1,09	2,56	3,65	4,22	0,80	2,45	2,15	2,70	2,63	0,84	0,82	3,13	53,90
	CASEIN PEPTONES																			
07-151	Casein Acid Hydrolysate	1,90	1,70	3,50	0,20	10,90	1,00	1,20	2,20	3,40	3,80	1,20	1,80	5,60	2,80	2,10	0	0,40	3,00	46,70
07-154	Casein Pancreatic Peptone	2,40	2,97	5,51	0,67	16,55	1,60	2,17	4,44	6,54	6,40	2,46	3,80	8,51	4,49	3,59	0,96	1,89	5,40	80,35
07-119	Casein Trypsic Peptone (Tryptone)	2,70	3,20	5,50	0,40	18,10	1,70	2,40	4,40	7,10	5,70	2,50	4,00	8,40	4,30	3,60	1.2	1,70	4,70	80,40
07-489	Peptone from casein	2,54	2,80	5,32	0,61	16,30	1,57	2,06	3,94	6,72	5,98	2,16	3,74	7,46	4,13	3,35	0,85	1,99	4,99	76,51
	VEGETAL PEPTONES																			
07-155	Soy Peptone	2,38	3,73	5,80	1,39	9,90	2,38	1,20	2,20	3,30	3,37	0,72	2,10	2,35	2,77	2,10	0,62	1,30	2,35	49,96
07-620	Potato Peptone	3,30	3,80	7,80	0	7,30	3,20	1,60	3,80	6,30	5,10	0	3,70	3,40	3,20	3,70	0	2,80	4,30	63,30
	ANIMAL PEPTONES																			
07-153	Gelatin Pancreatic Peptone	7,56	6,91	5,06	0,13	8,70	20,02	0,54	1,09	2,12	3,30	0,87	2,07	11,06	3,19	1,53	0,03	0,65	2,04	76,87
07-455	Lactalbumin hydrolysate	4,65	2,17	8,87	2,19	14,00	2,17	1,45	4,89	9,82	7,57	2,17	3,25	3,76	4,13	4,47	1,39	0,75	4,90	82,60
07-152	Meat Peptone	9,05	6,75	5,10	0,30	11,20	21,44	0,77	1,49	3,31	4,41	1,32	2,99	12,84	3,03	1,74	0,19	0,61	2,89	89,43
07-625	Proteose Peptone n. 3	3,97	1,49	5,91	0,64		2,82	1,80	3,41	5,28	5,34	1,95	2,19	5,41	3,45	3,21	0,50	1,57	4,16	53,10
07-197	Tryptose	2,89	2,76	5,27	0,45	14,26	1,72	1,74	3,25	5,60	3,92	1,65	3,22	5,80	3,50	2,96	1,20	1,59	3,85	65,63

Table 6. FREE AMINO ACIDS (g / 100 g) Typical Values

Art. No.	Product	ALA	ARG	ASP	CYS	GLU	GLY	HIS	ILE	LEU	LYS	MET	PHE	PRO	SER	THR	TRP	TYR	VAL	TOTAL
	EXTRACTS																			
07-515	Beef Extract	1,80	1,80	1,00	0	3,00	0,70	0,45	1,20	2,80	1,96	0,52	1,70	0,40	0,90	0,80	0,50	0,40	1,30	21,23
07-080	Malt Extract	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
07-075	Meat Extract	1,69	1,66	0,88	0,02	2,94	0,57	0,40	1,15	2,78	2,12	0,53	1,66	0,42	0,92	0,83	0,51	0,42	1,34	20,84
07-079	Yeast Extract	3,80	1,50	1,80	0,08	6,80	1,30	0,60	2,20	3,60	1,80	0,70	2,10	1,06	1,90	1,70	0,62	0,75	2,50	34,81
	CASEIN PEPTONES																			
07-151	Casein Acid Hydrolysate	1,90	1,70	3,80	0,20	10,9	1,00	1,20	1,60	3,40	3,80	1,20	1,80	5,60	2,80	2,10	0	0,20	2,40	45,60
07-154	Casein Pancreatic Peptone	0,22	2,30	0	0,21	0,06	0,02	0,18	0,50	2,72	3,43	0,21	1,81	0	0,15	0,36	0,88	0,24	6,11	19,40
07-119	Casein Trypsic Peptone (Tryptone)	0,20	3,00	0,01	0	0,40	0,30	0,30	0,60	3,50	3,10	0,70	2,50	0,30	0,40	0,50	1,20	0,10	0,90	18,01
07-489	Peptone from casein	0,47	1,90	0,19	0,15	0,80	0,13	0,22	0,46	3,20	2,60	0,57	1,90	0,15	0,31	0,42	0,64	0,30	7,61	22,02
	VEGETAL PEPTONES																			
07-155	Soy Peptone	0,84	1,19	0,42	0,16	1,51	0,64	0,28	0,47	1,44	0,81	0,30	0,66	0,21	0,62	0,25	0,24	0,34	5,41	15,79
07-620	Potato Peptone	1,50	2,30	1,30	0	1,90	0,80	1,60	2,20	4,90	2,90	0	2,80	0,20	3,20	2,50	0	2,30	2,80	33,20
	ANIMAL PEPTONES																			
07-153	Gelatin Pancreatic Peptone	0,09	3,76	0,10	0,13	0,10	0,18	0,11	0,20	0,59	1,31	0,21	0,73	0,10	0,06	0,36	0,03	0,45	0,17	8,68
07-455	Lactalbumin hydrolysate	3,42	1,90	1,01	2,19	2,80	0,62	0,96	3,91	9,44	5,73	2,12	3,25	0,56	2,34	2,67	1,39	0,54	4,62	49,46
07-152	Meat Peptone	1,00	2,56	0,43	0,21	1,10	1,15	0,15	0,46	1,15	1,18	0,28	0,85	0,30	0,37	0,25	0,11	0,53	4,61	16,69
07-625	Proteose Peptone n. 3	1,54	1,96	0,74	0,06	2,62	0,55	0,18	0,55	3,02	2,44	0,26	1,89	0,30	0,40	0,42	0,50	0,39	2,25	20,07
07-197	Tryptose	1,33	1,95	0,63	0,03	3,05	0,57	0,30	0,96	2,87	2,07	0,57	1,98	0,45	0,81	0,75	0,72	0,36	1,2	20,57

Agar-Agar
Art. No. 07-490

Specification

Gelling agent with a high gel strength but low degree of purification.

Description

Agar is the dried, hydrophilic, colloidal substance extracted from algae known as Agarophytes (several species and genera of the Class *Rhodophyceae*). It consists of two polysaccharides, agarose and agaropectin, in a variable proportions depending on the geographical zone of origin.

Agar-Agar is a solidifying agent with almost the same gelling strength as the Bacteriological Agar but with less purification. It has greater opacity and has a higher salt content. Its use in culture media is recommended only when transparency and clarity is not a critical requirement.

The most important characteristics are:

Physical data

Melting point	85 ± 5 °C
Gelling point	35 ± 5 °C
Gel strength (*Nikan*)	750 ± 50 g/cm^2
Dissolution time (at 100°C)	1,00 min
Turbidity	< 36 NTU

Chemical data

pH of 1,5% solution at 25°C	6,9 ± 0,1
Particle size	< 0,3 mm
Loss on drying	< 19,0 % (w/w)
Residue on ignition	6,5 % (w/w)
Acid insoluble ash	< 0,5 % (w/w)

Note: Data are average values, which may vary from batch to batch.

Microbiological limits

Total aerobic microbial count	< 5000 cfu/g
Heat resistant thermophiles	< 1 cfu/10 g
Heat resistant mesophiles	< 1 cfu/10 g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 500 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella spp	absent in 25 g

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Agar Bacteriological

Art. No. 07-004

Specification

Gelling agent selected for solidifying the microbiological culture media.

Description

Agar is the dried, hydrophilic, colloidal substance extracted from algae known as Agarophyites (several species and genera of the Class *Rhodophyceae*). It consists of two polysaccharides, agarose and agaropectine, in a variable proportion depending on the geographical zone of origin.

Bacteriological Agar is a solidifying agent selected and prepared by mixing different agars from several geographical zones of origin. It is especially recommended for gelling microbiological culture media where excellent transparency and clarity is required.

The most important characteristics are:

Physical data

Melting point	88 ± 3 °C
Gelling point	35 ± 3 °C
Gel strength (*Nikan*)	700 ± 50 g/cm^2
Dissolution time (at 100°C)	1,00 min
Turbidity	< 9 NTU

Chemical data

pH of 1,5% solution at 25°C	6,3 - 6,6
Particle size	< 0,3 mm
Loss on drying	< 11,00% (w/w)
Residue on ignition	< 3,50% (w/w)
Acid insoluble ash	< 0,03% (w/w)

Note: Data are average values, which may vary from batch to batch.

Microbiological limits

Total aerobic microbial count	< 1000 cfu/g
Heat resistant thermophiles	< 1 cfu/10 g
Heat resistant mesophiles	< 1 cfu/10 g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 500 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella spp	absent in 25 g

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Beef Extract

Art. No. 07-515

Specification

Culture Media Ingredient.

Description

For a long time beef extract has been the basic component of culture media, and initially it substituted meat infusions due to its ease-of-use. The trend now is to substitute it with peptones and different mixtures containing a more defined composition, as they allow for greater reproducibility. The dehydrated from is easier to use than the paste form, and requires a lesser amount to obtain the same effect.

Scharlau Microbiology Beef Extract is obtained from fat and tendon free beef muscle enzymatically predigested. Its production also includes the elimination of fermentable sugars. Beef extract solutions are clear, slightly coloured with a neutral pH value. In culture media they are used in concentrations varying from 0,3-0,5%.

The bovine constituents used in its preparation belong to Category 4 of the WHO classification. The bovine tissues are sourced from New Zealand, and come from herds accredited free from Bovine Spongiform Encephalopathy (BSE) and foot-and-mouth disease by the Veterinary Authorities. The enzymatic preparation is of porcine origin. The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC.

The manufacturing process includes heating treatment at 123°C for 30 minutes and instantaneous heating at 200°C on spray drying.

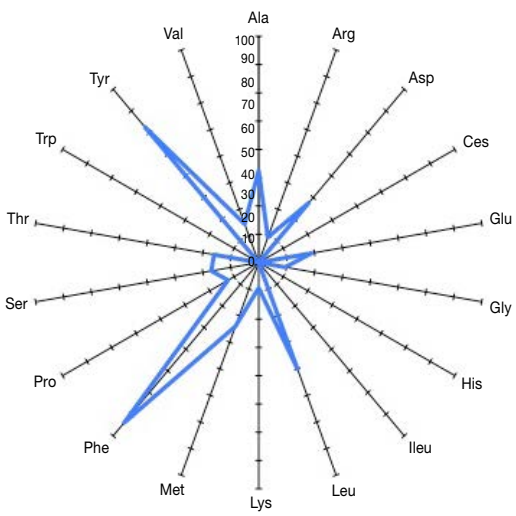
Microbiological limits

Total aerobic microbial count..... < 10000 cfu/g
Coliforms..... < 10 cfu/g
Moulds and yeasts..... < 20 cfu/g
Staphylococcus aureus..... absent in 10 g
Escherichia coli..... absent in 10 g
Salmonella spp..... absent in 25 g

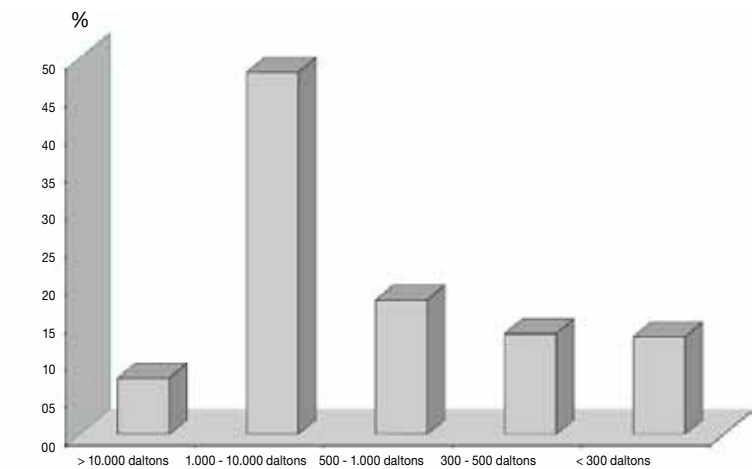
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-515 Beef Extract
Amino Acids (Free/Total) x 100



07-515 Beef Extract
Molecular weight distribution



Bile

Art. No. 07-039

Specification

Selective agent used in enterobacterial isolation media.

Description

Ox bile powder is obtained by spray drying fresh bile at high temperature, whilst ensuring the maintenance of the most important characteristics or properties of fresh bile. 1 g of ox bile powder corresponds to approx. 10 g of fresh bile. The manufacturing process includes rapid heating at 200°C by spray drying.

The bile used in this preparation is obtained from bovine herds free from Transmissible Spongiform Encephalopathy and Food-and-Mouth disease. It is derivated from animals which are raised and slaughtered in the same region, and which are in good health suitable for human consumption certified by Veterinary Authorities both *ante-mortem* and *post-mortem.*

In the culture media ox bile is employed at concentrations ranging from 1-2%. It acts as an inhibitor of non enteric microbial flora and is used as a selective agent in enterobacterial isolation media. Ox bile solutions at low concentrations are clear and have a slight colour, but at higher concentrations they are opalescent and are more darkly coloured.

Dry powder has a colour varying form yellow-beige to yellow-green, and it gives a transparent 5% solution in water, that has a pH between 6 and 7,5. In alcoholic solutions, (ethanol 84%) it provides less than 0,1% of insoluble substances.

Physical data

Solubility 5% in water	total
Solubility 5% in ethanol 84%	< 0,1% (w/w) of insoluble substances
Stability after autoclave	without visible precipitates

Chemical data

Loss on drying	< 6,0% (w/w)
Bile acids (in the dry matter)	> 4,5% (w/w)
pH solution 5%	6,0 - 7,5

Note: Data are average values, which may vary from batch to batch.

Microbiological limits

Total aerobic microbial count	<1000 cfu/g

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Bile Salts No.3

Art. No. 07-525

Specification

Used as a selective agent in enterobacterial isolation media.

Description

Bile salts for microbiological applications are obtained from fresh animal (sheep, pig) bile by precipitation with hydrochloric acid, in a process that removes pigments and other toxic substances and concentrates the bile salts. Nevertheless standardization is very difficult because the composition of the final mixture depends not only on the process, but on the raw material that can be variable.

In a normal preparation of bile salts several components such as gluconate, taurocholate, cholate, deoxycholate, and others, can be identified. The inhibitory character of the mixture depends on the relative ratios of all these different substances. Usually, in the microbiological culture media, bile salts are made up to a concentration of 0,5% (w/v) to inhibit the growth of Gram positive bacteria.

Bile salts No. 3 has a higher level of purification and concentration of actives substances and can be used at less than one third the concentration for microbiological applications. Bile Salts No. 3 is usually used in a concentration of 0,15% in culture media due to this increased efficacy.

Bile Salts No. 3 is derived from bovine bile sourced from Brazil, India and New Zealand, as well as from ovine bile sourced from New Zealand. Bile is not considered a TSE risk material according to EMEA/410/01 Rev. 2 (dated Oct. 2003), but all the material used for the production of bile salts are sourced from healthy animals which have passed *ante-mortem* and *post-mortem* inspection and have been found free of contagious diseases of quarantine concern by the Sanitary Authority in the country of origin. The manufacturing process includes an alkaline hydrolysis at high temperature (> 6% w/v NaOH at 125°C for > 8 hours). Subsequent purification steps involve prolonged boiling in organic solvents, addition of aqueous sodium hydroxide and boiling of the solution and spray drying by instantaneous heating at high temperature (> 140°C).

Each batch of The Scharlau Bile Salts No. 3 is standardized in order to supply a product as homogeneous as possible.

Dry powder is free flowing and white in appearance, with a bitter in odour and taste, but is a clear, transparent and pale yellow 2% solutions in water, with an alkaline pH (pH 8,0) and therefore its addition to culture media may require pH adjustment. When this product is used as a component in culture media it is advisable never to exceed 0,3% (w/v) concentration.

The most important characteristics:

Physical - chemical characteristics

Dry matter

- Appearance: White powder with a bitter odour/taste
- Water content: < 5 % (w/w)
- Solubility: Water-soluble

2% Solution

- Appearance: Transparent, with a pale yellow straw colour
- Reaction: Slightly alkaline (8,0 ± 0,2). Its addition to the culture media can require pH adjustment
- Bile salts content: > 45% (w/w, as cholic acid)
- Minimal effective concentration: 0,07-0,20% (w/w)

Note: Data are average values, which may vary from batch to batch.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Casein Acid Hydrolysate

Art. No. 07-151

Specification

Culture Media Ingredient.

Description

Casein Acid Hydrolysate is a protein hydrolysate obtained by acid digestion, where all the casein compounds are reduced to their amino acid, constituants, Tryptophan and most vitamins are destroyed due to the harsh acid digestion process.

Among the materials used in its preparation, the bovine constituents (Milk casein) belong to category C according to "*Note for Guidance*" EMEA/410/01 Rev. 2". The Milk casein is sourced from New-Zealand and Australia, from herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. Also it complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia. The manufacturing process includes a thermal treatment at 118°C for a minimum of 75 minutes and a heating at 107°C for 15 seconds on spray drying.

Note: The most important characteristics are shown in the following Figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

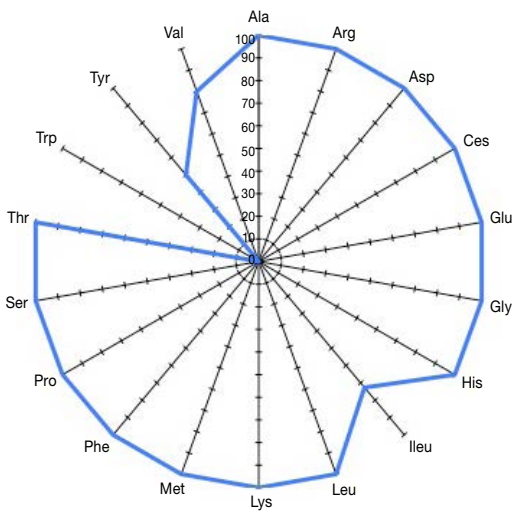
Microbiological limits

Total aerobic microbial count..... < 10000 cfu/g
Coliforms..... < 10 cfu/g
Moulds and yeasts..... < 20 cfu/g
Staphylococcus aureus..... absent in 10 g
Escherichia coli..... absent in 10 g
Salmonella spp..... absent in 25 g

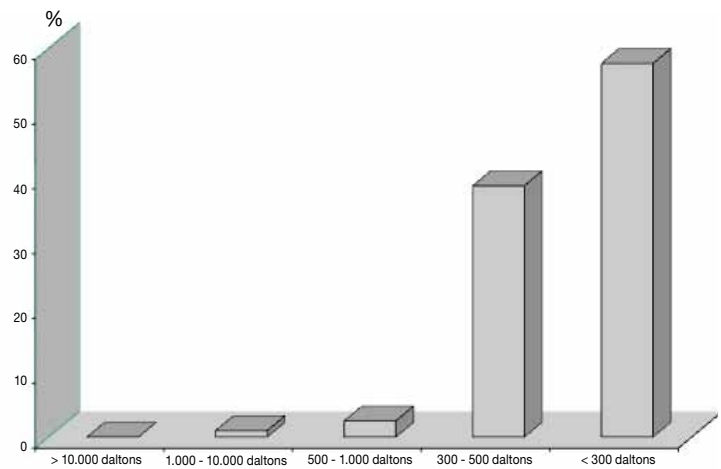
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-151 Casein Acid Hydrolysate
Amino Acids (Free/Total) x 100



07-151 Casein Acid Hydrolysate
Molecular weight distribution



Casein Pancreatic Peptone

Art. No. 07-154

Specification

Culture Media Ingredient.

Description

Casein Pancreatic Peptone is a protein hydrolysate, obtained by digestion with pancreatic enzyme extracts. It differs from Tryptone (Art. No. 07-119) only in the way it is obtained, which produces a different amino acid composition with the majority being of a smaller molecular size. It is the most commonly used peptone in industrial fermentation processes.

Among the raw materials used in its preparation the bovine constituents (Milk casein) belong to the category C according to "*Note for Guidance*" EMEA/410/01 Rev. 2". The Milk casein is sourced from New-Zealand, and comes from the herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. Also it complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia.

Other constituents used are of porcine origin. The manufacturing process includes boiling at 100°C for a minimum of 5 minutes and instantaneous heating at 170°C minimum on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

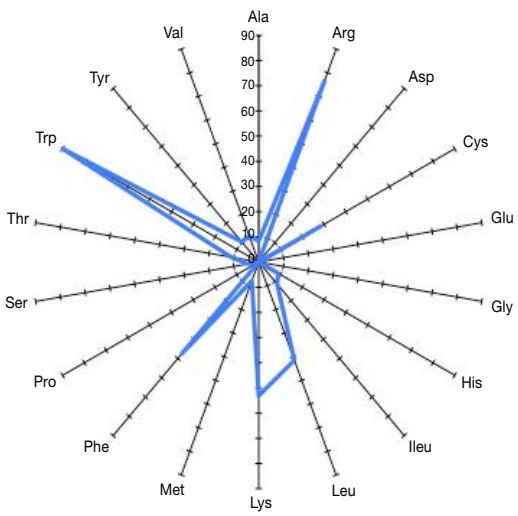
Microbiological limits

Total aerobic microbial count	< 10000 cfu/g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 20 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella spp	absent in 25 g

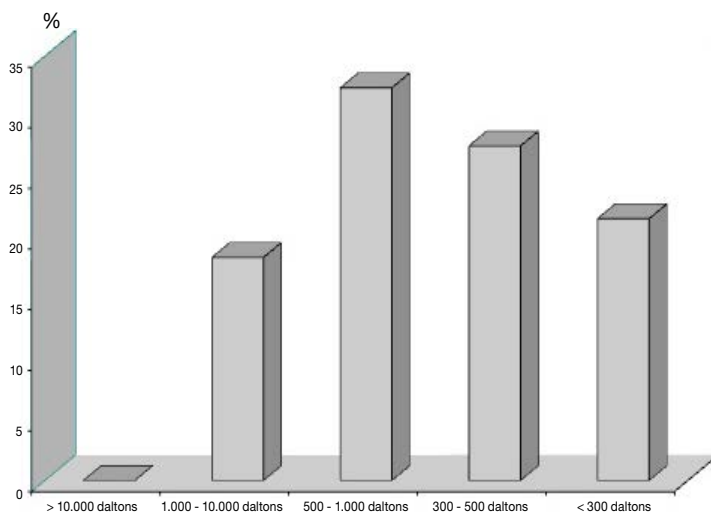
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-154 Casein Pancreatic Peptone
Amino Acids (Free/Total) x 100



07-154 Casein Pancreatic Peptone
Molecular weight distribution



Casein Trypsic Peptone (Tryptone)

Art. No. 07-119

Specification

Culture Media Ingredient.

Description

The Casein Trypsic Peptone or Tryptone is a protein hydrolysate obtained by the digest of casein with enriched porcine pancreatic enzymes, produced using a trypsin enriched preparation.

Both its nitrogen content and balanced amino-acid composition make it more suitable for use in culture media, producing exceptionally clear solutions.

Among the raw materials used in its preparation the bovine constituents (Milk casein) belong to the category C according to "*Note for Guidance*" EMEA /410/01 Rev. 2". The Milk casein is sourced from New-Zealand, and comes from the herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. Also it complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia.

The other constituents are of porcine origin. The manufacturing process includes boiling at 100ºC for a minimum of 5 minutes and instantaneous heating at 170ºC minimum on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

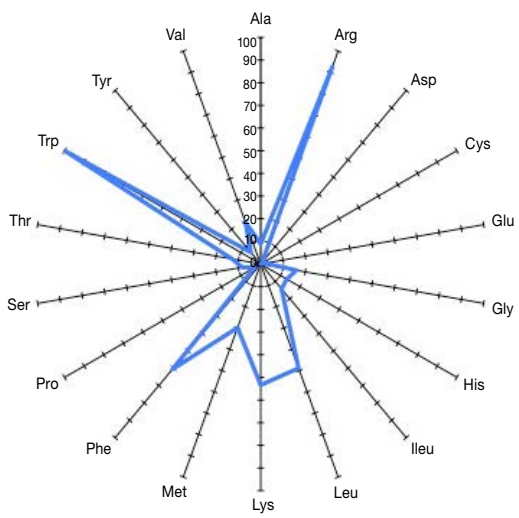
Microbiological limits

Total aerobic microbial count	< 10000 cfu/g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 20 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella spp	absent in 25 g

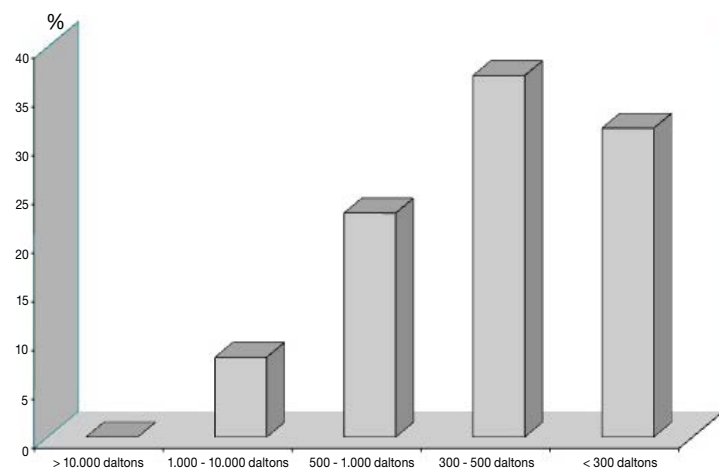
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

07-119 Casein Trypsic Peptone
Amino Acids (Free/Total) x 100



07-119 Casein Trypsic Peptone
Molecular weight distribution



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Gelatin Pancreatic Peptone

Art. No. 07-153

Specification

Culture Media Ingredient.

Description

Gelatin peptone is a cream coloured powder, with a characteristic odour, obtained by pancreatic digestion of gelatin.

The gelatin is obtained by boiling bones collagen from the connective tissue of healthy pigs, with *ante-mortem* and *post-mortem* sanitary certification by the Veterinary Authority in the country of origin. None of the raw materials used in its preparation are of bovine origin.

The Gelatin Pancreatic Peptone has a low tryptophan content, and has no fermentable sugars and no indol. Prepared solutions, even at high concentrations (10%), are light in colours, and without precipitate, due to an elaborate manufacturing process. Its nutritional capacity is lower, than other peptones, but it may be used for non fastidious microorganisms, and complies with the peptone specifications for fermentation studies.

The manufacturing process includes boiling at 100ºC for a minimum of 5 minutes and instantaneous heating at 200ºC on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

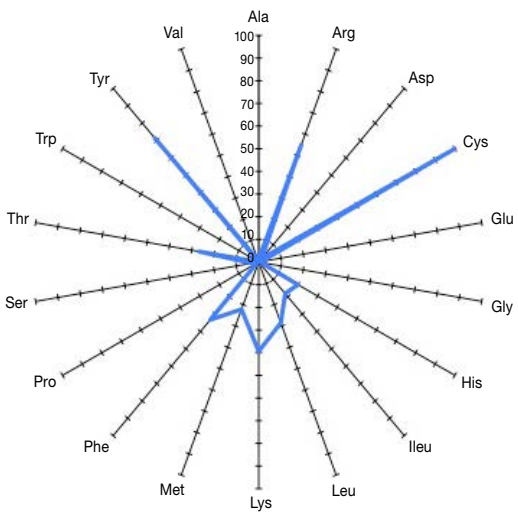
Microbiological limits

Total aerobic microbial count.....< 10000 cfu/g
Coliforms.....< 10 cfu/g
Moulds and yeasts.....< 20 cfu/g
Staphylococcus aureus.....absent in 10 g
Escherichia coli.....absent in 10 g
Salmonella spp.....absent in 25 g

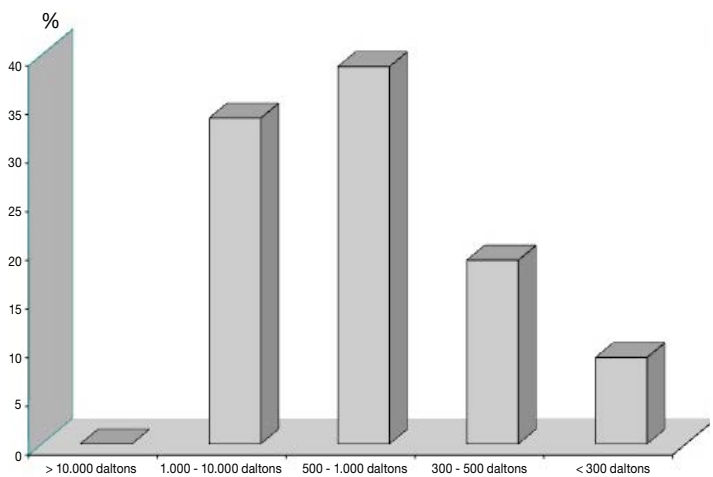
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

07-153 Gelatin Pancreatic Peptone
Amino Acids (Free/Total) x 100



07-153 Gelatin Pancreatic Peptone
Molecular weight distribution



Lactalbumin Hydrolysate

Art. No. 07-455

Specification

Culture Media Ingredient.

Description

The second major protein of milk is lactalbumin, a material less abundant than casein, but with a larger proportion of essential amino acids. Hydrolysis of this protein produces a mixture with an excellent nutritive value. Scharlau Lactalbumin Hydrolysate results from the pancreatic digestion of lactalbumin from cow's milk and gives a clear solution extremely effective as a nutrient source for microbiological systems.

Among the raw materials used in its preparation, bovine (milk lactalbumin) belongs to the category C according to "*Note for Guidance*" EMEA /410/01 Rev. 2". This milk casein is sourced from New-Zealand, and comes from herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. Also it complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia.

The other constituents are of porcine origin. The manufacturing process includes a thermal treatment at 70°C for 20 minutes and a heating at 129°C for 15 seconds on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

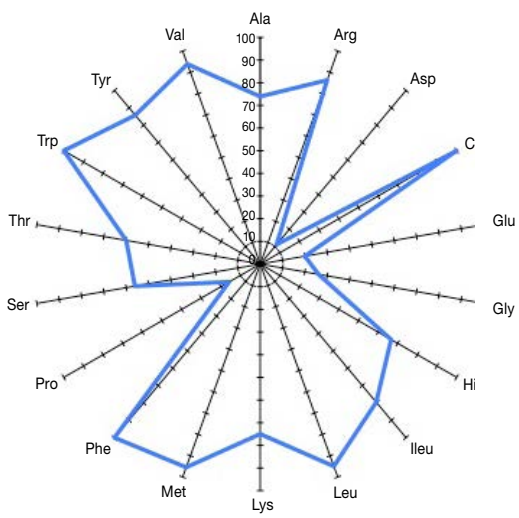
Microbiological limits

Total aerobic microbial count	< 1000 cfu/g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 20 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella spp	absent in 25 g

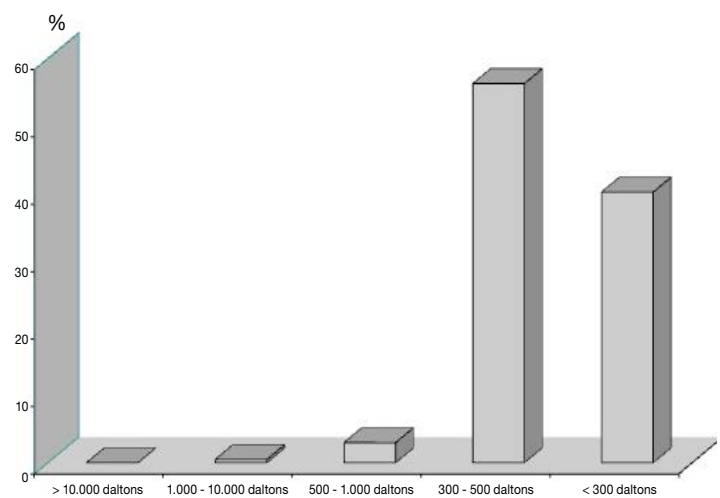
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-455 Lactalbumin Hydrolysate
Amino Acids (Free/Total) x 100



07-455 Lactalbumin Hydrolysate
Molecular weight distribution



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Lecithin

Art. No. 07-342

Specification

Culture Media ingredient.

Description

Phosphatidylcholines, are such a major component of lecithin, that the terms are often used synonymously. Phosphatidylcholine is a mixture of diglycerides of the stearic, palmitic and oleic acids, linked to the cholic ester of the phosphoric acid.

Scharlau's lecithin comes in powder form and is a rich brown colour. It is extracted from soybean seeds appropriately treated. It is made especially for inclusion culture media as an emulsifier or as a growth factor for lipophilic organisms.

The most important characteristics are:

Chemical data

Acetone solubility	< 3,0 % (w/v)
Water content	0,5 % (w/v)
Peroxide value	1,0 % (w/v)
Acid value	0,5 % (w/v)
C_{20}-C_{22} acids	5,5 % (w/v)
Linoleic acid	4,0 % (w/v)
Oleic acid	9,5 % (w/v)
Palmitoleic acid	8,5 % (w/v)
Stearic acid	4,0 % (w/v)
Palmitic acid	11,5 % (w/v)

Note: Data are average values, which may vary from batch to batch.

Microbiological limits

Total aerobic microbial count	< 10000 cfu/g
Heat resistant thermophiles	< 1 cfu/10 g
Heat resistant mesophiles	< 1 cfu/10 g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 500 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella spp	absent in 25 g

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Malt Extract

Art. No. 07-080

Specification

Culture Media ingredient.

Description

Malt extract is mostly used in the culture media for fungi, both as an enrichment and as a nutritive base. This is because of its ability to act as a substitute for peptone. It is obtained by extraction of the soluble fraction of malted barley, followed by a drying process at low temperature so that there is only minimal alteration in its nitrogen composition and high sugar content, especially maltose.

All the raw materials are of plant origin and are sourced from non GMO plants. This product complies with the European Regulation CE 1829/2003 and CE 1830/2003 regarding GMO.

Scharlau's Malt Extract has no diastatic activity and is very hygroscopic.

Malt extract solutions are usually opalescent or turbid. Should clear solutions be required, filtration is necessary, but this diminishes its nutritive potentiality.

Note: The most important characteristics are shown in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

Microbiological limits

Total aerobic microbial count	< 1000 cfu/g
Heat resistant thermophiles	< 1 cfu/10 g
Heat resistant mesophiles	< 1 cfu/10 g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 500 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella	absent in 25 g

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Meat Extract

Art. No. 07-075

Specification

Culture Media Ingredient.

Description

Meat extract has been the basic component of culture media for a long time, and initially it was substituted for meat infusions due to its ease-of-use. Being a refined and clarified extract with a more defined composition it provides greater reproducibility of results when used with other refined ingredients.

Meat extract is obtained from the tendon and fat free tissue of animals (sheep and pig), which are enzymatically pre-digested. Its production involves the elimination of fermentable sugars. The totally desiccated (dried) version is easier to use and requires a lesser quantity in order to obtain the same results. Meat extract solutions are clear, slightly coloured and with an almost neutral pH. In the culture media it is used in concentrations varying from 0,3-0,5%.

Among the raw materials used in its preparation, the bovine constituents belong to the category C, according to "*Note for Guidance* EMEA /410/01 Rev. 2". The bovine tissue is sourced from New-Zealand, and comes from herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC.

It is also complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia. The other constituents are of porcine origin. The manufacturing process includes boiling at 100°C for a minimum of 5 minutes and instantaneous heating at 170°C minimum on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

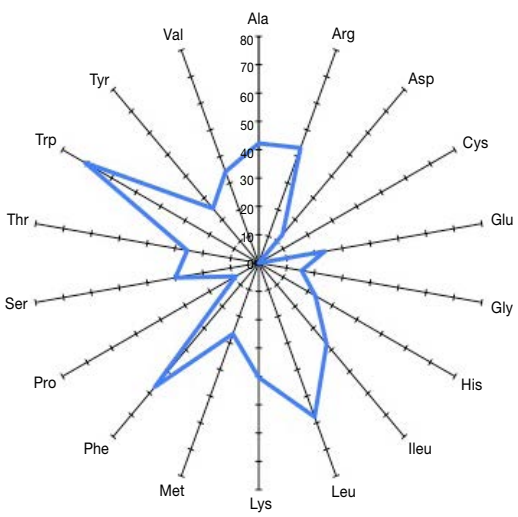
Microbiological limits

Total aerobic microbial count	< 10000 cfu/g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 20 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella spp	absent in 25 g

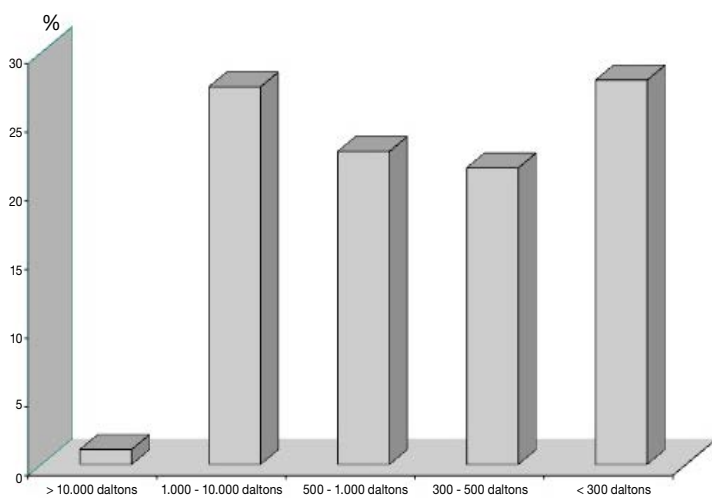
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-075 Meat Extract
Amino Acids (Free/Total) x 100



07-075 Meat Extract
Molecular Weight Distribution



Meat Peptone

Art. No. 07-152

Specification

Culture Media Ingredient.

Description

Meat peptone is a hydrolysate obtained by a partial digestion of meat by pepsin. It complies with the Pharmacopoeial specifications for the peptic digestion of animal tissues. It is a fine powder, cream to brown coloured, that gives very clear and plate coloured solution and is specially prepared for use in the culture media.

Among the raw materials used in its preparation, the bovine constituents belong to the category C according to "*Note for Guidance* EMEA /410/01 Rev. 2". The meat is sourced from New-Zealand, Australia and USA come from herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. Also it complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia.

The enzymatic preparation used in the digestion of the animal tissues is of porcine origin. The manufacturing process includes boiling at 100°C for a minimum of 5 minutes and instantaneous heating at 170°C minimum on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

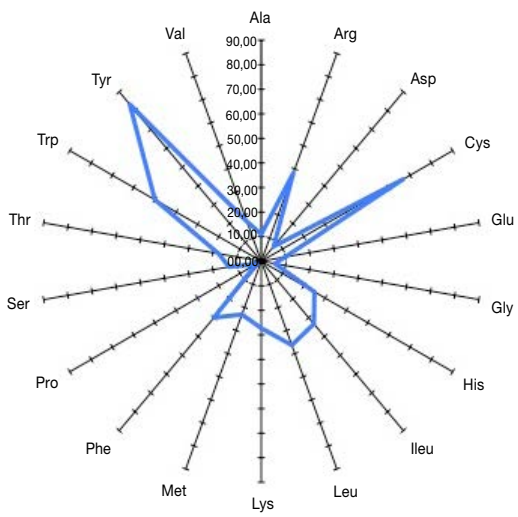
Microbiological limits

Total aerobic microbial count	< 10000 cfu/g
Coliforms	< 10 cfu/g
Moulds and yeasts	< 20 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella	absent in 25 g

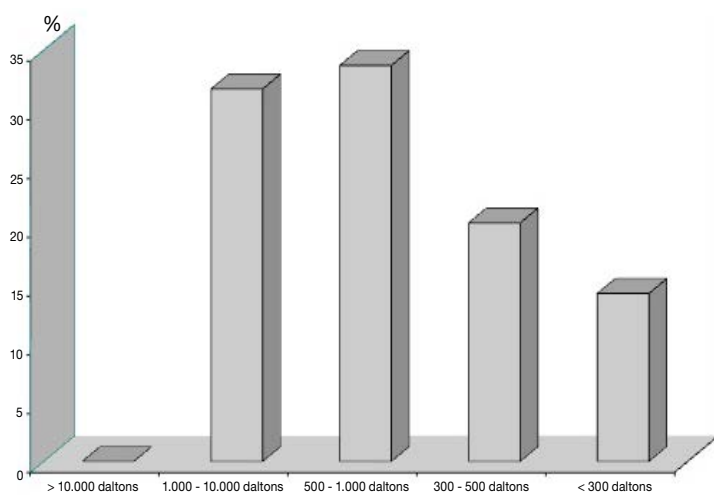
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-152 Meat Peptone
Amino Acids (Free/Total) x 100



07-152 Meat Peptone
Molecular Weight Distribution



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Peptone From Casein (Tryptone)

Art. No. 07-489

Specification

Culture Media Ingredient.

Description

The Peptone from Casein is a protein hydrolysate obtained from trypsin-digested casein according to the USP specifications. Both its nitrogen concentration and its balanced amino-acid composition provide an adequate support for microbial growyh and is used in the production of culture media, resulting in exceptionally clear solutions.

The digestion and production process uses an exceptionally trypsin-rich enzymatic preparation which produces a high content of tryptophane and ensures the absence of fermentable sugars and enzymatic activity.

Among the raw materials used in its preparation the bovine constituents (milk casein) belong to the category C according to "*Note for Guidance* EMEA /410/01 Rev. 2". The milk casein used is sourced from New-Zealand, and come from herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. Also it complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia.

The other constituents are of porcine origin. The manufacturing process includes boiling at 95°C for a minimum of 15 minutes and instantaneous heating at 200°C minimum on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

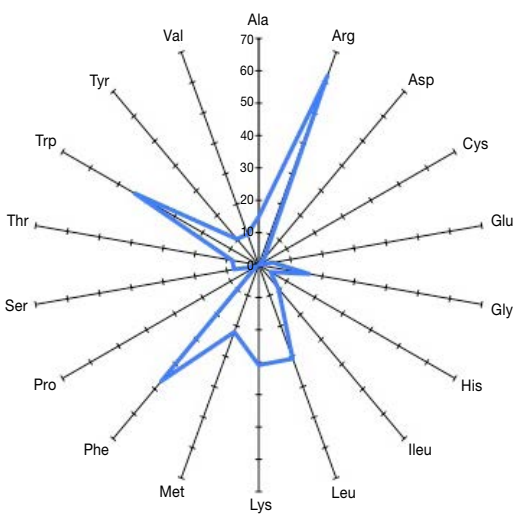
Microbiological limits

Total aerobic microbial count..... < 10000 cfu/g
Coliforms..... < 10 cfu/g
Moulds and yeasts..... < 20 cfu/g
Staphylococcus aureus..... absent in 10 g
Escherichia coli..... absent in 10 g
Salmonella spp..... absent in 25 g

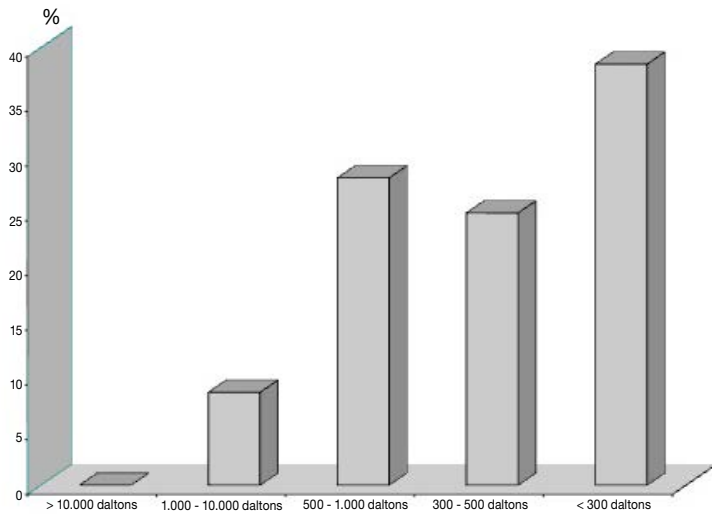
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-489 Peptone From Casein
Amino Acids (Free/Total) x 100



07-489 Peptone From Casein
Molecular Weight Distribution



Potato Peptone

Art. No. 07-620

Specification

Culture Media Ingredient.

Description

Vegetal Peptone is obtained by enzymatic digestion of potato proteins. In the culture media it is an advantageous substitute for the former Potato Extract. The Peptonization process results in a product that is both clear and transparent in solutions with a great uniformity and reproducibility from batch to batch. The microbial growth promoting ability of the Vegetal Peptone is also superior to the classical Potato Extract and its excellent results are particularly evident in the fungal culture media.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

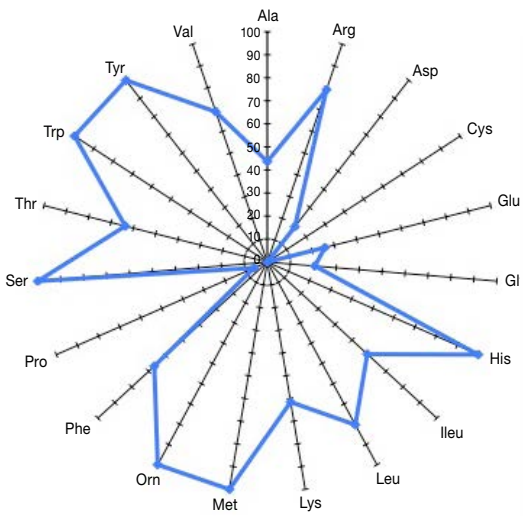
Microbiological limits

Total aerobic microbial count.....< 10000 cfu/g
Coliforms.....< 10 cfu/g
Moulds and yeasts.....< 20 cfu/g
Staphylococcus aureus.....absent in 10 g
Escherichia coli.....absent in 10 g
Salmonella spp.....absent in 25 g

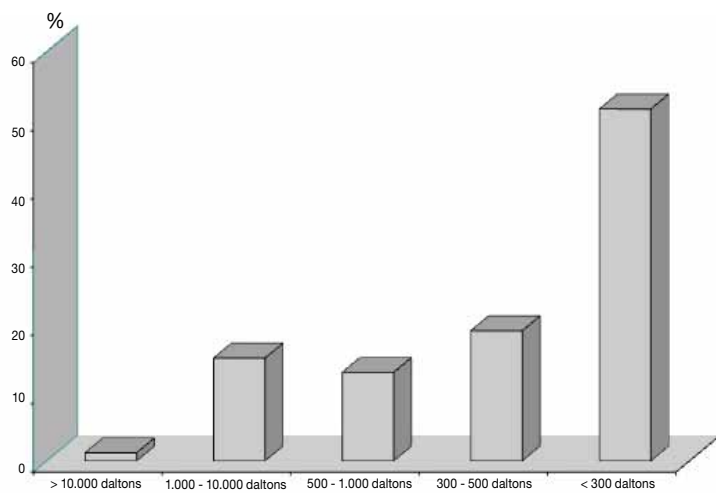
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-620 Potato Peptone
Amino Acids (Free/Total) x 100



07-620 Potato Peptone
Molecular Weight Distribution



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Proteose Peptone No. 3

Art. No. 07-625

Specification

Culture Media Ingredient.

Description

This Peptone is obtained after a partial enzymatic (peptic) digestion process of animal tissues. This process is carried out in such a way that it produces a high proportion of peptides of low molecular weight, free amino acids and other growth factors. Although all of these constituents make its definition very difficult, it has a high nutritive capacity that makes it suitable for supporting toxin production by microbes and as a basic nutrient for very fastidious microorganisms.

Among the raw materials used in its production, the bovine constituents belong to the category 4 of the WHO classification. The bovine tissues are sourced from New-Zealand, and come from herds of cattle which are declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities. The enzymatic preparations are of swine origin.

The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. The other constituents are of porcine origin.

The manufacturing process includes boiling at 100°C for a minimum of 5 minutes and instantaneous heating at 200°C on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

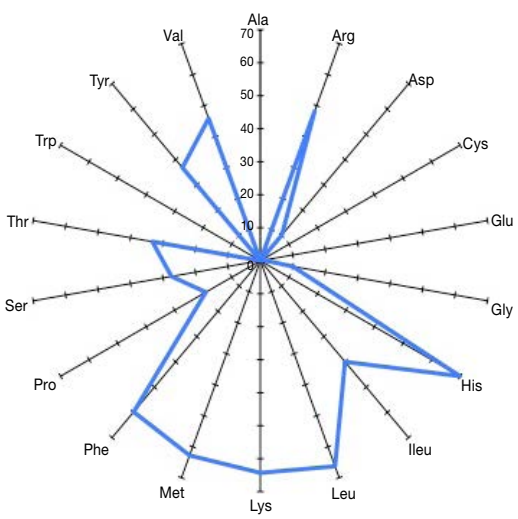
Microbiological limits

Total aerobic count.....< 10000 cfu/g
Coliforms.....< 10 cfu/g
Moulds and yeasts.....< 20 cfu/g
Staphylococcus aureus.....absent in 10 g
Escherichia coli.....absent in 10 g
Salmonella spp.....absent in 25 g

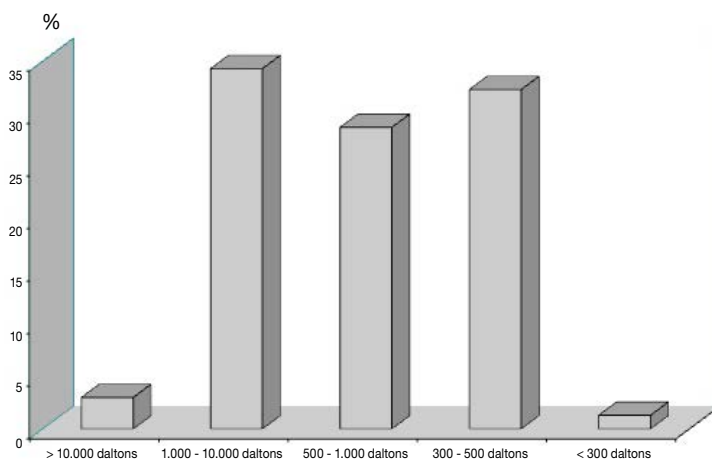
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-625 Proteose Peptone No.3
Amino Acids (Free/Total) x 100



07-625 Proteose Peptone No.3
Molecular Weight Distribution



Sodium Deoxycholate

Art. No. SO0257

WARNING

H: 3.1.0/4; H302

P: P264-P270-P301+P312-P330-P501a

Specification

Culture Media Ingredient.

Description

The Sodium Deoxycholate is the sodium salt of deoxycholic acid specially prepared for microbiological applications. The soluble form of the deoxycholic acid used in the microbiological culture media is in the concentration range 0,1 g/L to 5 g/L with no resulting turbidity. To distinguish streptococci from pneumococci a 10% solution of sodium deoxycholate is commonly used.

The exact function of bile in culture media has not been clearly defined, but can be related to the cell membrane permeability. The most inhibitory fraction of bile used in culture media is deoxycholic acid.

The action of deoxycholate can be modified by other ingredients in culture media, such sodium chloride and phosphate. Straight chain fatty acids such as acetic, propionic, butyric and citric acid will sharply enhance the inhibitory power of deoxycholate.

Scharlau Sodium Deoxycholate is manufactured from beef and sheep bile sourced in New Zealand, Brazil, Colombia and USA, which are TSE-free. The purification process involves use of more than 5% sodium hydroxide at greater than 110°C for more than 5 hours. Organic solvents are employed in the purification steps subsequent to hydrolysis and prior to obtaining the sodium salt of deoxycholic acid that is then spray dried at an inlet temperature >140°C and an outlet temperature >90°C.

Physical - chemical characteristics

Appearance	white powder
Assay (HPLC)	min. 98 %
Sodium cholate	max. 2 %
Heavy metals	max. 20 ppm
Loss on drying	max. 5 %
Solubility (2 % autoclaved solution)	clear
pH (2 % solution)	8,5 ± 1,0

Hazard identification

- R22 Harmful if swallowed
- R37 Irritating to the respiratory system
- S22 Do not breathe dust
- S36 Wear suitable protective clothing

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Sodium Pyruvate

Art. No. SO0590

Also known as

Pyruvic acid sodium Salt; 2-Oxopropanoic acid sodium salt; α-Ketopropionic acid sodium salt ($CH_3COCOONa$; $C_3H_3O_3Na$)

Specification

Culture Media Ingredient.

Description

- Sodium pyruvate improves coliform recovery when present in culture media.
- Scharlau's Sodium Pyruvate is checked and selected to yield the best results for microbiological culture media.
- It is not verified or tested for cell culture, (insect cell culture, embryo or hybridoma cell culture).

Physical - chemical characteristics

Contents	Puriss > 99 %
Molecular weight	110,04 g/mol
Appearance	White crystalline powder
Solubility	100 mg/mL
Transparency (525 nm)	90 % min.
Loss on drying	0,5 % max.
Sulfate	20 ppm max.
Chlorides	20 ppm max.
Arsenic	1 ppm max.
Heavy metals	10 ppm max.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Soy Peptone

Art. No. 07-155

Specification

Culture Media Ingredient.

Description

Soy peptone is a protein hydrolysate obtained by papaic digestion of soy flour. It complies with the Pharmacopeial specifications for these type of products, and it is a useful compound in laboratory culture media. However, due to its high sugar content it is not recommended for fermentation assays.
All raw materials are of plant origin.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

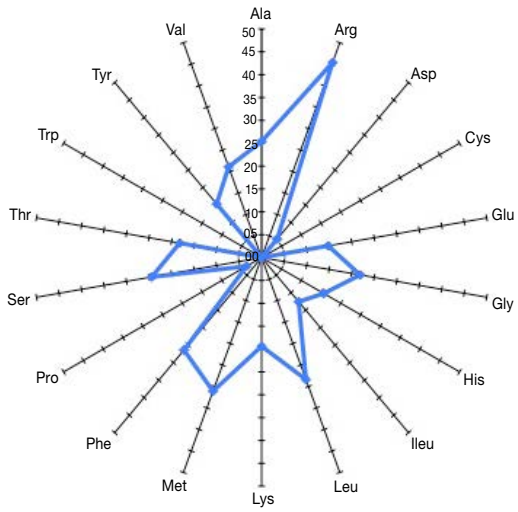
Microbiological limits

Total aerobic microbial count..... < 10000 cfu/g
Coliforms..... < 10 cfu/g
Moulds and yeasts..... < 20 cfu/g
Staphylococcus aureus..... absent in 10 g
Escherichia coli..... absent in 10 g
Salmonella..... absent in 25 g

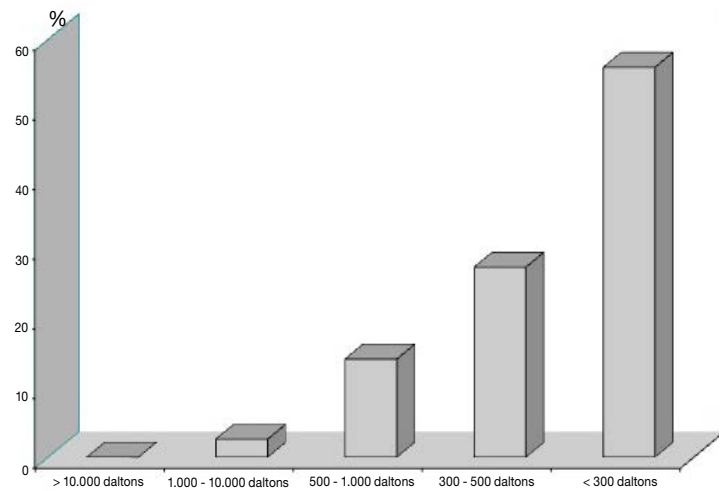
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

07-155 Soy Peptone
Amino Acids (Free/Total) x 100



07-155 Soy Peptone
Molecular Weight Distribution



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Tryptose
Art. No. 07-197

Specification
Culture Media Ingredient.

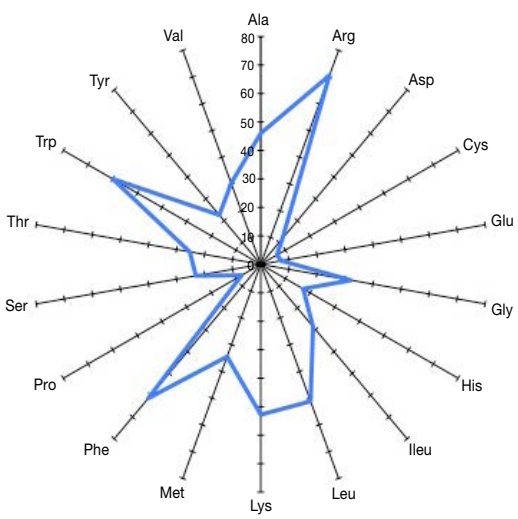
Description
Tryptose is a mixed peptone with high nutrient properties that make it appropriate for the use in culture media for very fastidious microorganisms. Among the raw materials used in its preparation, the bovine constituents (milk casein) belong to the category C according to "*Note for Guidance* EMEA /410/01 Rev. 2". The milk casein is sourced from New-Zealand, and comes from herds declared free from Bovine Spongiform Encephalopathy and Foot-and-Mouth disease after examination by the Veterinary Authorities.
The product does not contain and is not derived from specified risk material as defined in the European Commission Decision 97/534/EC. Also it complies with the General Monograph 1483 on "Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies" in the current European Pharmacopoeia.
The other constituents are of porcine origin. The manufacturing process includes boiling at 100°C for a minimum of 5 minutes and instantaneous heating at 170°C minimum on spray drying.

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

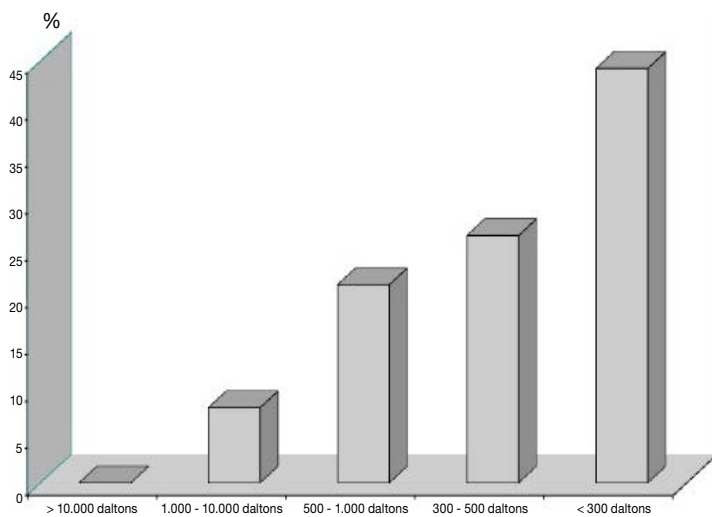
Microbiological limits
Total aerobic microbial count..... < 10000 cfu/g
Coliforms..... < 10 cfu/g
Moulds and yeasts..... < 20 cfu/g
Staphylococcus aureus..... absent in 10 g
Escherichia coli..... absent in 10 g
Salmonella spp..... absent in 25 g

Storage
For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-197 Tryptose
Amino Acids (Free/Total) x 100



07-197 Tryptose
Molecular Weight Distribution



Yeast Extract

Art. No. 07-079

Specification

Culture Media Ingredient.

Description

Yeast Extract is a water soluble extract of fresh autolysed yeast (*Saccharomyces cerevisiae*) cells prepared and standardized for use in microbiological culture media. It is a source of universal growth factors such as peptides, free amino acids, purine and pyrimidine nucleobases, trace elements and hydro soluble vitamins from the B complex. It is commonly added to culture media in concentrations between 0.2% and 1%.

Scharlau's Yeast Extract does not contain any component of animal origin and therefore has no risk of transmission of animal spongiform encephalopathies (TSE). It is not derived from or produced using GMOs or their derivatives and all reasonable steps have been taken to avoid contamination from GMOs or their derivatives. This has been verified by a declaration from previous suppliers.

Vitamin contents (Non exhaustive)

Thiamine (Vitamin B1)	85 mg/kg (ppm)
Riboflavin (Vitamin B2)	105 mg/kg (ppm)
Pantothenic acid (Vitamin B5)	300 mg/kg (ppm)
Pyridoxine (Vitamin B6)	65 mg/kg (ppm)
Biotin (Vitamin B8)	7 mg/kg (ppm)
Folic acid (Vitamin B9)	40 mg/kg (ppm)
Cyanocobalamin (Vitamin B12)	10 mcg/kg
Niacin (PP Factor)	800 mg/kg (ppm)

Note: The most important characteristics are shown in the following figures and in Tables 1 to 6 at the beginning of this chapter. Data are average values, which may vary from batch to batch.

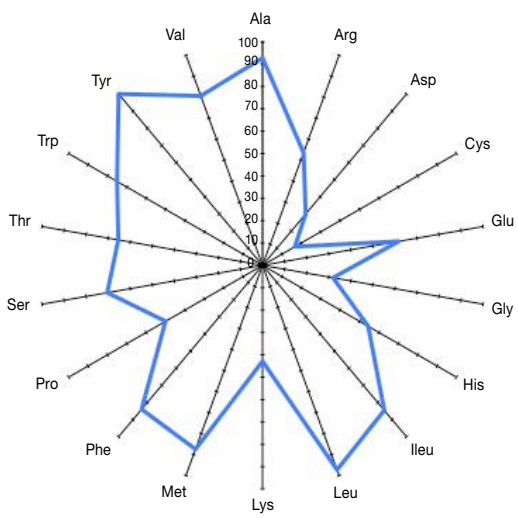
Microbiological limits

Total aerobic microbial count	< 1000 cfu/g
Coliforms	< 10 cfu/g
Moulds and yeasts	<500 cfu/g
Staphylococcus aureus	absent in 10 g
Escherichia coli	absent in 10 g
Salmonella	absent in 25 g

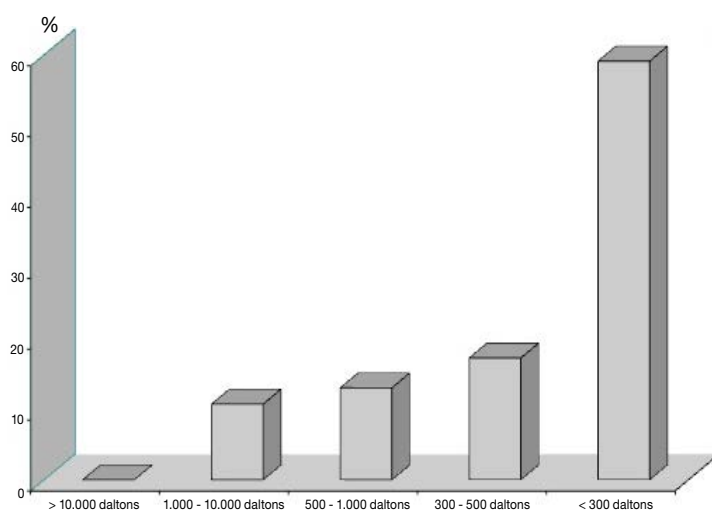
Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

07-079 Yeast Extract
Amino Acids (Free/Total) x 100



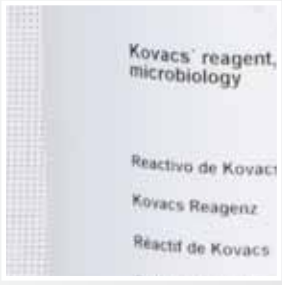
07-079 Yeast Extract
Molecular Weight Distribution



Scharlau










Reagents

Reagents are used for qualitative and quantitative test methods. These include chromatographic, electrophoretic, photometric and titrimetric techniques.

Barrit's Reagent (VP2)

Art. No. RE0100

DANGER

H: 2.6/2: H225-3.3/1: H318

P: P210-P241-P303+P361+P353-P305+P351+P338-P310-P501a

Also known as

VP 2 Reagent

Specification

Reagent used to verify the production of 2,3-butane-diol by Enterobacteriaceae. (Voges-Proskauer test).

Formula

α-naphthol.....50,00 g
Ethanol.....1000,00 mL
Filter- sterilized aqueous solution.

Description

All enterobacteria ferment glucose, some species such as *Klebsiella*, *Enterobacter*, etc..., ferment via the 2-3-butanediol path and other species such as *E coli*, *Salmonella*, etc..., ferment by the Mixed Acid pathway.
The Voges-Proskauer test demostrates the production of 2-3-butanediol and acetoin that is produced in great amounts in the 2-3-butanediol path. These compounds in the presence of air an alkaline medium, undergo oxidation and become diacetyl compounds, which react with guanidine producing very visible coloured compounds.
O'Meara, in 1931, observed that adding creatine to the alkaline solution (O'Meara's Reagent, Art. No. RE0060) aids the diacetyl reaction with guanidine, making it easier to detect the resulting red coloured compounds.
Later, in 1936, Barrit demonstrated that the addition of an alcoholic solution of α-naphthol 5% (Barrit's Reagent, Art. No. RE0100) increased the sensitivity of the test, and it was possible to obtain a positive reaction even when the final concentration of diacetyl was very low. It is important to add the Barrit's Reagent before the alkaline solution.

Technique

The microorganism to be tested is inoculated in Methyl Red Voges-Proskauer (MRVP) Broth (Art. No. 02-207) and is incubated at 30°C for a period between 3 and 5 days maximum.
Just before reading, add Barrit's Reagent (Art. No. RE0100) until the entire medium becomes milky in appearance. Then, add O'Meara's Reagent (Art. No. RE0060) until it no longer appears milky and shake briefly. Relative volumes of each reagent depend on the initial volume of inoculated medium.
When the test is positive a violet-pink colour appears before 5 minutes, starting from the top. When the test is negative there is no change in colour.
There is a quicker way to perform the Voges-Proskauer test, with very small volumes of medium and large inocula. Resulting in shorter incubation periods (18-20 hours) and the reading may be accelerated by heating the culture almost to boiling after adding the reagents. However, this method increases the possibility of getting false-results.

References

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- O'MEARA, R.A.Q. (1931) A Simple, Delicated and Rapid Method of Detecting the Formation of Acetylmethylcarbinol by Bacteria Fermenting Carbohydrate. J. Pathol. Bact. 34:401-406.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 12°C).

Quality control

Culture medium: Art. No. 02-207
Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 1.000-10.000 cfu

Microorganism	Growth	Remarks
Enterobacter aerogenes ATCC 13048	Good	VP (+)
Escherichia coli ATCC 25922	Good	VP (-)
Escherichia coli ATCC 8739	Good	VP (-)

Kovacs' Reagent

Art. No. RE0007

WARNING

H: 2.6/3; H226-3.1.O/4; H302-3.2/2; H315-3.3/2; H319-3.8/3; H335+H336

P: P210-P241-P303+P361+P353-P305+P351+P338-P405-P501a

Specification

Reagent for the Indol test in Enterobacteria.

Formula

4-Dimethyl-amino-benzaldehyde	50,00 g
Isoamyl alcohol	710,00 mL
Hydrochloric acid	240,00 mL

Description

The ability to produce indole from Tryptophan constitutes a classical test for the differentiation of *Escherichia* and *Enterobacter*, integrated in the Indole, Methyl Red Voges-Proskauer, Citrate (IMViC) tests. It is also widely used for the differentiation of other non enteric microorganisms.

Many microorganisms can produce indol (=benzopyrrole) from tryptophan thanks to tryptophanase, in a process enhanced by oxygen and inhibited by glucose. We therefore recommend that media used in this test contain no glucose, have a high tryptophan content and incubated aerobically.

The indol test can be conducted by various means, whilst the biochemical basis of the reaction remains the same. When a Pyrrole is mixed with a heated alcoholic p-dimethylaminobenzaldehyde solution, a unique cherry-red colouring (*Rosindole*) develops. If the reagent solution is prepared with concentrated hydrochloric acid heating is not necessary as is the case with Scharlau's reagent.

The reagent which was initially used was that of Ehrlich-Böhme which involved a prior solvent extraction and concentration. Later, Kovacs modified the original reagent by replacing the ethanol with amyl-alcohol, so that an initial extraction was no longer necessary. In 1956 Gadenbusch and Gabriel showed that Kovacs' Reagent was more stable if, butylic-alcohol or isoamylalcohol were used instead of amyl-alcohol.

The indol test reaction using para-diaminobenzaldehyde is not very specific since at least 17 compounds similar to indol are known to react in the same way. Although other reagents such as oxalic acid and hydroxylamine HCl have been proposed, their use has not been widespread.

Since indol is one of the most volatile compounds of the group, some authors choose to conduct the test using strips impregnated in reagent. Others suggest placing the reagent directly on the cap's swabs, avoiding all contact with the culture medium so that the reaction will occur with just the indol vapour. When the membrane filtration technique is used, the entire membrane without the medium is flooded or moistened with the Kovacs' Reagent. Isenberg and Suddenheim demonstrated that if a previous extraction with toluene was performed, only indol and alpha-methylindole (=indoleacetic acid) were detected. This is the most common practice nowadays when using Ehrlich-Böhme's Reagent, it differs from Kovacs method in the alcohol step only.

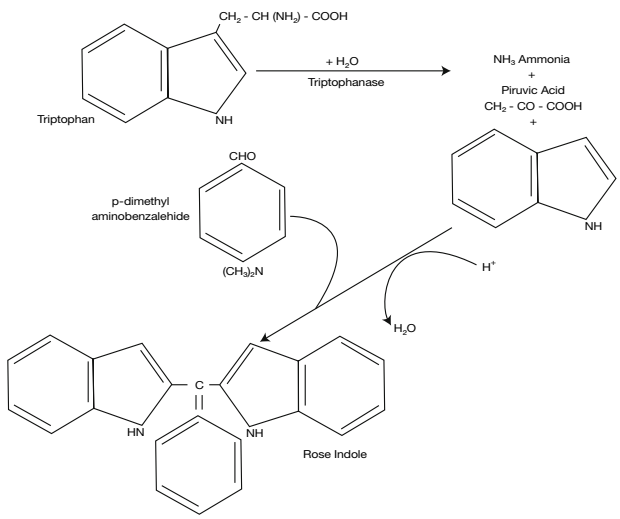
Technique

When conducting the indol production test on different groups of bacteria, an appropriate reagent for each group must be considered. Kovacs' Reagent (Art. No. RE0007) is recommended for enterobacteria while the Ehrlich test is recommended for non-fermenters and anaerobes. The test is carried out as follows:

Inoculate the pure culture to be verified in a high tryptophan content medium, for example Indol-Nitrite Fluid Medium (Art. No. 03-101), Sulfide-Indol-Motility (SIM) Medium (Art. No. 03-176) or a non-glucose tryptone broth. Incubate at 35°C for 48 hours. Incubation time can be reduced to 4 hours if solid medium is inoculated with a large inoculum followed by seeding of a small volume (0,5 mL) of broth medium.

In both cases, examination after incubation is carried out in the following way:

- Kovacs' Reagent (Art. No. RE0007, for enterobacteria): Add 0,5 mL of reagent to the broth's surface, shaking gently to help extraction. If a cherry-red colour develops in less than a minute it is considered a **positive** reaction. No change in the original colour constitutes a **negative** reaction.
- Ehrlich-Böhme Test: Add 1 mL of xylene or toluene to the broth and shake energetically to help extraction. Allow it to stand for 2 minutes until both layers separate. Then slide 0.5 mL of the Kovacs' reagent carefully down the sides of the tube, making sure there is no agitation. Should a dark red colour ring appear in the interface, it is considered a **positive** reaction. Spirillum, vibrio, rickettsia, chlamydia and most bacilli are Gram negative.



(continues on the next page)

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Kovacs' Reagent

Art. No. RE0007

WARNING

H: 2.6/3; H226-3.1.O/4; H302-3.2/2; H315-3.3/2; H319-3.8/3; H335+H336

P: P210-P241-P303+P361+P353-P305+P351+P338-P405-P501a

References

- BÖHME, A. (1906) Die Anwendung der Ehrlischen Indolreaktion für Bakteriologische Zwecke. Zentralbl. Bakt. Parasit. Abt 1, 40:129-133. Jena.
- CDC (1968) Identification of Unusual Pathogenic Bacteria. Atlanta. GE. USA.
- EDWARDS, P.R. & EWING, W.H. (1972) Identification of Enterobacteriaceae. 3rd ed. Burgess Pub. Co. Minneapolis.
- GADEBUSCH, H.H. & GABRIEL, S. (1956) Modified Stable Kovacs' Reagent for the deteccion of Indol. Am. J. Clin. Pathol. 26:1373-1375.
- ISENBERG, H.D. & SUNDHEIM, L.H. (1958) Indol Reactions in Bacteria. J. Bact. 75:682-690.
- ISO 9308-2 Standard (1990) Water Quality - Detection of coliformes, thermotolerant coliformes and presumptive *Escherichia coli* - MPN Method.
- ISO 21567 Standard (2004) Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella spp.*
- KOVACS, N. (1928) Eine vereinfachte Methode zum Nachweis der Indolbihndung durch Bakterien. Z. Immunitats. Forsch. Exp. Ther. 55:311-315.
- McFADDIN, J.F. (2000) Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott William & Wilkins. Philadelphia. USA.
- VPI (1972) Anaerobe Laboratory Manual. Blaksburg. VA. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 12°C).

Quality control

Culture medium: Art. No. 03-156
Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: The microorganisms grown in Peptone Water (Art. No. 03-156)

Microorganism	Growth	Remarks
E. coli ATCC 8739	Good	Indol (+)
Salmonella typhimurium ATCC 14028	Good	Indol (-)

Left: Uninoculated tube (Control)
Centre: *Salmonella typhimurium* ATCC 14028
Right: *Escherichia coli* ATCC 8739

Methyl Red Solution

Art. No. RE0057

DANGER

H: 2.6/2; H225

P: P210-P241-P280-P240-P303+P361+P353-P501a

Specification

Indicator solution used for the fermentation test in enterobacteria.

Formula

Methyl red	20,00 g
Ethanol	600,00 mL
Distilled water	400,00 mL

Description

Clark and Lubs, in 1915, described the Methyl Red test to distinguish between *E. coli* and *Enterobacter spp*.

All enteric bacteria ferment dextrose, some do it following the 2-3-butanediol pathway, like *Klebsiella*, *Enterobacter*, etc..., and others follow the Mixed Acid pathway, like *E. coli*, *Salmonella*, etc...In the latter case, there is an accumulation of acid products produced by the breakdown of sugar causes a decrease in pH, reaching a value up to 4 after incubation. On adding Methyl Red in an alcohol solution the following may be observed: it remains yellow above pH 5,1 and changes to red below pH 4,4. If the change is positive it means that the dextrose fermentation followed the Mixed Acid pathway, since in the 2-3-butanediol pathway, after 3 days of incubation, neutral or alkaline products are dominant. Too early readings may produce false positive results.

Technique

Inoculate a tube of Methyl Red Voges-Proskauer (MRVP) Broth (Art. No. 02-207) and incubate at 30°C for 3-5 days. Take 5 mL of culture and transfer it to a clean tube and then add 5 or 6 drops of indicator.

A positive reaction is shown by the presence of a red colour, whereas at negative reaction produces a yellow or orange colour.

In most cases, 48 hours incubation at 37°C is enough, but if the results are doubtful, the assay must be repeated incubating at 30°C for 5 days.

A quicker way to perform the test is to suspend a loop of bacterial growth from a solid medium in 0,5 mL of MRVP Broth (Art. No. 02-207) and incubate at 37°C for 18 hours. Add a couple of drops of indicator and read.

References

- BARRY, A.L., K.L. BERNSOHN, A.P. ADAMS, L.D. THRUPP (1970) Improved 18-hour methyl red test. Appl. Microbiol. 20:866-870.
- BLAZEVIC, D.J., G.M. EDERER (1975) Principles of Biochemical Tests in Diagnostic Microbiology. John Wiley and Sons. NY.
- CLARK, W.M. & LUBBS, H.A. (1915) The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J. Infect. Dis. 17:161-173.
- EDWARDS, P.R. y EWING, W.H. (1972) Identification of Enterobacteriaceae. 3rd ed. Burgess Pub. Co. Minneapolis.
- McFADDIN, J.F. (2000) Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott, William & Wilkins. Philadelphia.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C).

Quality control

Culture medium: Art. No. 02-207
Incubation temperature: 35°C ± 2,0
Incubation time: 24 h
Inoculum: 1.000-10.000 cfu

Microorganism	Growth	Remarks
Escherichia coli ATCC 25922	Good	MR (+)
Escherichia coli ATCC 8739	Good	MR (+)
Enterobacter aerogenes ATCC 13048	Good	MR (-)

Nitrate Reagents

Art. No. 06-003 - Nitrate Reagent A
Art. No. 06-004 - Nitrate Reagent B

DANGER

H: 3.2/1B; H314-2.6/3; H226
P: P210-P303+P361+P353-P305+P351+P338-P310-P405-P501a

Also known as

Griess-Ilosvay's Reagent A
Griess-Ilosvay's Reagent B

Specification

Griess-Ilosvay's Reagent for the verification of nitrate reduction through the determination of nitrites.

Formula

Nitrate Reagent A

Sulfanilic acid	8,00 g
Acetic acid	285,00 mL
Distilled water	707,00 mL

Nitrate Reagent B

N.N dimethyl-naphthylamina	6,00 mL
Acetic acid	285,70 mL
Distilled water	714,30 mL

Description

To use, mix equal parts of Nitrate A (Ref.06-003) and Nitrate B (Ref.06-004). Once they are mixed, the reagents are stable just for a few hours. Individually they may be stored for several months at room temperature. Nitrate reagent B may produce a slight crystallization that does not affect its efficacy. This process is accelerated with refrigeration, therefore it is recommended not to store them in the refrigerator.

Nitrate reduction in bacteria occurs in several ways and it can follow different pathways.

Nitrate assimilation involves a reduction to ammonia in several steps where nitrite may be detected. The ammonia that is produced is finally incorporated into the cellular material. However, in the dissimilation process, nitrite is used as the final receiver of electrons, and thus, more than an assimilation process it is an energetic respiration reaction without oxygen, and this fact allows the facultative growth of many aerobes in anaerobiosis. In this case, nitrite usually accumulates, which may be toxic for the microorganism. In other cases, nitrate may reduce itself to gas states and it is expelled as nitrogen bubbles. This process is called "denitrification", since it makes the active ion (NO_3) an inert gas (N_2).

The overall scheme of the process can be represented in the **Figure 1**.

Generally, Griess-Ilosvay's reagents detect the presence of nitrites with bacterial origin in a medium that initially has no nitrites (Indol Nitrite Fluid Medium, Art. No. 03-101 and Nitrate Broth, Art. No. 02-138).

The scheme for the complete reaction is represented in the **Figure 2**.

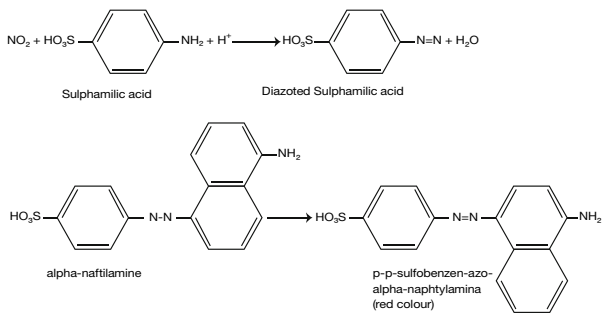
Thus, the appearance of a soluble red colour in the medium after the addition of reagents (alone or together) is accepted as positive proof of nitrite presence. The absence of colour after reagent addition does not involve necessarily an absence of nitrate reduction, since there may be three possibilities:

a) Nitrates have become ammonia and there is no nitrite in the medium.
b) Nitrates have become free nitrogen and there is no nitrite in the medium.
c) There has been no nitrite formation and the nitrate is still in the medium.

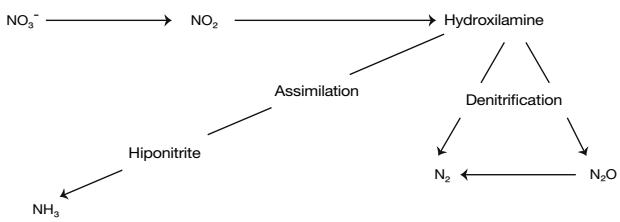
To verify nitrate reduction, add a little bit of zinc powder in the tube that gave the negative result. Zinc reduces nitrate to nitrite quickly and then produces a change of colour if there is still nitrate in the medium. In this case, the reaction must be considered negative. However, if there is no change of colour after the addition of zinc powder, the reaction must be considered positive, since (see above) there has been a reduction of nitrate to nitrite.

Should determination of the nitrite reaction be desired, start the culture medium with nitrite instead of nitrate and do the readings in the inverse way.

(Figure 1):



(Figure 2):



Technique

For gram-negative bacteria (enterobacteria and pseudomonas) it is recommended to use Nitrate Broth (Art. No. 02-138). For gram-positive bacteria this medium or Indol Nitrite Fluid Medium (Art. No. 03-101) may be used.

Inoculate the bacteria to test and incubate for 48 hours at the optimal temperature. A quick test may be carried out with a large inocula in a very small amount of medium and incubation at 37°C for 2 hours, but sometimes this may produce false negative results.

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Nitrate Reagents

Art. No. 06-003 - Nitrate Reagent A

Art. No. 06-004 - Nitrate Reagent B

DANGER

H: 3.2/1B; H314-2.6/3; H226

P: P210-P303+P361+P353-P305+P351+P338-P310-P405-P501a

The reading is carried out after incubation, adding a few drops of each reagent or, better still, at mixture both in equal parts. Cherry red is taken as a positive result. Absence of colour is a presumptive negative.

Verification is carried out adding a small amount of zinc powder: if a red colour appears, the test is negative, otherwise (there is no colour change) the test is positive.

An unstable red colour, that quickly turns brown/red, is due to excess nitrite, and has no effect on the test.

Negative cultures after 24 hours must be repeated after 48 and 72 hours in other tubes before discarding them as negative.

Gas production in non fermentative strains is used as a possible indicator of denitrification. With fermentative strains it may be CO_2 production.

It is advisable to inoculate with Durham tubes.

Nitrate reduction assays must always be performed with control tubes without inocula, since contamination of the test material with nitrous oxide or nitrite may produce a false positive reactions.

References

- BLAZEVIC, D.J.(1972) Laboratory Procedures in Diagnostic Microbiology. Telstar Productions Inc., St. Paul, Minn. pg 109.
- BLAZEVIC, D.J. y EDERER G.M. (1975) Principles of Biochemical Tests in Diagnostics Microbiology. John Wiley Sons N.Y.
- FORBES, B.A., D.F. SAMM y A.S. WEISSFELD (1998) Bailey & Scott's Diagnostics Microbiology. 10th. Ed. Mosby. St. Louis.
- GRIESS, P. (1879) Liebereinige Azoverbindungen. Ber. Deutsch. Chem. Gesellsch. 12:426-427.
- McFADDIN, J.F. (2000) Biochemical Tests for identification of medical bacteria. 3rd. Ed. Lippincott William and Wilkins. Philadelphia.
- WALLACE, G.I.y S.L. NEAVE (1927) The nitrite test as applied to bacterial cultures. J.Bact. 14:377-384.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+2°C to 12°C).

O'Meara's Reagent (VP1)

Art. No. RE0060

DANGER

H: 3.2/1A; H314-3.1.O/4; H302

P: P260-P303+P361+P353-P305+P351+P338-P310-P405-P501a

Also known as

VP1 Reagent

Specification

Reagent used to verify 2,3-butane-diol production (Voges-Proskauer Test) in Enterobacteriaceae.

Formula

Potassium hydroxide	400,00	g
Creatine	3,00	g
Distilled water	1000,00	mL

Description

All the enterobacteria ferment glucose but some species such as *Klebsiella*, *Enterobacter*, etc..., ferment via the 2-3-butanediol pathway and other species such as *Escherichia coli*, *Salmonella*..., ferment by the Mix Acid pathway.

Voges-Proskauer Test demostrates the production of 2-3-butanediol and acetoine, that are only produced in great amounts in the 2-3-butanediol path. These compounds, in the presence of air in alkaline environment, undergo oxidation and become diacethyl compounds, reacts with guanidine producing a very visible coloured compounds.

O'Meara, in 1931, observed that adding creatine to the alkaline solution (O'Meara's Reagent, Art. No. RE0060) aids diacethyl reaction with guanidine, making easier to detect the resulting red coloured compounds. Later, in 1936, Barrit demonstrated that the addition of an alcoholic solution of α-naphtol 5% (Barrit's Reagent, Art. No. RE0100) increased the sensitivity of the test, and it was possible to obtain a positive reaction even when the final concentration of diacethyl was very low. It is important to add the Barrit's Reagent before the alkaline solution.

Technique

The microorganism to be tested is inoculated in MRVP Broth (Art. No. 02-207) and is incubated at 30°C for a period between 3 and 5 days maximum.

Just before reading, add Barrit's Reagent (Art. No. RE0100) until all the medium becomes milky in appearance. Then, add O'Meara's Reagent (Art. No. RE0060) until it no longer appears milky and then shake briefly. Relative volumes of each reagent depend on the initial volume of inoculated medium.

When test is positive a violet pink colour appears before 5 minutes, starting from top. When the test is negative there is no change of colour.

There is a quicker way to perform the Voges-Proskauer test, with very little volumes of medium and a large inocula. This way allows very short incubations (18-20 hours) and the reading may be accelerated by heating up the culture almost to boiling after adding the reagents. However, this method increases the possibility of a false result.

References

- BARRITT, M.M. (1936) The Intensification of The Voges-Proskauer Reaction by the Addition of Ó-Naphtol J. Pathl. Bact.42:441-453.
- BARRY, A. L. & FEENEY, K.L. (1967)Two Quick Methods for the Voges-Proskauer test Appl. Microbiol. 15:1138-1141.
- BLAZEVIC, D.J. & EDERER G.M. (1975) Principles of Biochemical Tests in Diagnostic Microbiology. John Wiley Sons. New York.
- McFADDIN, J.F. (2000) Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott. William & Wilkins. Philadelphia.
- O'MEARA, R.A.Q.(1931) A Simple, Delicate and Rapid Method of Detecting the Formation of Acetylmethylcarbinol by Bacteria Fermenting Carbohydrate. J. Pathol. Bact. 34:401-406.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 12°C).

Quality control

Culture medium: Art. No. 02-207
Incubation temperature: 35°C
Incubation time: 24 - 48 h
Inoculum: 1.000-10.000 cfu

Microorganism	Growth	Remarks
Enterobacter aerogenes ATCC 13048	Good	VP (+)
Escherichia coli ATCC 25922	Good	VP (-)
Escherichia coli ATCC 8739	Good	VP (-)

Oxidase Reagent (Gordon & McLeod)

Art. No. RE0065

Also known as

4-amino-N,N dimethylaniline-2-HCl

Specification

Reagent for the detection and verification of bacterial cytochrome oxidase.

Formula

N,N-dimethyl-p-phenyldiamine-2-HCl

Directions

Prepare an aqueous solution of Oxidase Reagent 1% immediately before use. It is recommendable only to prepare the amount needed, since once diluted it can only be used for a week, even if kept at 4ºC, avoiding direct light. Self oxidation can be reduced with the addition of ascorbic acid 0,01%, if the liquid is dark **it must not be used.**
The solution is normally transparent or slightly pink.

Description

This reagent is very unstable in the presence of oxygen. Light acts as a catalyst in the self oxidation process and so, it is recommended that the reagent be stored in an amber flask. When the test reaction response is very slow, the sample colony has to be considered negative or very weak, since the late appearance of colour is more attributable to spontaneous oxidation than to the true presence of cytochrome. Generally, except in a few cases, oxidase production is linked to flagellation in the following way: Polar flagellation is related to an oxidase positive reaction; peritrichous flagellation is related to an oxidase negative reaction. The oxidation of cytochrome for an oxidase positive reaction is as follows and it may be observed that molecular oxygen is absolutely necessary:

$$2\ \underset{\text{c cytochrome}}{\text{reduced}} + 2H + \tfrac{1}{2}\,O_2 \xrightarrow[\text{oxidase}]{\text{c cytochrome}} 2\ \underset{\text{c cytochrome}}{\text{oxidized}} + H_2O$$

Technique

There are several techniques to determine cytrochrome-oxidase in different genera.
The more standardized techniques are as follows:

a) Soak a filtration paper disc/strip with reagent. Take a colony and spread it over the paper. This step must be performed with a platinum-iridium / plastic loop or a Pasteur pipette. The use of metallic objects (nichrome loop, etc...) may produce false positive results.

b) Flood the colony with reagent directly in the plate. Following this, colonies cannot be subcultured, but the test does not interfere with Gram staining and the colony may be observed under the microscope. A positive reaction is indicated by the development in a short time (1-3 minutes) of a pink colour, which becomes deep red and finally blue-black after 10-12 minutes.

References

- BLAZEVIC, D.J., G.M. EDERER (1975) Principles of Biochemical Test in Diagnostic Microbiology. John Wiley Sons. NY.
- EWING, W.H. & J.G. JOHNSON (1960) The differentiation of Aeromonas and C27 cultures from Enterobacteriaceae. Int. Bull. Bacteriol. Nomencl. Taxon. 10(3):223-230.
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- GABY W.L., C. MARTLEY (1957) Practical laboratory test for the identification of *Pseudomonas aeruginosa*. J. Bact. 74(3):356 358.
- GORDON, J. & J.W. McLEOD (1928) The practical application of the direct oxidase reaction in bacteriology. J. Pathol. Bacteriol 31(2):185-190.
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- McFADDIN, J.F. (1999) Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins. Philadelphia. USA.
- STEEL, K.J. (1962) The oxidase activity of staphylococci. J. Appl. Bacteriol. 25(3):297-306.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 12ºC).

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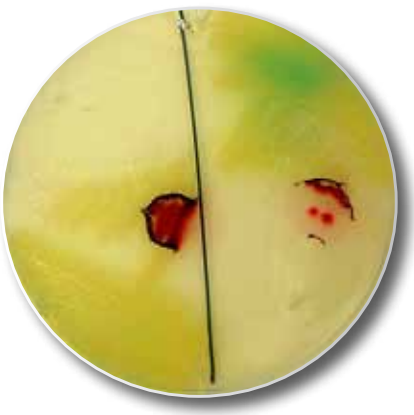
Oxidase Reagent (Gordon & McLeod)

Art. No. RE0065

Quality control

Culture medium: Art. No. 01-200
Incubation temperature: NA
Incubation time: NA
Inoculum: Pure culture

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC 10145	Good	Oxidase (+) . Dark red
Pseudomonas aeruginosa ATCC 27853	Good	Oxidase (+) . Dark red
Pseudomonas aeruginosa ATCC 9027	Good	Oxidase (+) . Dark red
Escherichia coli ATCC 25922	Good	Oxidase (-) . White to pink

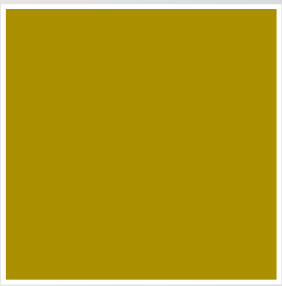


Left: *Pseudomonas aeruginosa* ATCC 10145
Right: *Pseudomonas aeruginosa* ATCC 25668

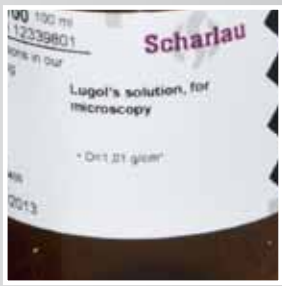
Pseudomonas aeruginosa ATCC 27853

Left: *Pseudomonas aeruginosa* ATCC 9027
Right: *Escherichia coli* ATCC 25922

Scharlau





Stains

Unstained bacteria have little contrast with their surroundings and are therefore difficult to see without staining. Staining is a simple procedure to color the microorganisms with a dye to emphasize certain structures. Stains can be broken down into 2 types – basic dyes and acidic dyes. Due to their negative charge at pH 7, most bacteria are stained by basic dyes such as crystal violet, methylene blue and safranin, whilst background tissue which is positively charged tends to be stained by acid dyes such as eosin.

The staining of bacteria can be broken down into three techniques: Simple staining, differential staining and special staining. A simple stain is a single basic dye used to highlight an entire microorganism so that its cellular shape and basic structures are visible. Differential stains react differently with different kinds of bacteria and thus can be used to distinguish them. The most frequently used differential stains are the Gram stain and the acid fast stain.

The Gram staining method is one of the most important staining techniques in Microbiology, allowing organisms to be divided into 2 broad groups: Gram negative and Gram positive, thus making further identification possible.

Special staining techniques such as spore staining and flegella staining are also valuable, and further aid in the identification of bacteria.

Fungi Stain

Art. No. AZ0175 - Lactophenol Blue



DANGER

H: 3.1.O/3; H301-3.1.D/3; H311-3.1.I/2; H330-3.2/1B; H314-3.5/2; H341-3.9/2; H373

P: P301+P310-P303+P361+P353-P305+P351+P338-P310-P320-P361-P405-P501a

Also Known as

Lactophenol Cotton Blue

Specification

For the staining of fungi in wet and fixed preparations.

Formula* in g/L

Phenol	260,00	g
Cotton Blue	260,00	g
Lactic acid	260,00	mL
Glycerol	520,00	mL
Distilled water	260,00	mL

* Adjusted and /or supplemented as required to meet performance criteria

Description

Lactophenol Blue is an excellent stain for the wet preparations of fungi. It combines in a single solution the following properties, mordant, fixative and stain. In the preparation of fungi for microscopic examination neither water nor aqueous stain solutions are used for mounting since the majority of moulds repel water and become encapsulated in micro-bubbles. For this reason lactophenol constitutes an ideal mounting fluid, wetting the structures whilst at the same time acting as a fixative and mordant. Due to its low evaporation rate the preparations remain usable for extended periods of time. This effect is further enhanced if the preparations are sealed with vaspar or nail varnish.

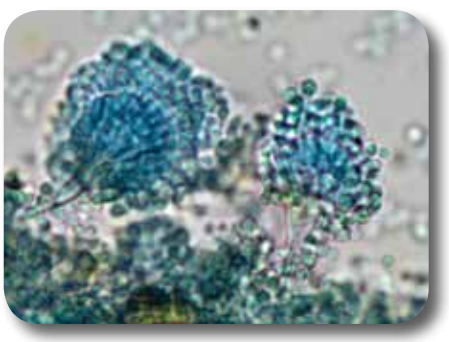
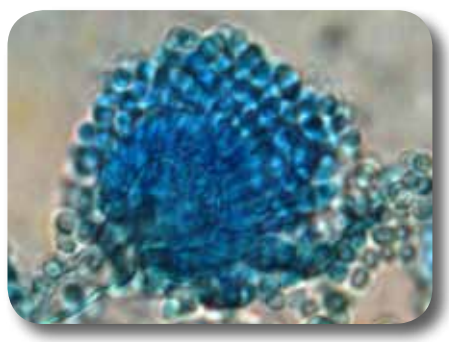
Cotton Blue, China Blue, or Soluble Aniline Blue is, a complex dye, but its effectiveness in the staining of fungi and vegetal material has been well demonstrated over time.

Recommended staining technique

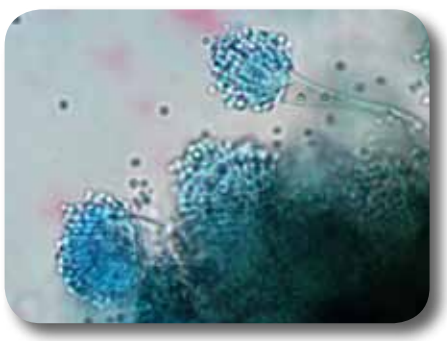
- On a clean slide place a small portion of the fungi to be examined and add one or two drops of Lactophenol blue.
- Gently tease the material, mixing it with the dye.
- Add two drops of water and gently mix before covering with the coverslip.
- The preparation is gently warmed using a flame until on the verge of bubbling. It is then pressed carefully to eliminate any excess liquid and the edges of the coverslip are sealed with nail varnish or vaspar.
- The preparation is now ready for microscopic examination.

Results

The mycelium of the fungus appears blue. The cellular protoplasts and other cellular structures appear in different tones of blue.

Aspergillus brasiliensis ATCC 16404, identification positive.



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Fungi Stain

Art. No. AZ0175 - Lactophenol Blue

DANGER

H: 3.1.O/3; H301-3.1.D/3; H311-3.1.I/2; H330-3.2/1B; H314-3.5/2; H341-3.9/2; H373

P: P301+P310-P303+P361+P353-P305+P351+P338-P310-P320-P361-P405-P501a

References

- CLARK, G. (1981) Staining Procedures. Biological Stain Commission. Williams & Wilkins. Baltimore and London.
- HARRIGAN, W.F. y M.E. McCANCE (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Pres. London.
- RAWLINS, T.E. (1933) Phytopathological and Botanical Research Methods. John Wiley & Sons. New York. USA
- SCHNEIDER, H. (1981) Pathological Anatomy and Mycology in "Staining Procedures" edited by G. Clark for the Biological Stain Commission. Williams & Wilkins. Baltimore. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).

Scharlau references

Cotton Blue (Aniline Blue C.I. 42755)..... AZO100
Glycerol (Anhydrous)..... GL0028
Phenol..... FE0484
L-(+)-Lactic Acid..... AC1380

Gram Stain

Art. No. VI0027 - Hucker¨s Crystal violet oxalate, solution according to Gram
Art. No. LU0010 - Lugol Iodine Solution for Gram Stain
Art. No. DE0010 - Bleaching agent, solution according to Gram (Decolourising)
Art. No. SA0042 - Safranin O Solution according to Gram

Stains needed for carrying out Gram Staining

Art. No. VI0027 Crystal violet oxalate, solution according to Gram Hucker's
Art. No. LU0010 Lugol Solution for Gram Stain
Art. No. DE0010 Bleaching agent, solution according to Gram (Decolourising)
Art. No. SA0042 Safranin O Solution according to Gram

Specification

Gram staining.

Dye formulas Scharlau microbiology

Art. No. VI0027 Crystal violet oxalate, solution according to Hucker for the Gram Stain

Formula* in g/L

Crystal violet	20,00 g
Ethanol	200,00 mL
Ammonium oxalate	8,00 g
Distilled Water	800,00 mL

WARNING
H: 2.6/3; H226-3.6/2; H351-3.3/2; H319-4.1.C/3; H412
P: P210-P241-P303+P361+P353-P305+P351+P338-P405-P501a

Art. No. LU0010 Lugol Iodine Solution for Gram Stain

Formula* in g/L

Iodine	3,00 g
Pottasium Iodide	6,00 g
Distilled Water	1000,00 mL

Art. No. DE0010 Bleaching agent, solution according to Gram

Formula* in g/L

Ethanol	500,00 mL
Acetone	500,00 mL

DANGER
H: 2.6/2; H225-3.3/2; H319-3.8/3; H336
P: P210-P241-P303+P361+P353-P305+P351+P338-P405-P501a

Art. No. SA0042 Safranin O Solution according to Gram

Formula* in g/L

Safranin	25,00 g
Ethanol	200,00 mL
Distilled Water	800,00 mL

Description

The Crystal violet solution for Gram staining, is prepared according to the Hucker formulation and is very stable, although it is possible that it may need to be filtered before use if left unused for too long. Aging does not harm its staining characteristics, but it can mean the decolourising time is extended slightly. The iodised-iodine solution is prepared according to Burke thus its stability is greater than Lugol's classic formulation, without this affecting the colouration. This solution can last several months at room temperature, but if on examination there is a loss of the characteristic amber colour, it must be discarded.
The decolourising agent for gram staining is made up of an alcohol-acetone mixture especially balanced to act quickly but gently on the base colours. Using the dropper bottle an addition of 15-20 drops is enough to obtain an effective decolourising of a smear stained correctly.
The counterstain is made up of a classic solution of safranin 0.25% which has been demonstrated to be much more effective than that of fuchsin used initially by Gram. This counterstain, is also used for the same purpose in many other differential staining methods.
All of these stains can be supplied in separate dropper bottles of 100 mL or in groups made up of each one of the reagents needed to carry out the stain. On request containers of greater capacity (1L) can be provided, but they do not include a dropper bottle.

Recommended staining technique

- Fix the smear.
- The slide is covered with Hucker's Crystal Violet (Art. No. VI0027) and left for 1 minute.
- Rinse off the excess dye with water. Do not rinse excessively. This step can be critical for the final colour contrast.
- Cover the preparation with the iodised-iodine solution (Art. No. LU0010) and drain immediately cover it again with new solution and allow it to act for 1 minute.
- Gently rinse again with water.
- Decolourise, pour the decolourising agent, (Art. No. DE0010) drop by drop, on an inclined slide, until no more colour is washed out.
  This stage should not last more than 60 seconds.

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Gram Stain

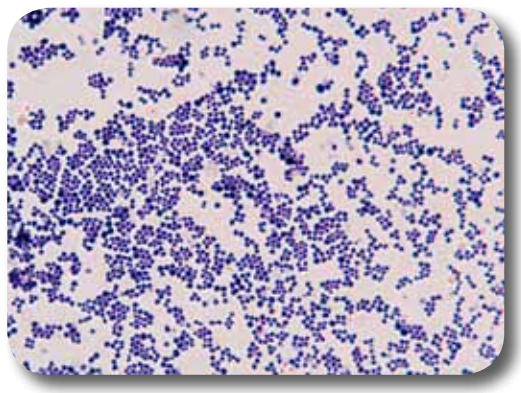
Art. No. VI0027 - Hucker¨s Crystal violet oxalate, solution according to Gram
Art. No. LU0010 - Lugol Iodine Solution for Gram Stain
Art. No. DE0010 - Bleaching agent, solution according to Gram (Decolourising)
Art. No. SA0042 - Safranin O Solution according to Gram

- Rinse with water to stop the action of the decolourising agent.
- Cover the preparation with the safranin solution (Art. No. SA0042) and allow it to act for 1 minute.
- Rinse gently to eliminate excess dye.
- Allow to dry and examine under oil immersion. If the presence of actinomycetes is suspected or that of microorganisms that are not easily stained Gram-positive, it is advisable to use crystal violet with aniline and to follow the same staining procedure.

Results

The microorganisms that are stained by the initial dye appear dark blue are called GRAM-POSITIVES (G +). Those that are stained with the counterstain, appear red in colour and are called GRAM-NEGATIVES (G –). The majority of eukaryotic cells, with the important exception of yeasts, are stained Gram-negative. For prokaryotes it constitutes one of the first levels of systematic identification: Amongst the bacteria, all the cocci except *Neisseria* and Veillonella are Gram-positive; as are all sporogenous bacilli. The spirochaetes, vibrios, rickettsias, chlamydiae and the great majority of other bacilli are Gram-negative.

Bacillus cereus ATCC 11778 , Gram (+)



Staphylococcus aureus ATCC 25923 , Gram (+)

Escherichia coli ATCC 25922 , Gram (-)

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Gram Stain

Art. No. VI0027 - Hucker¨s Crystal violet oxalate, solution according to Gram
Art. No. LU0010 - Lugol Iodine Solution for Gram Stain
Art. No. DE0010 - Bleaching agent, solution according to Gram (Decolourising)
Art. No. SA0042 - Safranin O Solution according to Gram

References

- BARTHOLOMEW, J.W. (1962) Variables influencing results, and the precise definition of steps in Gram staining as a means of standardizing the results obtained. Stain Technol. 37:139-155.
- BARTHOLOMEW, J.W. (1981) Stains for microorganisms in smears. In “Staining Procedures” edited by G. Clark for the Biological Stain Commission.
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- GRAM, C. (1884) Ueber die isolierte Färbung der Schyzomyceten in Schnitt und Trockenpräparaten. Fortschr.Med. 2:185-189
- GURR, E. (1973) Biological Staining Methods 8th edition. Searle Diagnostic Gurr Products. London
- HOROBIN, R.W. & J.A. KIERNAN (2002) Conn’s Biological Stains. 10th edition. Biological Stain Commission. Bios Sci. Pub. Ltd. London
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- LILLIE, R.D. (1969) H.J. Conn’s Biological Stains. 8th edition. Williams & Wilkins Co. Baltimore. USA
- PAIK, G. (1980) Reagents, Stain and Miscellaneous Procedures. In “Manual of Clinical Microbiology” 3rd Edition. Lennette, Balows, Hausler y Truant, editors. ASM. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C and <60 % RH).

Scharlau references

Acetone	AC0313
Ammonium Oxolate	AM0364
Cristal Violet	VI0025
Ethanol	ET0003
Potassium Iodide	PO0411
Safranin	SA0400
Iodine	YO0020

Spore Stain

Art. No. VE0101 - Malachite Green for Endospores

Specification

Dye for the staining of bacterial endospores.

Formula* in g/L

Malachite green oxalate	70,00 g
Distilled water	1000,00 mL

* Adjusted and /or supplemented as required to meet performance criteria

Description

Malachite Green stain for endospores is formulated according to Bartholomew and Mittwer (1950), who modified the procedure of Schaeffer and Fulton (1933), which was based on the original by Wirtz. The modification of the method consists of eliminating the step of heating the preparation, instead a more concentrated dye is prepared and left for a longer time, a gentler counterstain (safranin) is used. The true stain consists of a saturated aqueous solution of malachite green, when used in the Schaeffer technique it must be diluted in half to avoid the formation of precipitates.

Recommended staining technique

- A smear of the microorganism is prepared, and allowed to air dry.
- The smear is fixed by passing the slide over a flame approximately 20 times and allowed to cool.
- The smear is completely covered with malachite green and left to act for 10 minutes.
- It is rinsed with gentle running, water to eliminate any excess stain.
- The smear is then counterstained by covering with safranin solution (Art. No. SA0042), as used in Gram stains, and left for 15-30 seconds.
- It is washed gently, dried, and observed microscopically under oil immersion.

Results

The cellular bodies appear red or pink in colour whereas the endospores are stained green.

Bacillus subtilis ATCC 6633 (spores present)

References

- ALORS CORREDERAS, R. (2008) Técnicas de visualización microscópica de microorganismos. Revista Digital de Innovación y Experimentación Educativa Nº4. Granada
- BARTHOLOMEW, J.W. y T. MITTWER (1950). A simplified bacterial spore stain. Stain Technol. 24:153-156.
- BARTHOLOMEW, J.W. (1981) Stains for Microorganisms in Smears, in “Stain Procedures” edited by G. Clark para la Biological Stain Commission. Williams & Wilkins Co. Baltimore.
- CLARK, G. (1981) Staining procedures. Biological Stain Commission. Williams & Wilkins Co. Baltimore.
- GONZALEZ de BUITRAGO, J. M. (2004) Técnicas y Métodos de Laboratorio Clínico. Masson. Madrid.
- SCHAEFFER, A.B.y M. FULTON (1933). A simplified method of staining endospores. Science, 77, 194.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).

References for Scharlau products

Malachite Green oxalate C.I. 42000	VE0100

Scharlau

Most Probable Number (MPN) Technique

Most Probable Number (MPN) Technique

The Most Probable Number Technique (MPN) is one of the most commonly used methods for determining the number of viable bacteria in a sample. However, the MPN is only a statistical estimate of the microbial population of the sample, and therefore does not provide a precise enumeration. When the population is large better results are obtained with the plate enumeration technique, but the MPN is the best way to enumerate samples with small populations (less than 10 cells per g or mL), allowing the use of larger amounts sample.

The MPN is a statistical concept derived from the probability theory and certain important aspects of this should be taken into consideration before using this technique:

- It is assumed that microorganisms are spread evenly and randomly in the sample. If not, the sample should be thoroughly homogenised.
- Use a suitable culture medium for each organism, so that if only one cell grows, it will be detected.
- The method becomes less effective with cellular association, because the cells are considered as individual units.

The ideal approach is based on a choice of a liquid culture medium that helps the growth of bacteria, and in the preparation of a series of dilutions of the sample to be analyzed so as to allow observation of the growth in the lower dilution tubes and no growth in the higher dilution tubes. If the number of microorganisms in the sample is unknown, increase the number of dilutions to reduce the margin of error.

The more tubes inoculated for each dilution, the more precise the technique. The most common number is between 3 and 5 tubes per dilution.

The readings are carried out after incubation at the optimum temperature for the organism, taking as positive all the turbid tubes (growth), and as negative the tubes where there is no growth. Other reactions in addition to turbidity may be observed in the tubes and can be considered as positive (e.g., gas production in the coliform count.)

The approximate number of live microorganisms in the original suspension sample is calculated using the probability tables shown in the following pages.

The tables recommended below provide only the usual combinations of results, and have omitted inconsistent results.

Normally, the tables give results with a confidence level of 95% or 99%. The minimum error is obtained when the series used for the calculations has the same proportion of positive and negative tubes.

Although the tables most widely used are the 3 or 5 tubes per dilution (Tables 3, 5, 6, 7 and 8), for some special cases different combinations are used.

In the case of treated water for coliform and enterococci, a single set of 5 tubes with 10 mL of sample (Table 1) is used. However, when high or erratic values are obtained for the samples, or coliforms are detected repeatedly, inoculum volumes change significantly, and it is advisable to inoculate 100 mL of the sample, distributing this between a bottle containing 50 mL and 5 tubes with 10ml, always at double concentration (Table 2). If greater accuracy is required, add 1 mL of sample to 5 tubes (Table 4).

Tables 1 through 5 give results as the most probable number of cells per 100 mL of sample, and are typically used in water analysis. Tables 6 through 8 give the results per gram or millilitre, and is used in food microbiology.

(continues on the next page)

Most Probable Number (MPN) Technique

Table 1

Tubes whith positive reaction in 5 tubes of 10 mL	MPN / 100 mL Index	Confidence Limit 95%	
		Low Limit	High Limit
0	0,00	0,00	6,00
1	2,20	0,10	12,60
2	5,10	0,50	19,20
3	9,20	1,60	29,40
4	16,00	3,30	52,90
5	Infinite	8,00	Infinite

Table 2

Tubes whith positive reaction between		MPN / 100 mL Index	Confidence Limit 95%	
1 tube of 50 mL	5 tubes of 10 mL		Low Limit	High Limit
0	1	1	<0,50	4,00
0	2	2	<0,50	6,00
0	3	4	<0,50	11,00
0	4	5	1,00	13,00
1	0	2	0,50	6,00
1	1	3	<0,50	9,00
1	2	6	1,00	15,00
1	3	9	2,00	21,00
1	4	16	4,00	40,00

Most Probable Number (MPN) Technique

Table 3

Tubes whith positive reaction between			MPN / 100 mL Index	Confidence Limit 95%	
5 tubes of 10 mL	5 tubes of 1 mL	5 tubes of 0,1 mL		Low Limit	High Limit
0	0	1	1	<0,5	2
0	1	0	1	<0,5	2
0	1	1	1	<0,5	2
0	2	0	1	<0,5	2
0	3	0	1	<0,5	2
1	0	0	1	<0,5	2
1	0	1	1	<0,5	2
1	1	0	1	<0,5	2
1	1	1	1	<0,5	2
1	2	0	1	<0,5	2
1	2	1	2	<0,5	4
1	3	0	2	<0,5	4
2	0	0	1	<0,5	2
2	0	1	1	<0,5	2
2	1	0	1	<0,5	2
2	1	1	2	<0,5	4
2	2	0	2	<0,5	4
2	2	1	2	<0,5	4
2	3	0	2	<0,5	4
2	3	1	3	1	7
2	4	0	3	1	7
3	0	0	2	<0,5	4
3	0	1	2	<0,5	4
3	1	0	2	<0,5	4
3	1	1	2	<0,5	4
3	1	2	3	1	7
3	2	0	3	1	7
3	2	1	3	1	7
3	2	2	4	1	9
3	3	0	3	1	7
3	3	1	4	1	9
3	4	0	4	1	9
3	4	1	4	1	9
4	0	0	2	<0,5	4

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Most Probable Number (MPN) Technique

Table 3

Tubes whith positive reaction between			MPN / 100 mL Index	Confidence Limit 95%	
5 tubes of 10 mL	5 tubes of 1 mL	5 tubes of 0,1 mL		Low Limit	High Limit
4	0	1	3	1	7
4	0	2	3	1	7
4	1	0	3	1	7
4	1	1	4	1	9
4	1	2	4	1	9
4	2	0	4	1	9
4	2	1	4	1	9
4	2	2	5	2	12
4	3	0	5	2	12
4	3	1	5	2	13
4	3	2	6	2	14
4	4	0	6	2	14
4	4	1	7	3	17
4	5	0	7	3	17
4	5	1	8	3	19
5	0	0	4	1	9
5	0	1	4	1	9
5	0	2	6	2	14
5	1	0	5	2	12
5	1	1	6	2	14
5	1	2	7	3	17
5	2	0	6	2	14
5	2	1	8	3	19
5	2	2	10	4	23
5	2	3	12	4	28
5	3	0	9	3	21
5	3	1	11	4	26
5	3	2	14	5	34
5	3	3	18	6	53
5	4	0	13	5	31
5	4	1	17	6	47
5	4	2	22	7	70
5	4	3	28	9	85
5	4	4	35	11	101
5	5	0	24	8	75

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Most Probable Number (MPN) Technique

Table 3

Tubes whith positive reaction between			MPN / 100 mL Index	Confidence Limit 95%	
5 tubes of 10 mL	5 tubes of 1 mL	5 tubes of 0,1 mL		Low Limit	High Limit
5	5	1	35	11	101
5	5	2	54	18	140
5	5	3	92	27	218
5	5	4	161	39	424

Table 4

Tubes whith positive reaction between			MPN / 100 mL Index	Confidence Limit 95%	
1 tube of 50 mL	5 tubes of 10 mL	5 tubes of 1 mL		Low Limit	High Limit
0	0	1	1	<0,5	4
0	0	2	2	<0,5	6
0	1	0	1	<0,5	4
0	1	1	2	<0,5	6
0	1	2	3	<0,5	3
0	2	0	2	<0,5	6
0	2	1	3	<0,5	8
0	2	2	4	<0,5	11
0	3	0	3	<0,5	8
0	3	1	5	<0,5	13
0	4	0	5	<0,5	13
1	0	0	1	<0,5	4
1	0	1	3	<0,5	8
1	0	2	4	<0,5	11
1	0	3	6	<0,5	15
1	1	0	3	<0,5	8
1	1	1	5	<0,5	13
1	1	2	7	1	17
1	1	3	9	2	21
1	2	0	5	<0,5	13
1	2	1	7	1	17
1	2	2	10	3	23
1	2	3	12	3	28

(continues on the next page)

Most Probable Number (MPN) Technique

Table 4

Tubes whith positive reaction between			MPN / 100 mL Index	Confidence Limit 95%	
1 tube of 50 mL	5 tubes of 10 mL	5 tubes of 1 mL		Low Limit	High Limit
1	3	0	8	2	19
1	3	1	11	3	26
1	3	2	14	4	34
1	3	3	13	5	53
1	3	4	21	6	66
1	4	0	13	4	31
1	4	1	17	5	47
1	4	2	22	7	69
1	4	3	23	9	85
1	4	4	35	12	101
1	4	5	43	15	117
1	5	0	24	8	75
1	5	1	35	12	101
1	5	2	54	18	138
1	5	3	92	27	217
1	5	4	161	39	>450

Most Probable Number (MPN) Technique

Table 5

Tubes whith positive reaction between			MPN / 100 mL Index	Confidence Limit 95%	
3 tubes of 10 mL	3 tubes of 1 mL	3 tubes of 0,1 mL		Low Limit	High Limit
0	0	1	3	<0,5	9
0	1	0	3	<0,5	13
1	0	0	4	<0,5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	149
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	379
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800

Most Probable Number (MPN) Technique

Table 6

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
3 tubes of 1 mL	3 tubes of 0,1 mL	3 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
0	1	0	0,3		x	<0,1	2,3	<0,1	1,7
1	0	0	0,4	x		<0,1	2,8	0,1	2,1
1	0	1	0,7		x	0,1	3,5	0,2	2,7
1	1	0	0,7	x		0,1	3,6	0,2	2,8
1	2	0	1,1		x	0,2	4,4	0,4	3,5
2	0	0	0,9	x		0,1	5	0,2	3,8
2	0	1	1,4		x	0,3	6,2	0,5	4,8
2	1	0	1,5	x		0,3	6,5	0,5	5
2	1	1	2	x	x	0,5	7,7	0,8	6,1
2	2	0	2,1	x		0,5	8	0,8	6,3
3	0	0	2,3	x		0,4	17,7	0,7	12,9
3	0	1	4	x		1	23	1	18
3	1	0	4	x		1	29	2	21
3	1	1	7	x		2	37	2	28
3	2	0	9	x		2	52	3	39
3	2	1	15	x		3	66	5	51
3	2	2	21		x	5	82	8	64
3	3	0	20	x		<10	190	10	140
3	3	1	50	x		10	320	20	240
3	3	2	110	x		20	640	30	480

Note about Categories
(source J.C. de Man (1975) The probability of the Most Probable Number. Eur. J . Appl. Microbiol. 1: 72-77)
Category A: Normal results, obtained in the 95% of the cases.
Category B: Less probable results, obtained just in the 4% of the cases. They must not be uses in important decisions.
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Most Probable Number (MPN) Technique

Table 7

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
5 tubes of 1 mL	5 tubes of 0,1 mL	5 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
0	0	1	0,2		x	<0,1	1,3	<0,1	1
0	1	0	0,2	x		<0,1	1,4	<0,1	1
1	0	0	0,2	x		<0,1	1,4	<0,1	1
1	0	1	0,4		x	0,1	1,9	0,1	1,5
1	1	0	0,4	x		0,1	1,9	0,1	1,5
1	2	0	0,6		x	0,1	2,3	0,2	1,8
2	0	0	0,4	x		0,1	2,2	0,1	1,7
2	0	1	0,7		x	0,1	2,6	0,2	2,1
2	1	0	0,7	x		0,1	2,6	0,2	2,1
2	1	1	0,9		x	0,2	3,1	0,3	2,5
2	2	0	0,9	x		0,2	3,1	0,3	2,5
3	0	0	0,8	x		0,1	3,1	0,3	2,5
3	0	1	1,1	x		0,2	3,7	0,4	2,9
3	1	0	1,1	x		0,2	3,7	0,4	3
3	1	1	1,4		x	0,4	4,3	0,6	3,5
3	2	0	1,4	x		0,4	4,3	0,6	3,5
3	2	1	1,7		x	0,5	4,9	0,8	4,1
3	3	0	1,7		x	0,5	5	0,8	4,1
4	0	0	1,3	x		0,3	4,9	0,5	3,9
4	0	1	1,7	x		0,5	5,8	0,7	4,6
4	1	0	1,7	x		0,5	5,9	0,7	4,7
4	1	1	2,1	x		0,6	6,8	0,9	5,5
4	2	0	2,2	x		0,7	7,1	0,9	5,7
4	2	1	2,6		x	0,8	8,1	1,2	6,6
4	3	0	2,7	x		0,9	8,3	1,3	6,8
4	3	1	3,3		x	1,1	9,5	1,5	7,7
4	4	0	3,4		x	1,2	9,8	1,5	8,1
5	0	0	2,3	x		0,6	11,6	0,9	8,7
5	0	1	3,1	x		0,9	14,5	1,4	11,2
5	1	0	3	x		1	16	1	12
5	1	1	5	x		1	20	2	15
5	1	2	6	x		2	23	3	19
5	2	0	5	x		1	22	2	17

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Most Probable Number (MPN) Technique

Table 7

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
3 tubes of 1 mL	3 tubes of 0,1 mL	3 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
5	2	1	7	x		2	27	3	21
5	2	2	9		x	3	31	4	25
5	3	0	8	x		2	32	3	25
5	3	1	11	x		3	38	4	30
5	3	2	14	x		4	44	6	36
5	4	0	13	x		3	50	5	39
5	4	1	17	x		5	61	7	48
5	4	2	22	x		7	73	9	59
5	4	3	28		x	9	87	13	70
5	4	4	35		x	12	101	16	83
5	5	0	24	x		6	127	10	95
5	5	1	30	x		10	180	10	130
5	5	2	50	x		10	250	20	200
5	5	3	90	x		20	380	30	300
5	5	4	160	x		40	700	60	530

Note about Categories
(source J.C. de Man (1975) The probability of the Most Probable Number. Eur. J . Appl. Microbiol. 1: 72-77)
Category A: Normal results, obtained in the 95% of the cases.
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Most Probable Number (MPN) Technique

Table 8

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
10 tubes of 1 mL	10 tubes of 0,1 mL	10 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
0	0	1	0,09		x	<0,01	0,67	0,01	0,51
0	1	0	0,09	x		<0,01	0,68	0,01	0,51
0	2	0	0,18		x	0,02	0,85	0,05	0,67
1	0	0	0,09	x		<0,01	0,71	0,01	0,54
1	0	1	0,19		x	0,02	0,89	0,05	0,69
1	1	0	0,19	x		0,02	0,89	0,05	0,7
1	2	0	0,29		x	0,06	1,06	0,09	0,85
2	0	0	0,2	x		0,02	0,93	0,06	0,73
2	0	1	0,3		x	0,1	1,1	0,1	0,9
2	1	0	0,3	x		0,1	1,1	0,1	0,9
2	1	1	0,4		x	0,1	1,3	0,1	1
2	2	0	0,4	x		0,1	1,3	0,2	1
2	3	0	0,5		x	0,1	1,5	0,2	1,2
3	0	0	0,3	x		0,1	1,2	0,1	0,9
3	0	1	0,4	x		0,1	1,4	0,2	1,1
3	1	0	0,4	x		0,1	1,4	0,2	1,1
3	1	1	0,5		x	0,2	1,5	0,2	1,3
3	2	0	0,5	x		0,2	1,5	0,2	1,3
3	2	1	0,6		x	0,2	1,7	0,3	1,4
3	3	0	0,6		x	0,2	1,7	0,3	1,4
4	0	0	0,4	x		0,1	1,4	0,2	1,2
4	0	1	0,6	x		0,2	1,6	0,2	1,3
4	1	0	0,6	x		0,2	1,7	0,2	1,4
4	1	1	0,7	x		0,2	1,8	0,3	1,5
4	2	0	0,7	x		0,2	1,8	0,3	1,5
4	2	1	0,8	x		0,3	2	0,4	1,7
4	3	0	0,8	x		0,3	2,1	0,4	1,7
4	4	0	0,9		x	0,3	2,2	0,5	1,9
5	0	0	0,6	x		0,2	1,8	0,2	1,5
5	0	1	0,7	x		0,2	2	0,3	1,6
5	1	0	0,7	x		0,2	2	0,3	1,7
5	1	1	0,9	x		0,3	2,2	0,4	1,8
5	2	0	0,9	x		0,3	2,2	0,4	1,8

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Most Probable Number (MPN) Technique

Table 8

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
10 tubes of 1 mL	10 tubes of 0,1 mL	10 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
5	2	1	1		x	0,4	2,4	0,5	2
5	3	0	1	x		0,4	2,4	0,5	2
5	3	1	1,1		x	0,4	2,6	0,6	2,2
5	4	0	1,1		x	0,4	2,6	0,6	2,2
6	0	0	0,8	x		0,3	2,2	0,3	1,8
6	0	1	0,9	x		0	2,4	0,4	2
6	1	0	0,9	x		0,3	2,4	0,4	2
6	1	1	1,1	x		0,4	2,6	0,5	2,2
6	2	0	1,1	x		0,4	2,6	0,5	2,2
6	2	1	1,2	x		0,5	2,9	0,6	2,4
6	3	0	1,2	x		0,5	2,9	0,6	2,5
6	3	1	1,4		x	0,6	3,2	0,7	2,7
6	4	0	1,4		x	0,6	3,2	0,7	2,7
6	5	0	1,5		x	0,7	3,4	0,8	2,9
7	0	0	1	x		0,3	2,6	0,5	2,2
7	0	1	1,2	x		0,4	2,9	0,6	2,4
7	0	2	1,3		x	0,5	3,2	0,7	2,7
7	1	0	1,2	x		0,4	2,9	0,6	2,5
7	1	1	1,3	x		0,5	3,2	0,7	2,7
7	1	2	1,5		x	0,6	3,5	0,8	3
7	2	0	1,3	x		0,5	3,3	0,7	2,7
7	2	1	1,5	x		0,6	3,5	0,8	3
7	2	2	1,7		x	0,7	3,8	0,9	3,2
7	3	0	1,5	x		0,6	3,6	0,8	3
7	3	1	1,7	x		0,7	3,9	0,9	3,3
7	4	0	1,7	x		0,7	3,9	0,9	3,3
7	4	1	1,9		x	0,8	4,2	1	3,6
7	5	0	1,9		x	0,8	4,3	1	3,6
8	0	0	1,3	x		0,5	3,3	0,6	2,8
8	0	1	1,5	x		0,6	3,7	0,7	3,1
8	0	2	1,7		x	0,7	4,1	0,8	3,4
8	1	0	1,5	x		0,6	3,7	0,7	3,1
8	1	1	1,7	x		0,7	4,1	0,9	3,4

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Most Probable Number (MPN) Technique

Table 8

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
10 tubes of 1 mL	10 tubes of 0,1 mL	10 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
8	1	2	1,9		x	0,8	4,5	1	3,7
8	2	0	1,7	x		0,7	4,1	0,9	3,5
8	2	1	1,9	x		0,8	4,5	1	3,8
8	2	2	2,1		x	0,9	4,9	1,2	4,2
8	3	0	1,9	x		0,8	4,6	1	3,9
8	3	1	2,1	x		0,9	5	1,2	4,2
8	3	2	2,4		x	1	5,4	1,3	4,6
8	4	0	2,2	x		0,9	5,1	1,2	4,3
8	4	1	2,4	x		1	5,5	1,3	4,6
8	5	0	2,4	x		1,1	5,5	1,3	4,7
8	5	1	2,7		x	1,2	6	1,5	5,1
8	6	0	2,7		x	1,2	6,1	1,5	5,2
9	0	0	1,7	x		0,6	4,5	0,8	3,7
9	0	1	1,9	x		0,7	5	1	4,1
9	0	2	2,2		x	0,9	5,6	1,1	4,6
9	1	0	1,9	x		0,7	5,1	1	4,2
9	1	1	2,2	x		0,9	5,6	1,1	4,7
9	1	2	2,5		x	1	6,2	1,3	5,2
9	2	0	2,2	x		0,9	5,8	1,2	4,7
9	2	1	2,5	x		1	6,4	1,3	5,3
9	2	2	2,8		x	1,2	7	1,5	5,8
9	3	0	2,5	x		1,1	6,5	1,3	5,4
9	3	1	2,9	x		1,2	7,2	1,5	6
9	3	2	3,2		x	1,4	7,8	1,8	6,6
9	4	0	2,9	x		1,2	7,3	1,6	6,1
9	4	1	3,3	x		1,2	7,3	1,6	6,1
9	4	2	3,7		x	1,6	8,8	2	7,4
9	5	0	3,3	x		1,4	8,2	1,8	6,9
9	5	1	3,7	x		1,6	9	2	7,6
9	5	2	4,2		x	1,9	9,8	2,3	8,3
9	6	0	3,8	x		1,7	9,2	2,1	7,7
9	6	1	4,3		x	1,9	10	2,4	8,5
9	7	0	4,4		x	1,9	10,3	2,4	8,7

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Most Probable Number (MPN) Technique

Table 8

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
10 tubes of 1 mL	10 tubes of 0,1 mL	10 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
10	0	0	2,3	x		0,9	7,2	1,2	5,8
10	0	1	2,7	x		1,1	8,3	1,4	6,7
10	0	2	3,1		x	1,3	9,5	1,6	7,7
10	1	0	2,7	x		1,1	8,6	1,4	6,9
10	1	1	3,2	x		1,3	9,9	1,7	7,9
10	1	2	3,8	x		1,5	11,3	2	9,2
10	2	0	3,3	x		1,3	10,3	1,7	8,3
10	2	1	3,9	x		1,6	11,7	2	9,6
10	2	2	5	x		2	13	2	11
10	2	3	5		x	2	15	3	12
10	3	0	4	x		1,6	12,4	2	10
10	3	1	5	x		2	14	2	12
10	3	2	6	x		2	16	3	13
10	3	3	7		x	3	18	3	15
10	4	0	5	x		2	15	3	12
10	4	1	6	x		2	17	3	14
10	4	2	7	x		3	19	3	16
10	4	3	8		x	3	21	4	17
10	5	0	6	x		2	18	3	15
10	5	1	7	x		3	20	4	17
10	5	2	9	x		3	22	4	19
10	5	3	10	x		4	25	5	21
10	6	0	8	x		3	22	4	18
10	6	1	9	x		4	24	5	20
10	6	2	11	x		4	27	5	23
10	6	3	12	x		5	30	6	25
10	6	4	14		x	6	32	7	27
10	7	0	10	x		4	27	5	22
10	7	1	12	x		4	30	6	25
10	7	2	14	x		5	33	7	28
10	7	3	15	x		6	36	8	31
10	7	4	17		x	7	40	9	34
10	8	0	13	x		5	34	6	28

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Most Probable Number (MPN) Technique

Table 8

Tubes whith positive reaction between			MPN (g or mL)	Category		Confidence Limits			
						99%		95%	
10 tubes of 1 mL	10 tubes of 0,1 mL	10 tubes of 0,01 mL		A	B	Low Limit	High Limit	Low Limit	High Limit
10	8	1	15	x		6	38	8	32
10	8	2	17	x		7	42	9	36
10	8	3	20	x		8	47	10	40
10	8	4	22	x		10	52	12	44
10	8	5	25		x	11	57	14	48
10	9	0	17	x		6	47	9	38
10	9	1	20	x		8	53	10	43
10	9	2	23	x		9	60	12	49
10	9	3	26	x		11	68	14	56
10	9	4	0	x		13	77	16	64
10	9	5	35	x		15	86	18	72
10	9	6	40		x	17	97	21	82
10	10	0	24	x		9	77	12	61
10	10	1	29	x		11	94	15	75
10	10	2	35	x		13	114	17	91
10	10	3	40	x		20	140	20	110
10	10	4	50	x		20	170	30	140
10	10	5	70	x		30	210	30	170
10	10	6	90	x		30	260	40	210
10	10	7	120	x		40	30	60	270
10	10	8	160	x		60	450	80	370
10	10	9	230	x		80	760	110	600

Note about Categories
(source J.C. de Man (1975) The probability of the Most Probable Number. Eur. J . Appl. Microbiol. 1: 72-77)
Category A: Normal results, obtained in the 95% of the cases.
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Application Tables & Cross References

Application tables

Culture media for clinical analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
02-186	**Anaerobic/microaerophilic**	Thioglycollate Broth	AOAC/BAM/EP/USP	Sterility test
01-136	**Antibiotic assay**	Mueller-Hinton Agar	BAM/CLSI(NCCLS)/ISO/CE	Sensibility test
02-136		Mueller-Hinton Broth	CLSI(NCCLS)/CMPH	Sensibility test
01-262	***Bacillus cereus***	*Bacillus cereus* Agar	BAM/COMPF/IDF/ISO	Isolation/Enumeration
01-047	**Bacteria in urine**	CLED Agar	CE	Isolation
02-410	**Clostridia**	Differential Reinforced Clostridial Medium (DRCM)	DIN/ISO	Enumeration/Enrichment
01-289		Reinforced Clostridial Agar		Isolation/Enumeration/cutivation
01-068	**Coliforms**	Eosin Methylene Blue Agar (EMB Agar)	BAM/ISO/USP	Isolation/Enumeration
01-118		MacConkey Agar (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/ISO/SMWW/USP/CE	Isolation/Enumeration
02-041	**Coliforms and *E. coli***	Brilliant Green Bile 2% Broth	BAM/COMPF/ISO/SMWW	Detection/Enrichment
02-060		*E. coli* Broth	COMPF/ISO/SMWW	Detection/Enrichment
02-108		Tryptose Lauryl sulfate Broth	BAM/COMPF/IDF/ISO/SMD/SMWW	Detection/Enumeration/Enrichment
01-541	***E. coli* O157:H7**	MacConkey Sorbitol Agar	ISO	Isolation/Enumeration
02-691		Tryptic Soy Broth Modified	BAM/ISO	Enrichment
02-277	**Enterobacteria**	Buffered Peptone Water	ISO	Enrichment
01-103		Kligler Iron Agar (KIA)	ISO	Isolation/Enumeration
01-094		Lysine Iron Agar (LIA)	AOAC/COMPF/SMD	Isolation/Enumeration/Identification
02-207		Methyl Red Voges Proskauer Broth (MRVP)	BAM/IDF/ISO/SL	Identification
03-422		Motility Indol Ornithine Fluid Medium (MIO)	BAM	Confirmation
03-037		Oxidation-Fermentation Fluid Medium Base (O/F Medium)	CMPH/COMPF	Identification
01-083		Phenylalanine Agar (PPA)	CMPH	Identification/Differentation
03-176		SIM Medium		Isolation/Enumeration/Identification
01-177		Simmons Citrate Agar	AOAC/BAM/ISO/SMWW	Identification
01-192		Triple Sugar Iron Agar (TSI Agar)	AOAC/BAM/EP/IDF/ISO/USP	Isolation/Enumeration/Identification
01-261		Urea Agar Base	COMPF/DIN/IDF/ISO/SMD	Identification
02-202		Urea Broth Base	BAM/COMPF	Differentiation/Identification
01-057		Deoxycholate Lactose Agar	COMPF/SMWW	Isolation/Enumeration
02-105		Lactose Broth	AOAC/COMPF/EP/ISO/SMWW/USP	Enrichment
01-216	**Enterobacteria *(Salmonella spp./Shigella spp.)***	Hektoen Enteric Agar	AOAC/BAM/COMPF/ISO	Isolation/Enumeration
01-446	**Enterococci-Streptococci**	Liebermeister & Braveny Agar		Isolation
02-191		Todd-Hewitt Broth		Cultivation
01-352	**Fastidious Microorganisms**	Blood Agar Base	CE	Isolation/Enumeration/Cultivation
01-034		Blood Agar Base (Columbia)	ISO/CE	Cultivation
01-505		Blood Agar Base No. 2	BAM/COMPF/ISO	Isolation/Enrichment/Cultivation
01-310		GC Agar Base		Isolation/Cultivation
01-137	**Fungi *(Candida spp.)***	Nickerson Agar (Biggy Agar)		Isolation
01-442	**Fungi (Yeasts and moulds)**	Dermatophyte Selective Agar		Isolation
01-483		Potato Dextrose Agar (Eur. Pharm.)	HP/BAM/COMPF/USP	Isolation/Enumeration/Detection
01-166		Sabouraud Chloramphenicol Agar	ISO/EP/USP	Isolation/Enumeration/Identification
01-165		Sabouraud Dextrose Agar	HP/BAM/EP/ISO/USP	Enumeration
01-703	**Gram-positive cocci**	Columbia CNA Agar Base		Isolation
01-706	***Haemophilus***	HTM (Haemophilus Test Medium)		Susceptibility Test
02-135	**Lactic acid bacteria**	MRS Broth	IFU/ISO	Enrichment/Isolation/Cultivation
01-687	***Legionella***	*Legionella* BCYE Agar Base	ISO	Isolation/Enumeration
03-643	**Microorganism-Aerobic plate count**	Cary-Blair Transport Medium	CMPH	Transport/Preservation
01-140		Nutrient Agar	BAM/BP/ISO	Cultivation
01-161		Plate Count Agar (PCA)	BAM/COMPF/IDF/ISO/USP	Enumeration
02-032		Phenol Red Broth Base	BAM/COMPF/SMD/USP	Identification
01-200		Tryptic Soy Agar (TSA) (Eur. Pharm.)	HP/BAM/COMPF/ISO/EP/USP	General application
02-200		Tryptic Soy Broth (TSB) (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/USP	General application/Enrichment
02-599	**Pathogenic microorganisms**	Brain Heart Infusion Broth (BHI Broth)	BAM/COMPF/ISO/SMWW/CE	Enrichment/Cultivation
01-609	***Pseudomonas***	CN Selective Agar Base	EN/ISO	Isolation
01-203	***Salmonella***	Brilliant Green Agar (BGA)	EP/USP	Isolation/Enumeration
02-652		Mannitol Selenite Broth Base		Enrichment
02-335		Muller-Kauffmann Tetrathionate Broth Base	DIN/ISO	Enrichment
02-379		Rappaport Vassiliadis Broth	AOAC/BAM/COMPF/IDF/ISO	Enrichment
02-602		Selenite Cystine Broth Base	COMPF/ISO/USP/CE	Enrichment
01-555	***Salmonella spp./Shigella spp.***	*Salmonella*-Shigella Agar (SS Agar)	AOAC/COMPF/CE	Isolation/Enumeration
01-552		Xylose Lysine Deoxycholate Modified Agar	BAM/COMPF/ISO	Isolation/Enumeration
01-211		Xylose Lysine Deoxycholate Agar (Eur. Pharm.)	BAM/COMPF/ISO	Isolation
02-598		Selenite Broth Base	BAM/DIN/CE	Enrichment
01-030	**Staphylococci**	Baird Parker Agar Base	BAM/EP/ISO/USP	Isolation/Enumeration
01-116		Mannitol Salt Agar (Eur. Pharm.)	HP/BAM/ISO/USP	Isolation/Enumeration
01-206		Vogel Johnson Agar (VJ Agar)	BAM/ISO/USP	Isolation/Enumeration
01-567	***Vibrio***	TCBS Agar	AOAC/BAM/COMPF/ISO	Isolation
01-444	***Yersinia***	*Yersinia* CIN Agar Base	ISO	Isolation
01-655	**Others**	Sensitivity Test Agar (STA)	CLSI	Antibiotic Sensitivity
03-454		Stuart Ringertz Transport Medium	CE	Transport/Preservation

Application tables

Culture media for cosmetics analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
01-654	**Aerobic bacteria and *E. coli***	Eugon LT 100 Agar	ISO	Detection/Enumeration
02-200	**Aerobic bacteria colony count**	Tryptic Soy Broth (TSB) (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/USP	General application/Enrichment
01-050	**Clostridia**	*Clostridium perfringens*, Selective Agar (SPS Agar)	BAM	Isolation/Enumeration
01-068	**Coliforms**	Eosin Methylene Blue Agar (EMB Agar)	BAM/ISO/USP	Isolation/Enumeration
01-236	**Desinfection test**	Letheen Agar	AOAC/ASTM	Determination germicidal activity
02-236		Letheen Broth		Determination germicidal activity
02-583	**Diluents**	Neutralizing Special Broth		Neutralizing
02-257		Beerens Cosmetic Diluent		Neutralizing Diluter
02-539		Casein Lecithin Polysorbate Broth Base	USP	Dilute/Neutralize preservatives
01-237		Letheen Modified Agar	ASTM/BAM/ISO	Enrichment
02-237		Letheen Modified Broth	AOAC/ISO	Enrichment
02-666	***E. coli***	Lactose Neutralizing Broth	ISO	Enrichment
02-105	**Enterobacteria**	Lactose Broth	AOAC/COMPF/EP/ISO/SMWW/USP	Enrichment
01-137	**Fungi *(Candida spp.)***	Nickerson Agar (Biggy Agar)		Isolation
01-692	**Fungi (Yeasts and moulds)**	Glucose Peptone Chloramphenicol Agar (GP Agar+Antibiotic)	ISO	Isolation/Enumeration
01-165		Sabouraud Dextrose Agar	HP/BAM/EP/ISO/USP	Enumeration
01-219		Yeast Malt Agar		Isolation/Cultivation
01-144	**Microorganism**	Nutrient Agar (APHA)	COMPF/ISO	General application
02-613		Soybean Casein Lecithin Polysorbate 80 Medium	ISO	Detection
01-161	**Microorganism-Aerobic plate count**	Plate Count Agar (PCA)	BAM/COMPF/IDF/ISO/USP	Enumeration
01-200		Tryptic Soy Agar (TSA) (Eur. Pharm.)	HP/BAM/COMPF/ISO/EP/USP	General application
01-001	***Pseudomonas***	King A Agar (P Agar)	ISO	Detection/Enumeration
01-029		King B Agar (F Agar)	ISO	Detection/Enumeration
01-030	**Staphylococci**	Baird Parker Agar Base	BAM/EP/ISO/USP	Isolation/Enumeration
01-206		Vogel Johnson Agar (VJ Agar)	BAM/ISO/USP	Isolation/Enumeration
01-610	**Others**	D/E Neutralizing Agar	COMPF/ISO/SMD	Neutralization
02-610		D/E Neutralizing Broth	ISO/SMD	Neutralization

Culture media for environmental control analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
01-164	**Coliforms and *E. coli***	Violet Red Bile Agar (VRB Agar)	COMPF/IDF/ISO	Isolation/Enumeration
01-695		Chromogenic Coliform Agar	Spanish Health Ministery	Isolation/Enumeration
01-295	**Enterobacteria**	Violet Red Bile Dextrose Agar (VRBD Agar) (Eur. Pharm.)	HP/EP/ISO	Isolation/Enumeration
01-599	**Fastidious microorganisms**	Brain Heart Infusion Agar (BHI Agar)	COMPF/DIN/ISO/SMWW	Cultivation
01-573	**Fungi (Yeasts and moulds)**	Malt Extract Agar No. 2		Isolation/Enumeration
01-574		Malt Extract Agar No. 3		Isolation/Enumeration
01-301		Rose Bengal Agar	COMPF/SMWW	Isolation/Enumeration
01-166		Sabouraud Chloramphenicol Agar	ISO/EP/USP	Isolation/Enumeration/Identification
01-165		Sabouraud Dextrose Agar	HP/BAM/EP/ISO/USP	Enumeration
01-275		Sabouraud Oxytetracycline Agar Base (OGYEA)	EP/ISO/USP	Isolation/Enumeration
01-135	**Lactic acid bacteria**	MRS Agar	COMPF/IDF/IFU/ISO	Isolation/Enumeration
01-687	***Legionella***	*Legionella* BCYE Agar Base	ISO	Isolation/Enumeration
01-613	**Microorganism**	Microbial Content Test Agar	COMPF/SMD	Monitoring surfaces
01-161	**Microorganism-Aerobic plate count**	Plate Count Agar (PCA)	BAM/COMPF/IDF/ISO/USP	Enumeration
01-329		Plate Count Modified Agar		Enumeration
01-200		Tryptic Soy Agar (TSA) (Eur. Pharm.)	HP/BAM/COMPF/ISO/EP/USP	General application
01-609	***Pseudomonas***	CN Selective Agar Base	EN/ISO	Isolation
01-030	**Staphylococci**	Baird Parker Agar Base	BAM/EP/ISO/USP	Isolation/Enumeration
01-634	**Sulphite reducing bacteria**	Iron Sulfite Modified Agar	ISO/NMKL	Isolation/Enumeration

Application tables

Culture media for food and beverages analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
01-698	**Acid tolerant microorganisms**	Orange Serum Agar	IFU	Enumeration/Cultivation
01-675	**Acid tolerant microorganism *(Alicyclobacillus)***	BAT Agar	IFU	Isolation/Enumeration
02-675		BAT Broth	IFU	Isolation/Enrichment
01-674		K Agar	IFU	Isolation-DETECTION
01-673		Yeast Starch Glucose Agar	IFU	Detection/Isolation
02-673		Yeast Starch Glucose Broth	IFU	Isolation/Enrichment
02-200	**Aerobic bacteria colony count**	Tryptic Soy Broth (TSB) (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/USP	General application/Enrichment
03-187	**Anaerobic/microaerophilic**	Thioglycollate Fluid Medium	HP/AOAC/BAM/CMPH/COPF/EP/ISO/USP	Sterility test-
01-262	***Bacillus cereus***	*Bacillus cereus* Agar	BAM/COMPF/IDF/ISO	Isolation/Enumeration
01-487		*Bacillus cereus* Selective Agar	ISO	Enumeration
02-227	***Bacillus stearothermophilus***	Tryptic Soy Broth Without Dextrose	ISO	Sensitibity test
02-688		Bolton Enrichment Broth Base	BAM/ISO	Enrichment
01-685		Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base	BAM/ISO	Isolation
01-451		Preston *Campylobacter* Agar Base		Isolation
02-561		Preston *Campylobacter* Broth Base		Enrichment
01-556	**Canned Fod Spoiling Microorganisms**	Dextrose Tryptone Purple Bromcresol Agar		Cultivation
01-050	**Clostridia**	*Clostridium perfringens*, Selective Agar (SPS Agar)	BAM	Isolation/Enumeration
02-410		Differential Reinforced Clostridial Medium (DRCM)	DIN/ISO	Enumeration/Enrichment
03-632		Lactose Gelatin Medium	ISO	Confirmation
02-519		Lactose Sulfite Broth Base	EP/ISO	Identification
03-612		Motility Nitrate Medium	BAM/ISO	Identification
01-289		Reinforced Clostridial Agar		Isolation/Enumeration/cutivation
03-289		Reinforced Clostridial Medium (Eur. Pharm.)	HP/EP	Cultivation/Enumeration/Enrichment
01-195		Tryptone Sulfite Neomycin Agar (TSN Agar)		Isolation/Enumeration
01-278		Tryptose Sulfite Cycloserine Agar (TSC Agar)	BAM/COMPF/ISO	Isolation/Enumeration
01-068	**Coliforms**	Eosin Methylene Blue Agar (EMB Agar)	BAM/ISO/USP	Isolation/Enumeration
01-118		MacConkey Agar (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/ISO/SMWW/USP/CE	Isolation/Enumeration
02-041	**Coliforms and *E. coli***	Brilliant Green Bile 2% Broth	BAM/COMPF/ISO/SMWW	Detection/Enrichment
01-053		Chapman TTC Agar (Tergitol® 7 Agar)	ISO	Isolation/Enumeration
01-618		Chromogenic Colinstant Agar		Isolation/Enumeration
02-060		*E. coli* Broth	COMPF/ISO/SMWW	Detection/Enrichment
02-611		MacConkey Broth (Eur. Pharm.)	HP/EP	Enrichment/Isolation/Enumeration
01-619		Tryptone Bile Glucuronic Agar (TBX Agar)	ISO/SL	Detection/Isolation
02-108		Tryptose Lauryl sulfate Broth	BAM/COMPF/IDF/ISO/SMD/SMWW	Detection/Enumeration/Enrichment
01-164		Violet Red Bile Agar (VRB Agar)	COMPF/IDF/ISO	Isolation/Enumeration
01-695		Chromogenic Coliform Agar	Spanish Health Ministery	Isolation/Enumeration
02-510	**Diluents**	Maximum Recovery Diluent (MRD)	ISO	Dilution/Enrichment
02-631		Purple Maximum Recovery Diluent	ISO	General application
03-156		Tryptone Water (Peptone Water)	COMPF/ISO/SMWW	Enrichment/General application
01-541	***E. coli* 0157:H7**	MacConkey Sorbitol Agar	ISO	Isolation/Enumeration
02-691		Tryptic Soy Broth Modified	BAM/ISO	Enrichment
01-659	***E. coli***	Mineral Modified Glutamate Agar Base	COMPF/IDF/ISO	Recovery *E. coli* cells damaged
02-656		Mineral Modified Glutamate Medium Base	ISO	Enrichment
02-277	**Enterobacteria**	Buffered Peptone Water	ISO	Enrichment
02-336		Decarboxylase Lysine Broth (Taylor)	COMPF/IDF/ISO	Differentiation of Enterobacteria
01-589		Endo Agar Base	AOAC/COMPF/SMD	Isolation/Enrichment/Cultivation
02-064		Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)	HP/ISO/EP	Enrichment
01-502		Glucose Bromcresol Purple Agar	ISO	Confirmation
01-103		Kligler Iron Agar (KIA)	ISO	Isolation/Enumeration
01-094		Lysine Iron Agar (LIA)	AOAC/COMPF/SMD	Isolation/Enumeration/Identification
02-118		MacConkey Broth	AOAC/ISO/SMWW	Detection/Enumeration
02-207		Methyl Red Voges Proskauer Broth (MRVP)	BAM/IDF/ISO/SL	Identification
02-456		Methyl Red Voges Proskauer Saline Broth		Identification
02-138		Nitrate Borth	BAM/COMPF/ISO	Identification
02-568		Phosphate-Buffered Peptone Water	SL	Enrichment
03-176		SIM Medium		Isolation/Enumeration/Identification
01-192		Triple Sugar Iron Agar (TSI Agar)	AOAC/BAM/EP/IDF/ISO/USP	Isolation/Enumeration/Identification
01-261		Urea Agar Base	COMPF/DIN/IDF/ISO/SMD	Identification
01-295		Violet Red Bile Dextrose Agar (VRBD Agar) (Eur. Pharm.)	HP/EP/ISO	Isolation/Enumeration
01-057		Deoxycholate Lactose Agar	COMPF/SMWW	Isolation/Enumeration
02-093		Gram Negative Broth (GN Broth)	COMPF	Enrichment
02-105		Lactose Broth	AOAC/COMPF/EP/ISO/SMWW/USP	Enrichment
01-682	**Enterococci**	MacConkey No. 2 Agar		Isolation/Identification
02-027	**Enterococci-Streptococci**	Azide Dextrose Broth (Rothe)	COMPF	Isolation/Enumeration
01-592		Bile Esculin Azide Agar	BAM/ISO	Isolation/Enumeration/Confirmation
01-265		Bile Esculin Modified Agar	BAM/COMPF/ISO	Identification
01-263		Kanamycin Esculin Azide Agar (KAA Agar)	COMPF	Isolation/Enumeration
02-263		Kanamycin Esculin Azide Broth (KAA Broth)	COMPF	Isolation/Enumeration
01-294		Kenner Fecal Agar (KF Agar)	COMPF/SMWW	Isolation/Enumeration
01-579		Slanetz Bartley Agar Base	ISO	Isolation/Enumeration

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Application tables

Culture media for food and beverages analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
01-352	**Fastidious Microorganisms**	Blood Agar Base	CE	Isolation/Enumeration/Cultivation
01-505		Blood Agar Base No. 2	BAM/COMPF/ISO	Isolation/Enrichment/Cultivation
01-599		Brain Heart Infusion Agar (BHI Agar)	COMPF/DIN/ISO/SMWW	Cultivation
01-366	**Fungi (Yeasts and moulds)**	Chloramphenicol Glucose Agar (CGA)	DIN/IDF/ISO	Isolation/Enumeration
01-485		Dichloran Glycerin Selective Agar (DG18 Agar)		Isolation/Enumeration
01-657		Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)	ISO	Isolation/Enumeration
01-633		m-Green Agar	ISO	Enumeration
01-672		Malt Extract Agar (Blakeslee)	IFU	Cultivation
01-111		Malt Extract Agar No. 1	BAM/COMPF	Isolation/Enumeration
01-573		Malt Extract Agar No. 2		Isolation/Enumeration
01-574		Malt Extract Agar No. 3		Isolation/Enumeration
02-111		Malt Extract Broth No. 1	BAM/COMPF	Cultivation
02-491		Malt Extract Broth No. 2		Cultivation
01-483		Potato Dextrose Agar (Eur. Pharm.)	HP/BAM/COMPF/USP	Isolation/Enumeration/Detection
02-483		Potato Dextrose Broth	BAM/COMPF/SMD/USP	Enrichment/Cultivation
01-301		Rose Bengal Agar	COMPF/SMWW	Isolation/Enumeration
02-165		Sabouraud Broth	HP/USP	Sterility test/Enrichment
01-166		Sabouraud Chloramphenicol Agar	ISO/EP/USP	Isolation/Enumeration/Identification
01-165		Sabouraud Dextrose Agar	HP/BAM/EP/ISO/USP	Enumeration
01-275		Sabouraud Oxytetracycline Agar Base (OGYEA)	EP/ISO/USP	Isolation/Enumeration
01-210		WL Nutrient Agar		Isolation/Enumeration/Culture
01-132		Wort Agar		Isolation/Enumeration/Cultivation
02-132		Wort Broth		Isolation/Enumeration/Cultivation
01-219		Yeast Malt Agar		Isolation/Cultivation
01-026	**Lactic acid bacteria**	APT Agar	COMPF	Isolation/Enumeration
01-135		MRS Agar	COMPF/IDF/IFU/ISO	Isolation/Enumeration
02-135		MRS Broth	IFU/ISO	Enrichment/Isolation/Cultivation
02-496	***Listeria spp.***	*Listeria* Enrichment Broth Base (Fraser)	ISO	Enrichment
02-498		*Listeria* Enrichment Broth Base (Lovett)	BAM/IDF	Enrichment
02-472		*Listeria* Enrichment Broth Base (UVM)	AOAC	Enrichment
01-471		Oxford Agar Base	ISO	Isolation/Enumeration
01-470		Palcam Agar Base	ISO	Isolation/Enumeration
01-144	**Microorganism-Aerobic plate count**	Nutrient Agar (APHA)	COMPF/ISO	General application
01-635		Nutrient Agar (ISO)	ISO	General application
02-144		Nutrient Broth (APHA)	COMPF/EP/SMD/SMWW	Enrichment/Cultivation
01-161		Plate Count Agar (PCA)	BAM/COMPF/IDF/ISO/USP	Enumeration
01-412		Plate Count Skim Milk Agar	DIN/IDF	Enumeration
01-200		Tryptic Soy Agar (TSA) (Eur. Pharm.)	HP/BAM/COMPF/ISO/EP/USP	General application
01-082		Tryptone Glucose Extract Agar (TGE Agar)	BAM/COMPF/SMD	Enumeration
02-599	**Pathogenic microorganisms**	Brain Heart Infusion Broth (BHI Broth)	BAM/COMPF/ISO/SMWW/CE	Enrichment/Cultivation
01-160	***Pseudomonas***	Cetrimide Agar (Pseudomonas Selective Agar) (Eur. Pharm.)	HP/AOAC/EP/USP	Isolation/Enumeration
01-609		CN Selective Agar Base	EN/ISO	Isolation
01-001		King A Agar (P Agar)	ISO	Detection/Enumeration
01-203	***Salmonella***	Brilliant Green Agar (BGA)	EP/USP	Isolation/Enumeration
02-335		Muller-Kauffmann Tetrathionate Broth Base	DIN/ISO	Enrichment
02-379		Rappaport Vassiliadis Broth	AOAC/BAM/COMPF/IDF/ISO	Enrichment
03-376		Rappaport Vassiliadis Modified Semisolid Medium Base	ISO	Isolation/Enrichment
02-602		Selenite Cystine Broth Base	COMPF/ISO/USP/CE	Enrichment
01-309	***Salmonella spp./Shigella spp.***	Brilliant Green Modified Agar (BGA Modified)	DIN/IDF/ISO	Isolation/Enumeration
01-216		Hektoen Enteric Agar	AOAC/BAM/COMPF/ISO	Isolation/Enumeration
01-555		*Salmonella*-Shigella Agar (SS Agar)	AOAC/COMPF/CE	Isolation/Enumeration
02-598		Selenite Broth Base	BAM/DIN/CE	Enrichment
02-629		Tetrationate Bile Brilliant Green Broth Base	EP	Enrichment
01-552		Xylose Lysine Deoxycholate Modified Agar	BAM/COMPF/ISO	Isolation/Enumeration
01-665	***Shigella spp.***	Acetate Differential Agar	BAM/ISO	Differentation
01-664		Christensen's Citrate Agar	BAM/ISO	Detection/Identification
02-663		Purple Broth Base	ISO	Confirmation
02-662		Shigella Broth	BAM/ISO	Enrichment
01-030	***Staphylococci***	Baird Parker Agar Base	BAM/EP/ISO/USP	Isolation/Enumeration
01-346		DNase Agar		Isolation/Enumeration/Identification
02-230		Giolitti-Cantoni Broth	EN/IDF/ISO	Enumeration/Enrichment
01-116		Mannitol Salt Agar (Eur. Pharm.)	HP/BAM/ISO/USP	Isolation/Enumeration
01-206		Vogel Johnson Agar (VJ Agar)	BAM/ISO/USP	Isolation/Enumeration
01-634	**Sulphite reducing bacteria**	Iron Sulfite Modified Agar	ISO/NMKL	Isolation/Enumeration
02-468	***Vibrio***	Alkaline Peptone Water		Enrichment/Cultivation
02-697		Alkaline Saline Peptone Water	COMPF/ISO/SMWW	Enrichment/Cultivation
01-291		Marine Agar		Isolation/Enumeration/Maintenance
01-567		TCBS Agar	AOAC/BAM/COMPF/ISO	Isolation
01-444	***Yersinia***	*Yersinia* CIN Agar Base	ISO	Isolation

Application tables

Culture media for molecular biology analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
01-118	**Coliforms**	MacConkey Agar (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/ISO/SMWW/USP/CE	Isolation/Enumeration
01-385	***E. coli***	LB Agar (Miller)		Cultivation
02-384		LB Broth		Cultivation
02-406		LB Broth (Lennox)		Cultivation
02-385		LB Broth (Miller)		Cultivation
02-118	**Enterobacteria**	MacConkey Broth	AOAC/ISO/SMWW	Detection/Enumeration
02-473	**Fungi (Yeasts and moulds)**	Yeast Extract Peptone Dextrose Broth (YEPD)		Cultivation

Culture media for pharma analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
02-186	**Anaerobic/microaerophilic**	Thioglycollate Broth	AOAC/BAM/EP/USP	Sterility test
03-187		Thioglycollate Fluid Medium	HP/AOAC/BAM/CMPH/COPF/EP/ISO/USP	Sterility test
01-009	**Antibiotic assay**	Antibiotic Medium A pH 6.6 (Eur. Pharm.)	HP/EP/USP	Antibiotic assay
01-017		Antibiotic Medium A pH 7.9 (Eur. Pharm.)	HP/EP/USP	Antibiotic assay
01-016		Antibiotic Medium B (Eur. Pharm.)	HP/EP/USP	Antibiotic assay
02-011		Antibiotic Medium C (Eur. Pharm.)	HP/EP/USP	Antibiotic Assay
01-430		Antibiotic Medium E	HP/EP/USP	Antibiotic assay
01-434		Antibiotic Medium F (Eur. Pharm.)	HP/EP/USP	Antibiotic assay
01-545		Antibiotic Medium NO. 35 (Eur. Phar. Antibiotic Medium G)	HP/EP/USP	Antibiotic assay
01-136		Mueller-Hinton Agar	BAM/CLSI(NCCLS)/ISO/CE	Sensibility test
02-519	**Clostridia**	Lactose Sulfite Broth Base	EP/ISO	Identification
03-289		Reinforced Clostridial Medium (Eur. Pharm.)	HP/EP	Cultivation/Enumeration/Enrichment
01-068	**Coliforms**	Eosin Methylene Blue Agar (EMB Agar)	BAM/ISO/USP	Isolation/Enumeration
01-118		MacConkey Agar (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/ISO/SMWW/USP/CE	Isolation/Enumeration
02-611	**Coliforms and *E. coli***	MacConkey Broth (Eur. Pharm.)	HP/EP	Enrichment/Isolation/Enumeration
01-164		Violet Red Bile Agar (VRB Agar)	COMPF/IDF/ISO	Isolation/Enumeration
02-583	**Diluents**	Neutralizing Special Broth		Neutralizing
02-494		Buffered Peptone Water (Eur.Pharm.)	HP/EP/ISO	Homogenizations of samples
02-539		Casein Lecithin Polysorbate Broth Base	USP	Dilute/Neutralize preservatives
02-510		Maximum Recovery Diluent (MRD)	ISO	Dilution/Enrichment
02-512		Neutralizing Fluid	EP	Neutralizing Diluter
02-064	**Enterobacteria**	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)	HP/ISO/EP	Enrichment
01-192		Triple Sugar Iron Agar (TSI Agar)	AOAC/BAM/EP/IDF/ISO/USP	Isolation/Enumeration/Identification
01-295		Violet Red Bile Dextrose Agar (VRBD Agar) (Eur. Pharm.)	HP/EP/ISO	Isolation/Enumeration
01-220		Violet Red Bile Lactose Dextrose Agar	EP	Isolation/Detection
02-105		Lactose Broth	AOAC/COMPF/EP/ISO/SMWW/USP	Enrichment
02-027	**Enterococci-Streptococci**	Azide Dextrose Broth (Rothe)	COMPF	Isolation/Enumeration
01-263		Kanamycin Esculin Azide Agar (KAA Agar)	COMPF	Isolation/Enumeration
01-034	**Fastidious Microorganisms**	Blood Agar Base (Columbia)	ISO/CE	Cultivation
01-680		Columbia Agar (Eur. Pharm.)	HP	Cultivation/Study non-sterile products
01-483	**Fungi (Yeasts and moulds)**	Potato Dextrose Agar (Eur. Pharm.)	HP/BAM/COMPF/USP	Isolation/Enumeration/Detection
02-483		Potato Dextrose Broth	BAM/COMPF/SMD/USP	Enrichment/Cultivation
02-165		Sabouraud Broth	HP/USP	Sterility test/Enrichment
01-166		Sabouraud Chloramphenicol Agar	ISO/EP/USP	Isolation/Enumeration/Identification
01-165		Sabouraud Dextrose Agar	HP/BAM/EP/ISO/USP	Enumeration
01-275		Sabouraud Oxytetracycline Agar Base (OGYEA)	EP/ISO/USP	Isolation/Enumeration
01-140	**Microorganism-Aerobic plate count**	Nutrient Agar	BAM/BP/ISO	Cultivation
02-140		Nutrient Broth	BP	Cultivation
02-144		Nutrient Broth (APHA)	COMPF/EP/SMD/SMWW	Enrichment/Cultivation
02-032		Phenol Red Broth Base	BAM/COMPF/SMD/USP	Identification
01-161		Plate Count Agar (PCA)	BAM/COMPF/IDF/ISO/USP	Enumeration
01-540		R2A Agar	HP(WATER MONOGRAPH 6TH ED.)/EP/SMWW	Enumeration
01-200		Tryptic Soy Agar (TSA) (Eur. Pharm.)	HP/BAM/COMPF/ISO/EP/USP	General application
02-200		Tryptic Soy Broth (TSB) (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/USP	General application/Enrichment
02-575		Tryptic Soy Broth Irradiated	HP/EP/ISO	General application
01-160	***Pseudomonas***	Cetrimide Agar (Pseudomonas Selective Agar) (Eur. Pharm.)	HP/AOAC/EP/USP	Isolation/Enumeration
01-001		King A Agar (P Agar)	ISO	Detection/Enumeration
01-029		King B Agar (F Agar)	ISO	Detection/Enumeration
01-203	***Salmonella***	Brilliant Green Agar (BGA)	EP/USP	Isolation/Enumeration
02-668		Rappaport Vassiliadis *Salmonella* Enrichment Broth	HP/ISO	Enrichment
02-602		Selenite Cystine Broth Base	COMPF/ISO/USP/CE	Enrichment
01-211	***Salmonella spp./Shigella spp.***	Xylose Lysine Deoxycholate Agar (Eur. Pharm.)	BAM/COMPF/ISO	Isolation
02-629		Tetrationate Bile Brilliant Green Broth Base	EP	Enrichment
01-069	**Spores suspensions**	Spoulating Agar (USP Antibiotic Medium 32)	USP	Antibiotic assay
01-030	**Staphylococci**	Baird Parker Agar Base	BAM/EP/ISO/USP	Isolation/Enumeration
01-116		Mannitol Salt Agar (Eur. Pharm.)	HP/BAM/ISO/USP	Isolation/Enumeration
01-206		Vogel Johnson Agar (VJ Agar)	BAM/ISO/USP	Isolation/Enumeration

Application tables

Culture media for water analysis

Art. No.	Parameter	Description	Regulations	Enrichment/Isolation...
02-654	**Aerobic bacteria/*E. coli***	Eugon LT 100 Broth	ISO	Enrichment
01-007	**Algae**	Algae Agar	SMWW	Isolation/Cultivation
02-007		Algae Broth	SMWW	Cultivation
02-227	***Bacillus stearothermophilus***	Tryptic Soy Broth Without Dextrose	ISO	Sensitibity test
01-050	**Clostridia**	*Clostridium perfringens*, Selective Agar (SPS Agar)	BAM	Isolation/Enumeration
01-513		m-CP Agar Base	DIRECTIVE 98/83/CE	Isolation/Enumeration
02-410		Differential Reinforced Clostridial Medium (DRCM)	DIN/ISO	Enumeration/Enrichment
01-289		Reinforced Clostridial Agar		Isolation/Enumeration/cutivation
01-195		Tryptone Sulfite Neomycin Agar (TSN Agar)		Isolation/Enumeration
01-278		Tryptose Sulfite Cycloserine Agar (TSC Agar)	BAM/COMPF/ISO	Isolation/Enumeration
01-068	**Coliforms**	Eosin Methylene Blue Agar (EMB Agar)	BAM/ISO/USP	Isolation/Enumeration
01-118		MacConkey Agar (Eur. Pharm.)	HP/AOAC/BAM/COMPF/EP/ISO/SMWW/USP/CE	Isolation/Enumeration
02-041	**Coliforms and *E. coli***	Brilliant Green Bile 2% Broth	BAM/COMPF/ISO/SMWW	Detection/Enrichment
01-053		Chapman TTC Agar (Tergitol® 7 Agar)	ISO	Isolation/Enumeration
01-695		Chromogenic Coliform Agar	Spanish Health Ministery	Isolation/Enumeration
01-618		Chromogenic Colinstant Agar		Isolation/Enumeration
02-060		*E. coli* Broth	COMPF/ISO/SMWW	Detection/Enrichment
01-524		m-Lauryl sulfate Agar		Isolation/Enumeration
02-611		MacConkey Broth (Eur. Pharm.)	HP/EP	Enrichment/Isolation/Enumeration
01-619		Tryptone Bile Glucuronic Agar (TBX Agar)	ISO/SL	Detection/Isolation
02-418		Tryptophan Broth	ISO	Confirmation
03-577		Tryptone Water pH 7,5		Differntiation/Identification
02-108		Tryptose Lauryl sulfate Broth	BAM/COMPF/IDF/ISO/SMD/SMWW	Detection/Enumeration/Enrichment
02-460		Tryptose Lauryl sulfate Mannitol Tryptophan Broth	ISO	Confirmation
01-164		Violet Red Bile Agar (VRB Agar)	COMPF/IDF/ISO	Isolation/Enumeration
02-510	**Diluents**	Maximum Recovery Diluent (MRD)	ISO	Dilution/ Enrichment
01-526	***E. coli***	Tryptone Bile Agar	ISO	Enumeration
01-589	**Enterobacteria**	Endo Agar Base	AOAC/COMPF/SMD	Isolation/Enrichment/Cultivation
02-118		MacConkey Broth	AOAC/ISO/SMWW	Detection/Enumeration
01-057		Deoxycholate Lactose Agar	COMPF/SMWW	Isolation/Enumeration
02-105		Lactose Broth	AOAC/COMPF/EP/ISO/SMWW/USP	Enrichment
01-592	**Enterococci-Streptococci**	Bile Esculin Azide Agar	BAM/ISO	Isolation/Enumeration/Confirmation
02-028		Ethyl Violet Azide Broth (EVA Broth)	COMPF/SMWW	Confirmation
01-263		Kanamycin Esculin Azide Agar (KAA Agar)	COMPF	Isolation/Enumeration
01-294		Kenner Fecal Agar (KF Agar)	COMPF/SMWW	Isolation/Enumeration
01-682		MacConkey No. 2 Agar		Isolation/Identification
01-579		Slanetz Bartley Agar Base	ISO	Isolation/Enumeration
01-599	**Fastidious microorganisms**	Brain Heart Infusion Agar (BHI Agar)	COMPF/DIN/ISO/SMWW	Cultivation
01-633	**Fungi (Yeasts and moulds)**	m-Green Agar	ISO	Enumeration
02-633		m-Green Broth	ISO	Enumeration
01-301		Rose Bengal Agar	COMPF/SMWW	Isolation/Enumeration
01-166		Sabouraud Chloramphenicol Agar	ISO/EP/USP	Isolation/Enumeration/Identification
01-165		Sabouraud Dextrose Agar	HP/BAM/EP/ISO/USP	Enumeration
02-135	**Lactic acid bacteria**	MRS Broth	IFU/ISO	Enrichment/Isolation/Cultivation
01-687	***Legionella***	*Legionella* BCYE Agar Base	ISO	Isolation/Enumeration
01-144	**Microorganism-Aerobic plate count**	Nutrient Agar (APHA)	COMPF/ISO	General application
01-140		Nutrient Agar	BAM/BP/ISO	Cultivation
02-144		Nutrient Broth (APHA)	COMPF/EP/SMD/SMWW	Enrichment/Cultivation
01-161		Plate Count Agar (PCA)	BAM/COMPF/IDF/ISO/USP	Enumeration
01-540		R2A Agar	HP(WATER MONOGRAPH 6TH ED.)/EP/SMWW	Enumeration
01-200		Tryptic Soy Agar (TSA) (Eur. Pharm.)	HP/BAM/COMPF/ISO/EP/USP	General application
01-082		Tryptone Glucose Extract Agar (TGE Agar)	BAM/COMPF/SMD	Enumeration
01-590		Tryptone Yeast Extract Agar	ISO	Enumeration
02-599	**Pathogenic microorganisms**	Brain Heart Infusion Broth (BHI Broth)	BAM/COMPF/ISO/SMWW/CE	Enrichment/Cultivation
01-160	***Pseudomonas***	Cetrimide Agar (Pseudomonas Selective Agar) (Eur. Pharm.)	HP/AOAC/EP/USP	Isolation/Enumeration
01-609		CN Selective Agar Base	EN/ISO	Isolation
01-001		King A Agar (P Agar)	ISO	Detection/Enumeration
01-029		King B Agar (F Agar)	ISO	Detection/Enumeration
03-428	***Pseudomonas aeruginosa***	Acetamide Medium	DIN/EN/ISO	Enrichment/Confirmation
02-271		Asparagine Broth		MPN Enumeration
02-668	***Salmonella spp.***	Rappaport Vassiliadis *Salmonella* Enrichment Broth	HP/ISO	Enrichment
02-602		Selenite Cystine Broth Base	COMPF/ISO/USP/CE	Enrichment
01-309	***Salmonella spp./ Shigella spp.***	Brilliant Green Modified Agar (BGA Modified)	DIN/IDF/ISO	Isolation/Enumeration
01-211		Xylose Lysine Deoxycholate Agar (Eur. Pharm.)	BAM/COMPF/ISO	Isolation
01-116	**Staphylococci**	Mannitol Salt Agar (Eur. Pharm.)	HP/BAM/ISO/USP	Isolation/Enumeration
02-697	***Vibrio***	Alkaline Saline Peptone Water	COMPF/ISO/SMWW	Enrichment/Cultivation
01-291		Marine Agar		Isolation/Enumeration/Maintenance
01-567		TCBS Agar	AOAC/BAM/COMPF/ISO	Isolation

Cross References

Cross-references: DIFCO - SCHARLAU (alphabetical)

Art. No. DIFCO	Description DIFCO	Art. No. SCHARLAU	Description SCHARLAU
274210	Acetate Differential Agar	01-665	Acetate Differential Agar
226340	Antibiotic Medium 1	01-009	Antibiotic Medium A pH 6.6 (Eur. Pharm.)
246310	Antibiotic Medium 10	01-016	Antibiotic Medium B (Eur. Pharm.)
259310	Antibiotic Medium 11	01-017	Antibiotic Medium A pH 7.9 (Eur. Pharm.)
243100	Antibiotic Medium 19	01-434	Antibiotic Medium F (Eur. Pharm.)
232681	Antimicrobic vial P	06-021	Polymyxin B Sulfate Selective Supplement
265430	APT Agar	01-026	APT Agar
214010	Bacto Agar	07-004	Agar Bacteriological
211520	Bacto Beef extract, desiccated	07-515	Beef Extract
225930	Bacto casitone	07-154	Casein Pancreatic Peptone
243620	Bacto Soytone	07-155	Soy Peptone
249240	Bacto Todd Hewitt Broth	02-191	Todd-Hewitt Broth
211705	Bacto tryptone	07-119	Casein Trypsic Peptone (Tryptone)
211705	Bacto tryptone	07-489	Peptone From Casein (Tryptone)
211713	Bacto Tryptose	07-197	Tryptose
212750	Bacto Yeast Extract	07-079	Yeast Extract
276840	Baird Parker Agar Base	01-030	Baird Parker Agar Base
269620	Blood Agar Base N.2	01-505	Blood Agar Base No. 2
241830	Brain Heart Infusion Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
256120	Brain Heart Infusion porcine	02-599	Brain Heart Infusion Broth (BHI Broth)
228530	Brilliant green Agar	01-203	Brilliant Green Agar (BGA)
218801	Brilliant Green Agar Modified	01-309	Brilliant Green Modified Agar (BGA Modified)
274000	Brilliant green Bile Broth 2%	02-041	Brilliant Green Bile 2% Broth
218105	Buffered peptone Water	02-568	Phosphate-Buffered Peptone Water
214891	*Campylobacter* Amtimicrobic Supplement Skirrow	06-132	*Campylobacter* Skirrow Selective Supplement
285420	Cetrimide Agar Base	01-160	Cetrimide Agar (Pseudomonas Selective Agar)
279240	Columbia Blood Agar Base	01-034	Blood Agar Base (Columbia)
268620	D/E Neutralizing Agar	01-610	D/E Neutralizing Agar
281910	D/E Neutralizing Broth	02-610	D/E Neutralizing Broth
242010	Desoxycholate Lactose Agar	01-057	Deoxycholate Lactose Agar
214530	Difco Agar granulated	07-490	Agar-Agar
263220	DNase Test Agar	01-346	DNase Agar
258710	DRBC Agar Base	01-657	Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)
231430	EC Medium	02-060	*E. coli* Broth
256620	EE Broth Mossel	02-064	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)
233472	Egg-Yolk Enrichment 50%	06-016	Sterile Egg Yolk Emulsion
274620	m-Enteroccocus AGAR	01-579	Slanetz Bartley Agar Base
259010	Eugon Broth	02-654	Eugon LT 100 Broth
212107	EVA Broth	02-028	Ethyl Violet Azide Broth (EVA Broth)
267720	m-FC Agar	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
264210	Fluid Sabouraud Medium	02-165	Sabouraud Broth
225650	Fluid Thioglycollate Medium	03-187	Thioglycolate Fluid Medium
211767	Fraser Broth Base	02-496	*Listeria* Enrichment Broth Base (Fraser)
228950	GC MEDIUM BASE	01-310	GC Agar Base
218091	Giolitti Cantoni Broth Base	02-230	Giolitti-Cantoni Broth
248610	GN broth, Hajna	02-093	Gram Negative Broth (GN Broth)
285340	Hektoen Eneteric Agar	01-216	Hektoen Enteric Agar
261185	Indol Reagent	RE0007	Kovacs' Reagent
249610	KF Streptococcus Agar	01-294	Kenner Fecal Agar (KF Agar)
288210	Lactobacilli MRS Agar	01-135	MRS Agar
288130	Lactobacilli MRS Broth	02-135	MRS Broth
211835	Lactose Broth	02-105	Lactose Broth
224150	Lauryl tryptose Broth	02-108	Tryptose Lauryl sulfate Broth
244520	LB Agar, Miller	01-385	LB Agar (Miller)

Cross References

Cross-references: DIFCO - SCHARLAU (alphabetical)

Art. No. DIFCO	Description DIFCO	Art. No. SCHARLAU	Description SCHARLAU
240230	LB Broth, Lennox	02-406	LB Broth (Lennox)
244620	LB Broth, Miller	02-385	LB Broth (Miller)
268010	Letheen Agar	01-236	Letheen Agar
263110	Letheen Agar Modified	01-237	Letheen Modified Agar
268110	Letheen Broth	02-236	Letheen Broth
263010	Letheen Broth Modified	02-237	Letheen Modified Broth
222220	*Listeria* Enrichment Broth	02-498	*Listeria* Enrichment Broth Base (Lovett)
241420	Luria Broth Base, Miller	02-384	LB Broth
284920	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
212123	MacCONKEY Agar	01-118	MacConkey Agar (Eur. Pharm.)
220100	MaCConkey Broth	02-611	MacConkey Broth (Eur. Pharm.)
279100	MacConkey Sorbitol Agar	01-541	MacConkey Sorbitol Agar
230650	Mannitol Salt Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
212185	Marine Agar 2216	01-291	Marine Agar
218971	Maximum recovery Diluent	02-510	Maximum Recovery Diluent (MRD)
255320	Microbial content Test Agar	01-613	Microbial Content Test Agar
218501	Mineral Modified Glutamate Broth	02-656	Mineral Modified Glutamate Medium Base
273520	MIO Medium	03-422	Motility Indol Ornithine Fluid Medium (MIO)
216300	MR-VP Medium	02-207	Methyl Red Voges Proskauer Broth (MRVP)
225250	Mueller Hinton Agar	01-136	Mueller-Hinton Agar
275730	Mueller Hinton Broth (not Cation-Adjusted)	02-136	Mueller-Hinton Broth
218531	Muller Kauffman tetrathionate Broth Base	02-335	Muller-Kauffmann Tetrathionate Broth Base
281010	MYP Agar	01-262	*Bacillus cereus* Agar
225710	NIH Thioglycollate Broth	02-186	Thioglycollate Broth
261197	Nitrate A Reagent	06-003	Nitrate Reagent A
261198	Nitrate B Reagent	06-004	Nitrate Reagent B
226810	Nitrate Broth	02-138	Nitrate Broth
213000	Nutrient Agar (APHA)	01-144	Nutrient Agar (APHA)
234000	Nutrient Broth	02-144	Nutrient Broth (APHA)
218111	O.G.Y.E Agar Base	01-275	Sabouraud Oxytetracycline Agar Base (OGYEA)
268820	O/F Basal Medium	03-037	Oxidation-Fermentation Fluid Medium Base (O/F Medium)
222530	Oxford Medium Base	01-471	Oxford Agar Base
263620	Palcam Medium Base	01-470	Palcam Agar Base
274520	Phenylalanine Agar	01-083	Phenylalanine Agar (PPA)
247940	Plate Count Agar (Tryptone Glucose Yeast Agar)	01-161	Plate Count Agar (PCA)
213400	Potato Dextrose Agar	01-483	Potato Dextrose Agar (Eur. Pharm.)
254920	Potato Dextrose Broth	02-483	Potato Dextrose Broth
211693	Proteose Peptone n 3	07-625	Proteose Peptone No. 3
244820	Pseudomonas Agar F	01-029	King B Agar (F Agar)
244910	Pseudomonas Agar P	01-001	King A Agar (P Agar)
218263	R2A Agar	01-540	R2A Agar
218681	Rappaport Vassiliadis MSRV medium Semi-solid modification	03-376	Rappaport Vassiliadis Modified Semisolid Medium Base
218581	Rappaport Vassiliadis R10 Broth	02-379	Rappaport Vassiliadis Broth
218081	Reinforced Cloristidial Medium	01-289	Reinforced Clostridial Agar
218081	Reinforced Clostridial Medium (RCM)	03-289	Reinforced Clostridial Medium (Eur. Pharm.)
218312	Rose Bengal Agar Base	01-301	Rose Bengal Agar
210950	Sabouraud Dextrose Agar	01-165	Sabouraud Dextrose Agar
274500	*Salmonella* Shigella Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)
227540	Selenite Broth	02-598	Selenite Broth Base
268740	Selenite Cystine Broth	02-602	Selenite Cystine Broth Base
281110	SFP Agar Base	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
232100	Skim Milk	06-019	Skimmed Milk Powder
284530	SPS Agar	01-050	*Clostridium perfringens*, Selective Agar (SPS Agar)
265020	TCBS Agar	01-567	TCBS AGAR

Cross References

Cross-references: DIFCO - SCHARLAU (alphabetical)

Art. No. DIFCO	Description DIFCO	Art. No. SCHARLAU	Description SCHARLAU
226540	Triple Sugar Iron Agar	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
236950	Tryptic Soy Agar (Soy Bean Casein Agar)	01-200	Tryptic Soy Agar (TSA)
223000	Tryptone Glucose Extract Agar	01-082	Tryptone Glucose Extract Agar (TGE Agar)
264410	Tryptone water	03-156	Tryptone Water (Peptone Water)
211825	Trytic Soy Broth	02-200	Tryptic Soy Broth (TSB)
286220	Trytic Soy Broth w/o Dextrose	02-227	Tryptic Soy Broth without Dextrose
231121	TTC Solution 1%	06-023	TTC Sterile Solution 1%
227210	Urea Broth	02-202	Urea Broth Base
222330	UVM modified *Listeria* Enrichment Broth	02-472	*Listeria* Enrichment Broth Base (UVM)
211695	Violet Red Bile Agar	01-164	Violet Red Bile Agar (VRB Agar)
218661	Violet Red Bile Glucose Agar	01-295	Violet Red Bile Dextrose Agar (VRBD Agar)
256220	VJ Agar (Vogel Johnson Agar)	01-206	Vogel Johnson Agar (VJ Agar)
261193	VP Reagent B	RE0060	O'Meara's Reagent (VP1)
242420	WL Nutrient Medium	01-210	WL Nutrient Agar
278850	XLD Agar	01-211	Xylose Lysine Deoxycholate Agar (Eur. Pharm.)
219001	Yeast Extract Glucose Chloramphenicol Agar	01-366	Chloramphenicol Glucose Agar (CGA)
231961	*Yersinia* Antimicrobic Supplement CN	06-143	*Yersinia* Selective Supplement
218172	*Yersinia* Selective Agar	01-144	Nutrient Agar (APHA)
271210	YM Agar	01-219	Yeast Malt Agar
271120	YM Broth	02-219	Yeast Malt Broth
223420	XLT4 Agar Base	01-708	XLT4 Agar
235310	XLT4 Agar Supplement	06-709	XLT4 Agar Selective Supplement

Cross References

Cross-references: DIFCO - SCHARLAU (numeric)

Art. No. DIFCO	Description DIFCO	Art. No. SCHARLAU	Description SCHARLAU
210950	Sabouraud Dextrose Agar	01-165	Sabouraud Dextrose Agar
211520	Bacto Beef extract, desiccated	07-515	Beef Extract
211693	Proteose Peptone n 3	07-625	Proteose Peptone No. 3
211695	Violet Red Bile Agar	01-164	Violet Red Bile Agar (VRB Agar)
211705	Bacto tryptone	07-119	Casein Trypsic Peptone (Tryptone)
211705	Bacto tryptone	07-489	Peptone From Casein (Tryptone)
211713	Bacto Tryptose	07-197	Tryptose
211767	Fraser Broth Base	02-496	*Listeria* Enrichment Broth Base (Fraser)
211825	Trytic Soy Broth	02-200	Tryptic Soy Broth (TSB)
211835	Lactose Broth	02-105	Lactose Broth
212107	EVA Broth	02-028	Ethyl Violet Azide Broth (EVA Broth)
212123	MacCONKEY Agar	01-118	MacConkey Agar (Eur. Pharm.)
212185	Marine Agar 2216	01-291	Marine Agar
212750	Bacto Yeast Extract	07-079	Yeast Extract
213000	Nutrient Agar (APHA)	01-144	Nutrient Agar (APHA)
213400	Potato Dextrose Agar	01-483	Potato Dextrose Agar (Eur. Pharm.)
214010	Bacto Agar	07-004	Agar Bacteriological
214530	Difco Agar granulated	07-490	Agar-Agar
216300	MR-VP Medium	02-207	Methyl Red Voges Proskauer Broth (MRVP)
218081	Reinforced Cloristidial Medium	01-289	Reinforced Clostridial Agar
218081	Reinforced Clostridial Medium (RCM)	03-289	Reinforced Clostridial Medium (Eur. Pharm.)
218091	Giolitti Cantoni Broth Base	02-230	Giolitti-Cantoni Broth
218105	Buffered peptone Water	02-568	Phosphate-Buffered Peptone Water
218111	O.G.Y.E Agar Base	01-275	Sabouraud Oxytetracycline Agar Base (OGYEA)
218263	R2A Agar	01-540	R2A Agar
218312	Rose Bengal Agar Base	01-301	Rose Bengal Agar
218501	Mineral Modified Glutamate Broth	02-656	Mineral Modified Glutamate Medium Base
218531	Muller Kauffman tetrathionate Broth Base	02-335	Muller-Kauffmann Tetrathionate Broth Base
218581	Rappaport Vassiliadis R10 Broth	02-379	Rappaport Vassiliadis Broth
218661	Violet Red Bile Glucose Agar	01-295	Violet Red Bile Dextrose Agar (VRBD Agar)
218681	Rappaport Vassiliadis MSRV medium Semi-solid modification	03-376	Rappaport Vassiliadis Modified Semisolid Medium Base
218801	Brilliant Green Agar Modified	01-309	Brilliant Green Modified Agar (BGA Modified)
218971	Maximum recovery Diluent	02-510	Maximum Recovery Diluent (MRD)
219001	Yeast Extract Glucose Chloramphenicol Agar	01-366	Chloramphenicol Glucose Agar (CGA)
220100	MaCConkey Broth	02-611	MacConkey Broth (Eur. Pharm.)
222220	*Listeria* Enrichment Broth	02-498	*Listeria* Enrichment Broth Base (Lovett)
222330	UVM modified *Listeria* Enrichment Broth	02-472	*Listeria* Enrichment Broth Base (UVM)
222530	Oxford Medium Base	01-471	Oxford Agar Base
223000	Tryptone Glucose Extract Agar	01-082	Tryptone Glucose Extract Agar (TGE Agar)
223420	XLT4 Agar Base	01-708	XLT4 Agar
224150	Lauryl tryptose Broth	02-108	Tryptose Lauryl sulfate Broth
225250	Mueller Hinton Agar	01-136	Mueller-Hinton Agar
225650	Fluid Thioglycollate Medium	03-187	Thioglycollate Fluid Medium
225710	NIH Thioglycollate Broth	02-186	Thioglycollate Broth
225930	Bacto casitone	07-154	Casein Pancreatic Peptone
226340	Antibiotic Medium 1	01-009	Antibiotic Medium A pH 6.6 (Eur. Pharm.)
226540	Triple Sugar Iron Agar	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
226810	Nitrate Broth	02-138	Nitrate Borth
227210	Urea Broth	02-202	Urea Broth Base
227540	Selenite Broth	02-598	Selenite Broth Base
228530	Brilliant green Agar	01-203	Brilliant Green Agar (BGA)
228950	GC MEDIUM BASE	01-310	GC Agar Base
230650	Mannitol Salt Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
231121	TTC Solution 1%	06-023	TTC Sterile Solution 1%

Cross References

Cross-references: DIFCO - SCHARLAU (numeric)

Art. No. DIFCO	Description DIFCO	Art. No. SCHARLAU	Description SCHARLAU
231430	EC Medium	02-060	*E. coli* Broth
232100	Skim Milk	06-019	Skimmed Milk Powder
232681	Antimicrobic vial P	06-021	Polymyxin B Sulfate Selective Supplement
233472	Egg-Yolk Enrichment 50%	06-016	Sterile Egg Yolk Emulsion
234000	Nutrient Broth	02-144	Nutrient Broth (APHA)
235310	XLT4 Agar Supplement	06-709	XLT4 Agar Selective Supplement
236950	Tryptic Soy Agar (Soy Bean Casein Agar)	01-200	Tryptic Soy Agar (TSA)
240230	LB Broth, Lennox	02-406	LB Broth (Lennox)
241420	Luria Broth Base, Miller	02-384	LB Broth
241830	Brain Heart Infusion Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
242010	Desoxycholate Lactose Agar	01-057	Deoxycholate Lactose Agar
242420	WL Nutrient Medium	01-210	WL Nutrient Agar
243100	Antibiotic Medium 19	01-434	Antibiotic Medium F (Eur. Pharm.)
243620	Bacto Soytone	07-155	Soy Peptone
244520	LB Agar, Miller	01-385	LB Agar (Miller)
244620	LB Broth, Miller	02-385	LB Broth (Miller)
244820	Pseudomonas Agar F	01-029	King B Agar (F Agar)
244910	Pseudomonas Agar P	01-001	King A Agar (P Agar)
246310	Antibiotic Medium 10	01-016	Antibiotic Medium B (Eur. Pharm.)
247940	Plate Count Agar (Tryptone Glucose Yeast Agar)	01-161	Plate Count Agar (PCA)
248610	GN broth, Hajna	02-093	Gram Negative Broth (GN Broth)
249610	KF Streptococcus Agar	01-294	Kenner Fecal Agar (KF Agar)
254920	Potato Dextrose Broth	02-483	Potato Dextrose Broth
255320	Microbial content Test Agar	01-613	Microbial Content Test Agar
256120	Brain Heart Infusion porcine	02-599	Brain Heart Infusion Broth (BHI Broth)
256220	VJ Agar (Vogel Johnson Agar)	01-206	Vogel Johnson Agar (VJ Agar)
256620	EE Broth Mossel	02-064	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)
258710	DRBC Agar Base	01-657	Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)
259010	Eugon Broth	02-654	Eugon LT 100 Broth
259310	Antibiotic Medium 11	01-017	Antibiotic Medium A pH 7.9 (Eur. Pharm.)
261185	Indol Reagent	RE0007	Kovacs' Reagent
261193	VP Reagent B	RE0060	O'Meara's Reagent (VP1)
261197	Nitrate A Reagent	06-003	Nitrate Reagent A
261198	Nitrate B Reagent	06-004	Nitrate Reagent B
263010	Letheen Broth Modified	02-237	Letheen Modified Broth
263110	Letheen Agar Modified	01-237	Letheen Modified Agar
263220	DNase Test Agar	01-346	DNase Agar
263620	Palcam Medium Base	01-470	Palcam Agar Base
264210	Fluid Sabouraud Medium	02-165	Sabouraud Broth
264410	Tryptone water	03-156	Tryptone Water (Peptone Water)
265020	TCBS Agar	01-567	TCBS AGAR
265430	APT Agar	01-026	APT Agar
267720	m-FC Agar	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
268010	Letheen Agar	01-236	Letheen Agar
268110	Letheen Broth	02-236	Letheen Broth
268620	D/E Neutralizing Agar	01-610	D/E Neutralizing Agar
268740	Selenite Cystine Broth	02-602	Selenite Cystine Broth Base
268820	O/F Basal Medium	03-037	Oxidation-Fermentation Fluid Medium Base (O/F Medium)
269620	Blood Agar Base N.2	01-505	Blood Agar Base No. 2
271210	YM Agar	01-219	Yeast Malt Agar
273520	MIO Medium	03-422	Motility Indol Ornithine Fluid Medium (MIO)
274000	Brilliant green Bile Broth 2%	02-041	Brilliant Green Bile 2% Broth
274210	Acetate Differential Agar	01-665	Acetate Differential Agar
274500	*Salmonella* Shigella Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)

Cross References

Cross-references: DIFCO - SCHARLAU (numeric)

Art. No. DIFCO	Description DIFCO	Art. No. SCHARLAU	Description SCHARLAU
274520	Phenylalanine Agar	01-083	Phenylalanine Agar (PPA)
274620	m-Enteroccocus Agar	01-579	Slanetz Bartley Agar Base
275730	Mueller Hinton Broth (not Cation-Adjusted)	02-136	Mueller-Hinton Broth
276840	Baird Parker Agar Base	01-030	Baird Parker Agar Base
278850	XLD Agar	01-211	Xylose Lysine Deoxycholate Agar (Eur. Pharm.)
279100	MacConkey Sorbitol Agar	01-541	MacConkey Sorbitol Agar
279240	Columbia Blood Agar Base	01-034	Blood Agar Base (Columbia)
281010	MYP Agar	01-262	*Bacillus cereus* Agar
281110	SFP Agar Base	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
281910	D/E Neutralizing Broth	02-610	D/E Neutralizing Broth
284530	SPS Agar	01-050	*Clostridium perfringens*, Selective Agar (SPS Agar)
284920	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
285340	Hektoen Eneteric Agar	01-216	Hektoen Enteric Agar
285420	Cetrimide Agar Base	01-160	Cetrimide Agar (Pseudomonas Selective Agar)
286220	Trytic Soy Broth w/o Dextrose	02-227	Tryptic Soy Broth without Dextrose
288130	Lactobacilli MRS Broth	02-135	MRS Broth
288210	Lactobacilli MRS Agar	01-135	MRS Agar
214891	*Campylobacter* Amtimicrobic Supplement Skirrow	06-132	*Campylobacter* Skirrow Selective Supplement
218172	*Yersinia* Selective Agar	01-144	Nutrient Agar (APHA)
231961	*Yersinia* Antimicrobic Supplement CN	06-143	*Yersinia* Selective Supplement
249240	Bacto Todd Hewitt Broth	02-191	Todd-Hewitt Broth
271120	YM Broth	02-219	Yeast Malt Broth

Cross References

Cross references: BBL - SCHARLAU (alphabetical)

Art. No. BBL	Description BBL	Art. No. SCHARLAU	Description SCHARLAU
212304	Agar grade A	07-004	Agar Bacteriological
210912	AK Agar # 2	01-069	Spoulating Agar (USP Antibiotic Medium 32)
210986	Antibiotic Medium 13	02-165	Sabouraud Broth
212303	Beef Extract powder	07-515	Beef Extract
211027	Biggy Agar	01-137	Nickerson Agar (Biggy Agar)
299068	Bile Esculin Agar	01-265	Bile Esculin Modified Agar
211037	Blood Agar Base (Infusion Agar)	01-352	Blood Agar Base
211065	Brain Heart Infusion Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
211059	Brain Heart Infusion Broth	02-599	Brain Heart Infusion Broth (BHI Broth)
212367	Buffered peptone Water	02-568	Phosphate-Buffered Peptone Water
211102	Cary & Blair Transport Medium	03-643	Cary-Blair Transport Medium
212309	CIN Agar (*Yersinia* Selective Agar)	01-144	Nutrient Agar (APHA)
212218	CLED Agar	01-047	CLED Agar
211124	Columbia Agar Base	01-034	Blood Agar Base (Columbia)
212104	Columbia CNA Agar	01-703	Columbia CNA Agar Base
212330	Dermatophyte Test medium Base	01-442	Dermatophyte Selective Agar
211179	DNase Test Agar	01-346	DNase Agar
297005	EE Broth Mossel Enrichment	02-064	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)
211199	Endo Agar	01-589	Endo Agar Base
212205	Enterococcosel	01-592	Bile Esculin Azide Agar
211221	Eosine Methylene Blue Agar , LEVINE	01-068	Eosin Methylene Blue Agar (EMB Agar)
211260	Fluid Thioglycallate Medium	03-187	Thioglycollate Fluid Medium
211287	m-Green Yeast & Mold Broth	02-633	m-Green Broth
212211	Hektoen Eneteric Agar	01-216	Hektoen Enteric Agar
211317	Kligler Iron Agar	01-103	Kligler Iron Agar (KIA)
211333	Lactose Broth	02-105	Lactose Broth
211338	Lauryl Sulfate Broth	02-108	Tryptose Lauryl sulfate Broth
211363	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
211387	MacConkey Agar	01-118	MacConkey Agar (Eur. Pharm.)
299769	MacConkey II Agar w. Sorbitol	01-541	MacConkey Sorbitol Agar
211407	Mannitol Salt Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
296309	Motility Nitrate (MN) Medium	03-454	Stuart Ringertz Transport Medium
211438	Mueller Hinton II Agar	01-136	Mueller-Hinton Agar
211443	Mueller Hinton Broth	02-136	Mueller-Hinton Broth
211486	Orange Serum Agar	01-698	Orange Serum Agar
211506	Phenol-Red Broth Base	02-032	Phenol Red Broth Base
211537	Phenylalanine Agar	01-083	Phenylalanine Agar (PPA)
211906	Phytone peptone	07-155	Soy Peptone
211558	Purple Broth Base	02-663	Purple Broth Base
211584	Sabouraud Dextrose Agar	01-165	Sabouraud Dextrose Agar
211597	*Salmonella* Shigella Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)
211578	SIM Medium	03-176	SIM Medium
211620	Simmons Citrate Agar	01-177	Simmons Citrate Agar
211638	Standard Methods Agar (Tryptone Glucose Yeast Agar)	01-161	Plate Count Agar (PCA)
211651	Sterility Test Broth	02-186	Thioglycollate Broth
296004	Tech Agar	01-001	King A Agar (P Agar)
211917	Tellurite Solution 1%	06-089	Potassium Tellurite Sterile Solution 1%
211736	Todd hewith Broth	02-191	Todd-Hewitt Broth
211921	Trypticase peptone	07-119	Casein Trypsic Peptone (Tryptone)
211921	Trypticase peptone	07-489	Peptone From Casein (Tryptone)
211043	Trypticase Soy Agar (Soy bean casein Digest Agar)	01-200	Tryptic Soy Agar (TSA)
211764	Trypticase Soy Agar w. Polysorbate 80 and Lecithin	01-613	Microbial Content Test Agar
211768	Trypticase Soy Broth	02-200	Tryptic Soy Broth (TSB)
296264	Trypticase Soy Broth, Sterile	02-575	Tryptic Soy Broth Irradiated

Cross References

Cross references: BBL - SCHARLAU (alphabetical)

Art. No. BBL	Description BBL	Art. No. SCHARLAU	Description SCHARLAU
211749	TSI Agar	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
211690	TSN Agar	01-195	Tryptone Sulfite Neomycin Agar (TSN Agar)
211795	Urea Agar Base	01-261	Urea Agar Base
211838	XLD Agar	01-211	Xylose Lysine Deoxycholate Agar (Eur. Pharm.)
211929	Yeast Extract	07-079	Yeast Extract

Cross References

Cross references: BBL - SCHARLAU (numerical)

Art. No. BBL	Description BBL	Art. No. SCHARLAU	Description SCHARLAU
210912	AK Agar # 2	01-069	Spoulating Agar (USP Antibiotic Medium 32)
210986	Antibiotic Medium 13	02-165	Sabouraud Broth
211027	Biggy Agar	01-137	Nickerson Agar (Biggy Agar)
211037	Blood Agar Base (Infusion Agar)	01-352	Blood Agar Base
211043	Trypticase Soy Agar (Soy bean casein Digest Agar)	01-200	Tryptic Soy Agar (TSA)
211059	Brain Heart Infusion Broth	02-599	Brain Heart Infusion Broth (BHI Broth)
211065	Brain Heart Infusion Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
211102	Cary & Blair Transport Medium	03-643	Cary-Blair Transport Medium
211124	Columbia Agar Base	01-034	Blood Agar Base (Columbia)
211179	DNase Test Agar	01-346	DNase Agar
211199	Endo Agar	01-589	Endo Agar Base
211221	Eosine Methylene Blue Agar , LEVINE	01-068	Eosin Methylene Blue Agar (EMB Agar)
211260	Fluid Thioglycallate Medium	03-187	Thioglycollate Fluid Medium
211287	m- Green Yeast & Mold Broth	02-633	m-Green Broth
211317	Kligler Iron Agar	01-103	Kligler Iron Agar (KIA)
211333	Lactose Broth	02-105	Lactose Broth
211338	Lauryl Sulfate Broth	02-108	Tryptose Lauryl sulfate Broth
211363	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
211387	MacConkey Agar	01-118	MacConkey Agar (Eur. Pharm.)
211407	Mannitol Salt Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
211438	Mueller Hinton II Agar	01-136	Mueller-Hinton Agar
211443	Mueller Hinton Broth	02-136	Mueller-Hinton Broth
211486	Orange Serum Agar	01-698	Orange Serum Agar
211506	Phenol-Red Broth Base	02-032	Phenol Red Broth Base
211537	Phenylalanine Agar	01-083	Phenylalanine Agar (PPA)
211558	Purple Broth Base	02-663	Purple Broth Base
211578	SIM Medium	03-176	SIM Medium
211584	Sabouraud Dextrose Agar	01-165	Sabouraud Dextrose Agar
211597	*Salmonella* Shigella Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)
211620	Simmons Citrate Agar	01-177	Simmons Citrate Agar
211638	Standard Methods Agar (Tryptone Glucose Yeast Agar)	01-161	Plate Count Agar (PCA)
211651	Sterility Test Broth	02-186	Thioglycollate Broth
211690	TSN Agar	01-195	Tryptone Sulfite Neomycin Agar (TSN Agar)
211736	Todd hewith Broth	02-191	Todd-Hewitt Broth
211749	TSI Agar	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
211764	Trypticase Soy Agar w. Polysorbate 80 and Lecithin	01-613	Microbial Content Test Agar
211768	Trypticase Soy Broth	02-200	Tryptic Soy Broth (TSB)
211795	Urea Agar Base	01-261	Urea Agar Base
211838	XLD Agar	01-211	Xylose Lysine Deoxycholate Agar (Eur. Pharm.)
211906	Phytone peptone	07-155	Soy Peptone
211917	Tellurite Solution 1%	06-089	Potassium Tellurite Sterile Solution 1%
211921	Trypticase peptone	07-119	Casein Trypsic Peptone (Tryptone)
211921	Trypticase peptone	07-489	Peptone From Casein (Tryptone)
211929	Yeast Extract	07-079	Yeast Extract
212104	Columbia CNA Agar	01-703	Columbia CNA Agar Base
212205	Enterococcosel	01-592	Bile Esculin Azide Agar
212211	Hektoen Eneteric Agar	01-216	Hektoen Enteric Agar
212218	CLED Agar	01-047	CLED Agar
212303	Beef Extract powder	07-515	Beef Extract
212304	Agar grade A	07-004	Agar Bacteriological
212309	CIN Agar (*Yersinia* Selective Agar)	01-144	Nutrient Agar (APHA)
212330	Dermatophyte Test medium Base	01-442	Dermatophyte Selective Agar
212367	Buffered peptone Water	02-568	Phosphate-Buffered Peptone Water
296004	Tech Agar	01-001	King A Agar (P Agar)

Cross References

Cross references: BBL - SCHARLAU (numerical)

Art. No. BBL	Description BBL	Art. No. SCHARLAU	Description SCHARLAU
296264	Trypticase Soy Broth, Sterile	02-575	Tryptic Soy Broth Irradiated
296309	Motility Nitrate (MN) Medium	03-454	Stuart Ringertz Transport Medium
297005	EE Broth Mossel Enrichment	02-064	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)
299068	Bile Esculin Agar	01-265	Bile Esculin Modified Agar
299769	MacConkey II Agar w. Sorbitol	01-541	MacConkey Sorbitol Agar

Cross References

Cross-references: MERCK - SCHARLAU (alphabetical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.01614	Agar agar granulated	07-490	Agar-Agar
1.01800	Alkaline peptone water	02-468	Alkaline Peptone Water
1.05272	Antibiotic Agar n. 1	01-009	Antibiotic Medium A pH 6.6 (Eur. Pharm.)
1.05269	Antibiotic Agar n. 11	01-017	Antibiotic Medium A pH 7.9 (Eur. Pharm.)
1.05273	Antibiotic Broth (Medium No. 3)	02-011	Antibiotic Medium C (Eur. Pharm.)
1.10453	APT Agar	01-026	APT Agar
1.01590	Azide Dextrose Broth	02-027	Azide Dextrose Broth (Rothe)
1.09875	*Bacillus cereus* Selective Supplement (Polimixine B 50000u)	06-021	Polymyxin B Sulfate Selective Supplement
1.05406	Baird Parker Agar	01-030	Baird Parker Agar Base
1.07994	BAT medium	01-675	BAT Agar
1.00072	Bile Aesculin Azide Agar	01-265	Bile Esculin Modified Agar
1.00072	Bile Aesculin Azide Agar	01-592	Bile Esculin Azide Agar
1.04054	Bile salt mixture for microbiology	07-525	Bile Salts No.3
1.10886	Blood Agar Base	01-352	Blood Agar Base
1.10328	Blood Agar Base N.2	01-505	Blood Agar Base No. 2
1.00079	Bolton Broth Selective supplement	06-131	*Campylobacter* Bolton Selective Supplement
1.00068	Bolton Selective Enrichment Broth	02-688	Bolton Enrichment Broth Base
1.10747	BPLS Agar, mod	01-309	Brilliant Green Modified Agar (BGA Modified)
1.07232	BPLS Agar (USP) (Brilliant green Phenol red Lactose Sucrose Agar)	01-203	Brilliant Green Agar (BGA)
1.13825	Brain Heart Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
1.10493	Brain Heart Broth	02-599	Brain Heart Infusion Broth (BHI Broth)
1.05454	Brilliant-green-2%-Bile Broth	02-041	Brilliant Green Bile 2% Broth
1.01638	Brolacin Agar (CLED Agar)	01-047	CLED Agar
1.07228	Buffered Peptone Water	02-277	Buffered Peptone Water
1.00070	*Campylobacter* Blood-Free Selective Agar (modified CCDA)	01-685	Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base
1.02249	*Campylobacter* Supplement Skirrow	06-139	Novobiocin Selective Supplement (10 mg)
1.10456	*Candida* Selective Agar acc. to Nickerson	01-137	Nickerson Agar (Biggy Agar)
1.02245	Caseinhydrolysate (acid hydrolyzed)	07-151	Casein Acid Hydrolysate
1.00071	CCDA Selective supplement	06-133	Campylobater CCDA Selective Supplement
1.10426	Chromocult Coliform Agar	01-695	Chromogenic Coliform Agar
1.16122	Chromocult TBX (tryptone Bile X-glucoronide) Agar	01-619	Tryptone Bile Glucuronic Agar (TBX Agar)
1.10455	Columbia Agar Base	01-034	Blood Agar Base (Columbia)
1.02894	Deoxycholate Lactose Agar	01-057	Deoxycholate Lactose Agar
1.08960	Dermatophyte selective Agar (DTM) acc. To Taplin	01-442	Dermatophyte Selective Agar
1.10860	Dextrose Tryptone Agar	01-556	Dextrose Tryptone Purple Bromcresol Agar
1.00465	Dichloran Glycerol (DG18) Agar	01-485	Dichloran Glycerin Selective Agar (DG18 Agar)
1.00466	Dichloran Rose Bengal Chloramphenicol (DRBC) Agar	01-657	Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)
1.10449	DNase Test Agar	01-346	DNase Agar
1.00898	*E. coli*/Coliform Selective Supplement	06-140	Coliform CV Selective Supplement
1.10765	EC Broth	02-060	*E. coli* Broth
1.03784	Egg-Yolk Emulsion Sterile	06-016	Sterile Egg Yolk Emulsion
1037850	Egg-Yolk Tellurite Emulsion 20% Steril	06-026/064-BA1018	Sterile Egg Yolk Tellurite Emulsion
1.04044	Endo Agar	01-589	Endo Agar Base
1.05394	Enterobacteriaceae Enrichment Broth acc. Mossel	02-064	Enrichment EnterobacteriaceaeBroth (EE Broth)(Eur. Pharm.)
1.05289	mf-*Enterococcus* Selective Agar Base acc. to Slanetz Bartley	01-579	Slanetz Bartley Agar Base
1.08191	Fluid Thioglycollate Medium	03-187	Thioglycollate Fluid Medium
1.16761	Fluid Thioglycollate Medium G	03-187	Thioglycollate Fluid Medium
1.10398	Fraser *Listeria* Selective Enrichment broth Base	02-496	*Listeria* Enrichment Broth Base (Fraser)
1.10675	Giolitti Cantoni Broth	02-230	Giolitti-Cantoni Broth
1.10756	GN Enrichment Broth acc. To Hajna	02-093	Gram Negative Broth (GN Broth)
1.11885	Gram stainig set	RE0100	Barrit's Reagent (VP2)
1.11885	Gram stainig set	VI0027	Gram Stain
1.11885	Gram stainig set	LU0010	Gram Stain
1.11885	Gram stainig set	DE0010	Gram Stain

Cross References

Cross-references: MERCK - SCHARLAU (alphabetical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.11885	Gram stainig set	DE0010	Gram Stain
1.09023	Griess-ilosvay´s Reagent	06-003	Nitrate Reagent A
1.09023	Griess-ilosvay´s Reagent	06-004	Nitrate Reagent B
1.11681	HEKTOEN Enteric Agar	01-216	Hektoen Enteric Agar
1.05222	Kanamycin Esculine Azide Agar	01-263	Kanamycin Esculin Azide Agar (KAA Agar)
1.10707	KF Streptococcus Agar Base	01-294	Kenner Fecal Agar (KF Agar)
1.03913	KLIGLER Agar	01-103	Kligler Iron Agar (KIA)
1.09293	Kovacs Indol Reagent	RE0007	Kovacs' Reagent
1.12523	Lactalbumin hydrolisate	07-455	Lactalbumin Hydrolysate
1.07680	Lactosa TTC Agar With TERGITOL R 7	01-053	Chapman TTC Agar (Tergitol® 7 Agar)
1.07661	Lactose Broth	02-105	Lactose Broth
1.10266	Lauryl Sulfate Broth	02-108	Tryptose Lauryl sulfate Broth
1.10283	LB-Agar (Miller)	01-385	LB Agar (Miller)
1.10285	LB-Broth (Miller)	02-385	LB Broth (Miller)
1.10240	*Legionella* BCYE Growth Supplement	06-137	*Legionella* BCYE Growth Supplement
1.10242	*Legionella* CYE Agar	01-687	*Legionella* BCYE Agar Base
1.10241	*Legionella* GVPC Selective Supplement	06-138	*Legionella* GVPC Selective Supplement
1.10404	Letheen Agar Base Modified	01-237	Letheen Modified Agar
1.10405	Letheen Broth Modified	02-237	Letheen Modified Broth
1.01342	Levine EMB Agar	01-068	Eosin Methylene Blue Agar (EMB Agar)
1.10549	*Listeria* Enrichment Broth (LEB) acc. To FDA/IDF-FIL	02-498	*Listeria* Enrichment Broth Base (Lovett)
1.11781	*Listeria* selective Enrichment Supplement acc. To FDA / BAM	06-107	*Listeria* Selective Supplement for Enrichment (FDA & IDF/FIL)
1.11640	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
1.05465	MacCONKEY Agar	01-118	MacConkey Agar (Eur. Pharm.)
1.05396	MaCConkey Broth	02-118	MacConkey Broth
1.05396	MaCConkey Broth	02-611	MacConkey Broth (Eur. Pharm.)
1.05391	Malt extract	07-080	Malt Extract
1.05398	Malt Extract Agar	01-573	Malt Extract Agar No. 2
1.05397	Malt Extract Broth	02-491	Malt Extract Broth No. 2
1.05404	Mannitol Salt Phenol Red Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
1.12535	Maximum recovery Diluent	02-510	Maximum Recovery Diluent (MRD)
1.03979	Meat extract	07-075	Meat Extract
1.05712	MR VP Broth	02-207	Methyl Red Voges Proskauer Broth (MRVP)
1.10660	MRS Agar	01-135	MRS Agar
1.10661	MRS Broth	02-135	MRS Broth
1.09878	MSRV Medium, Base Modified	03-376	Rappaport Vassiliadis Modified Semisolid Medium Base
1.10293	Mueller Hinton Broth	02-136	Mueller-Hinton Broth
1.05435	Mueller-Hinton Agar acc. to NCCLS	01-136	Mueller-Hinton Agar
1.05878	Muller KauffMann tetrathionate-Novobiocin Broth (MKTTn)	02-335	Muller-Kauffmann Tetrathionate Broth Base
1.05267	MYP Agar	01-262	*Bacillus cereus* Agar
1.05450	Nutrient Agar (APHA)	01-144	Nutrient Agar (APHA)
1.05443	Nutrient Broth	02-144	Nutrient Broth (APHA)
1.10282	O/F Basal Medium acc. To Hugh & Leifson	03-037	Oxidation-Fermentation Fluid Medium Base (O/F Medium)
1.09877	OGYE Selective Supplement	06-114	Sodium Disulfite (Sodium Meta-Bisulfite) for Bacteriology
1.10673	Orange Serum Agar	01-698	Orange Serum Agar
1.03756	Ox bile dried	07-039	Bile
1.07004	Oxford *Listeria* Selective Agar Base	01-471	Oxford Agar Base
1.07006	Oxford *Listeria* Selective Supplement	06-109	Oxford Agar Selective Supplement
1.05978	Oxytetracyclin Glucose Yeast Extract Agar (O.G.Y.E Agar)	01-275	Sabouraud Oxytetracycline Agar Base (OGYEA)
1.11755	Palcam *Listeria* Selective Agar Base acc. to Van Netten	01-470	Palcam Agar Base
1.12122	Palcam *Listeria* Selective Supplement acc. to Van Netten	06-110	Palcam Agar Selective Supplement
1.07213	Peptone from casein, pancreatic	07-154	Casein Pancreatic Peptone
1.02239	Peptone from caseine (Tryptone)	07-119	Casein Trypsic Peptone (Tryptone)
1.02239	Peptone from caseine (Tryptone)	07-489	Peptone From Casein (Tryptone)

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Cross References

Cross-references: MERCK - SCHARLAU (alphabetical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.07284	Peptone from Gelatin (pancreatic)	07-153	Gelatin Pancreatic Peptone
1.07224	Peptone from meat (peptic), granulated	07-152	Meat Peptone
1.07212	Peptone from Soyameal (papainic),granulated	07-155	Soy Peptone
1.10987	Phenol-Red Broth Base	02-032	Phenol Red Broth Base
1.05463	Plate Count Agar (Casein Peptone Dextrose Yeast Agar)	01-161	Plate Count Agar (PCA)
1.15338	Plate Count Skim Milk Agar	01-412	Plate Count Skim Milk Agar
1.10130	Potato Dextrose Agar	01-483	Potato Dextrose Agar (Eur. Pharm.)
1.07229	Proteose peptone	07-625	Proteose Peptone No. 3
1.10989	Pseudomonas Agar F Base	01-029	King B Agar (F Agar)
1.10988	Pseudomonas Agar P Base	01-001	King A Agar (P Agar)
1.07620	Pseudomonas Selective Agar Base	01-609	CN Selective Agar Base
1.05284	Pseudomonas Selective Agar Base (Cetrimide Agar)	01-160	Cetrimide Agar (Pseudomonas Selective Agar)
1.00416	R2A Agar	01-540	R2A Agar
1.07700	Rappaport Vassiliadis Broth	02-379	Rappaport Vassiliadis Broth
1.07700	Rappaport Vassiliadis Broth	02-668	Rappaport Vassiliadis *Salmonella* Enrichment Broth
1.05410	Reinforced Clostridial Agar	01-289	Reinforced Clostridial Agar
1.05411	Reinforced Clostridial Medium (RCM)	03-289	Reinforced Clostridial Medium (Eur. Pharm.)
1.00467	Rose Bengal Chloramphenicol (RBC) Agar	01-301	Rose Bengal Agar
1.08339	Sabouraud 2% Dextrose Broth	02-165	Sabouraud Broth
1.05438	Sabouraud 4% Dextrose Agar	01-165	Sabouraud Dextrose Agar
1.07667	*Salmonella*-Shigella Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)
1.07709	Selenite Cystine Broth	02-602	Selenite Cystine Broth Base
1.07717	Selenite Enrichment Broth Leifson	02-598	Selenite Broth Base
1.05470	SIM Medium	03-176	SIM Medium
1.02501	Simmons Citrate Agar	01-177	Simmons Citrate Agar
1.15363	Skim Milk Powder	06-019	Skimmed Milk Powder
1.10582	Sodium Chloride peptone Broth Buffered	02-494	Buffered Peptone Water (Eur. Pharm.)
1.06504	Sodium Deoxycholate for microbiology	07-644	Sodium Deoxycholate
1.0634	Sodium hydrogen selenite	06-607	Basic Fuchsin (250) Selective Supplement
1.09207	Sorbitol MacConkey (SMAC) Agar	01-541	MacConkey Sorbitol Agar
1.10235	SPS Agar	01-050	*Clostridium perfringens*, Selective Agar (SPS Agar)
1.01621	Standard Count Agar	01-635	Nutrient Agar (ISO)
1.11723	TAT Broth Base	02-539	Casein Lecithin Polysorbate Broth Base
1.05178	TBG-Broth modified	02-629	Tetrationate Bile Brilliant Green Broth Base (Eur. Pharm.)
1.10263	TCBS Agar (Vibrio Selective Agar)	01-567	TCBS Agar
1.10128	TGE Agar	01-082	Tryptone Glucose Extract Agar (TGE Agar)
1.08190	Thioglycollate Broth	02-186	Thioglycollate Broth
1.08100	Thioglycollate Broth	02-186	Thioglycollate Broth
1.03915	Triple Sugar Iron Agar (TSI Agar)	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
1.07324	Tryptic Soy Agar (CASO) w. Polysorbate 80 and Lecithin	01-613	Microbial Content Test Agar
1.05458	Tryptic Soy Agar (TSA) (CASO Agar)	01-200	Tryptic Soy Agar (TSA)
1.05459	Tryptic Soy Broth (CASO Broth)	02-200	Tryptic Soy Broth (TSB)
1.10859	Tryptone Water	03-156	Tryptone Water (Peptone Water)
1.10213	Tryptose	07-197	Tryptose
1.11972	TSC Agar	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
1.05264	TSN Agar (Perfringens Selective Agar acc. to Marshall)	01-195	Tryptone Sulfite Neomycin Agar (TSN Agar)
1.08492	Urea Agar Base acc CHRISTENSEN	01-261	Urea Agar Base
1.08483	Urea Broth	02-202	Urea Broth Base
1.05405	Vogel-Johnson Agar Base	01-206	Vogel Johnson Agar (VJ Agar)
1.01406	VRB Agar (violet Red Bile Agar)	01-164	Violet Red Bile Agar (VRB Agar)
1.10275	VRBD (Violet Red Bile Dextrose) agar acc. to Mossel	01-295	Violet Red Bile Dextrose Agar (VRBD Agar)
1.10866	WL Nutrient Agar	01-210	WL Nutrient Agar
1.05448	Wort Agar	01-132	Wort Agar
1.05449	Wort Broth Base	02-132	Wort Broth

Cross References

Cross-references: MERCK - SCHARLAU (alphabetical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.05287	XLD (Xylose Lysine Deoxycholate) Agar	01-552	Xylose Lysine Deoxycholate Modified Agar
1.05287	XLD Agar	01-211	Xylose Lysine Deoxycholate Agar (Eur. Pharm.)
1.13919	XLT4 Agar	01-708	XLT4 Agar
1.08981	XLT4 Agar Supplement 4,6mL	06-709	XLT4 Agar Selective Supplement
1.03753	Yeast extract, granulated	07-079	Yeast Extract
1.16434	*Yersinia* Selective agar acc. to Schiemann (CIN-Agar)	01-144	Nutrient Agar (APHA)
1.16466	*Yersinia* Selective Supplement (CIN)	06-143	*Yersinia* Selective Supplement
1.16000	YGC Agar (Yeast Extract Glucose Chloramphenicol Agar FIL-IDF)	01-366	Chloramphenicol Glucose Agar (CGA)

Cross References

Cross-references: MERCK - SCHARLAU (numerical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.00068	Bolton Selective Enrichment Broth	02-688	Bolton Enrichment Broth Base
1.00070	*Campylobacter* Blood-Free Selective Agar (modified CCDA)	01-685	Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base
1.00071	CCDA Selective supplement	06-133	Campylobater CCDA Selective Supplement
1.00072	Bile Aesculin Azide Agar	01-265	Bile Esculin Modified Agar
1.00072	Bile Aesculin Azide Agar	01-592	Bile Esculin Azide Agar
1.00079	Bolton Broth Selective supplement	06-131	*Campylobacter* Bolton Selective Supplement
1.00416	R2A Agar	01-540	R2A Agar
1.00465	Dichloran Glycerol (DG18) Agar	01-485	Dichloran Glycerin Selective Agar (DG18 Agar)
1.00466	Dichloran Rose Bengal Chloramphenicol (DRBC) Agar	01-657	Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)
1.00467	Rose Bengal Chloramphenicol (RBC) Agar	01-301	Rose Bengal Agar
1.00898	*E. coli*/Coliform Selective Supplement	06-140	Coliform CV Selective Supplement
1.01342	Levine EMB Agar	01-068	Eosin Methylene Blue Agar (EMB Agar)
1.01406	VRB Agar (violet Red Bile Agar)	01-164	Violet Red Bile Agar (VRB Agar)
1.01590	Azide Dextrose Broth	02-027	Azide Dextrose Broth (Rothe)
1.01614	Agar agar granulated	07-490	Agar-Agar
1.01621	Standard Count Agar	01-635	Nutrient Agar (ISO)
1.01638	Brolacin Agar (CLED Agar)	01-047	CLED Agar
1.01800	Alkaline peptone water	02-468	Alkaline Peptone Water
1.02239	Peptone from caseine (Tryptone)	07-119	Casein Trypsic Peptone (Tryptone)
1.02239	Peptone from caseine (Tryptone)	07-489	Peptone From Casein (Tryptone)
1.02245	Caseinhydrolysate (acid hydrolyzed)	07-151	Casein Acid Hydrolysate
1.02249	*Campylobacter* Supplement Skirrow	06-139	Novobiocin Selective Supplement (10 mg)
1.02501	Simmons Citrate Agar	01-177	Simmons Citrate Agar
1.02894	Deoxycholate Lactose Agar	01-057	Deoxycholate Lactose Agar
1.03753	Yeast extract, granulated	07-079	Yeast Extract
1.03756	Ox bile dried	07-039	Bile
1.03784	Egg-Yolk Emulsion Sterile	06-016	Sterile Egg Yolk Emulsion
1.03913	Kligler Agar	01-103	Kligler Iron Agar (KIA)
1.03915	Triple Sugar Iron Agar (TSI Agar)	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
1.03979	Meat extract	07-075	Meat Extract
1.04044	Endo Agar	01-589	Endo Agar Base
1.04054	Bile salt mixture for microbiology	07-525	Bile Salts No.3
1.05178	TBG-Broth modified	02-629	Tetrationate Bile Brilliant Green Broth Base (Eur. Pharm.)
1.05222	Kanamycin Esculine Azide Agar	01-263	Kanamycin Esculin Azide Agar (KAA Agar)
1.05264	TSN Agar (Perfringens Selective Agar acc. to Marshall)	01-195	Tryptone Sulfite Neomycin Agar (TSN Agar)
1.05267	MYP Agar	01-262	*Bacillus cereus* Agar
1.05269	Antibiotic Agar n. 11	01-017	Antibiotic Medium A pH 7.9 (Eur. Pharm.)
1.05272	Antibiotic Agar n. 1	01-009	Antibiotic Medium A pH 6.6 (Eur. Pharm.)
1.05273	Antibiotic Broth (Medium No. 3)	02-011	Antibiotic Medium C (Eur. Pharm.)
1.05284	Pseudomonas Selective Agar Base (Cetrimide Agar)	01-160	Cetrimide Agar (Pseudomonas Selective Agar)
1.05287	XLD (Xylose Lysine Deoxycholate) Agar	01-552	Xylose Lysine Deoxycholate Modified Agar
1.05287	XLD Agar	01-211	Xylose Lysine Deoxycholate Agar (Eur. Pharm.)
1.05289	mf-*Enterococcus* Selective Agar Base acc. to Slanetz Bartley	01-579	Slanetz Bartley Agar Base
1.05391	Malt extract	07-080	Malt Extract
1.05394	Enterobacteriaceae Enrichment Broth acc. Mossel	02-064	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)
1.05396	MaCConkey Broth	02-118	MacConkey Broth
1.05396	MaCConkey Broth	02-611	MacConkey Broth (Eur. Pharm.)
1.05397	Malt Extract Broth	02-491	Malt Extract Broth No. 2
1.05398	Malt Extract Agar	01-573	Malt Extract Agar No. 2
1.05404	Mannitol Salt Phenol Red Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
1.05405	VOGEL-JOHNSON Agar Base	01-206	Vogel Johnson Agar (VJ Agar)
1.05406	Baird Parker Agar	01-030	Baird Parker Agar Base
1.05410	Reinforced Clostridial Agar	01-289	Reinforced Clostridial Agar
1.05411	Reinforced Clostridial Medium (RCM)	03-289	Reinforced Clostridial Medium (Eur. Pharm.)

Cross References

Cross-references: MERCK - SCHARLAU (numerical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.05435	Mueller-Hinton Agar acc. to NCCLS	01-136	Mueller-Hinton Agar
1.05438	Sabouraud 4% Dextrose Agar	01-165	Sabouraud Dextrose Agar
1.05443	Nutrient Broth	02-144	Nutrient Broth (APHA)
1.05448	Wort Agar	01-132	Wort Agar
1.05449	Wort Broth Base	02-132	Wort Broth
1.05450	Nutrient Agar (APHA)	01-144	Nutrient Agar (APHA)
1.05454	Brilliant-green-2%-Bile Broth	02-041	Brilliant Green Bile 2% Broth
1.05458	Tryptic Soy Agar (TSA) (CASO Agar)	01-200	Tryptic Soy Agar (TSA)
1.05459	Tryptic Soy Broth (CASO Broth)	02-200	Tryptic Soy Broth (TSB)
1.05463	Plate Count Agar (Casein Peptone Dextrose Yeast Agar)	01-161	Plate Count Agar (PCA)
1.05465	MacCONKEY Agar	01-118	MacConkey Agar (Eur. Pharm.)
1.05470	SIM Medium	03-176	SIM Medium
1.05712	MR VP Broth	02-207	Methyl Red Voges Proskauer Broth (MRVP)
1.05878	Muller KauffMann tetrathionate-Novobiocin Broth (MKTTn)	02-335	Muller-Kauffmann Tetrathionate Broth Base
1.05978	Oxytetracyclin Glucose Yeast Extract Agar (O.G.Y.E Agar)	01-275	Sabouraud Oxytetracycline Agar Base (OGYEA)
1.0634	Sodium hydrogen selenite	06-607	Basic Fuchsin (250) Selective Supplement
1.06504	Sodium Deoxycholate for microbiology	07-644	Sodium Deoxycholate
1.07004	Oxford *Listeria* Selective Agar Base	01-471	Oxford Agar Base
1.07006	Oxford *Listeria* Selective Supplement	06-109	Oxford Agar Selective Supplement
1.07212	Peptone from Soyameal (papainic),granulated	07-155	Soy Peptone
1.07213	Peptone from casein, pancreatic	07-154	Casein Pancreatic Peptone
1.07224	Peptone from meat (peptic), granulated	07-152	Meat Peptone
1.07228	Buffered Peptone Water	02-277	Buffered Peptone Water
1.07229	Proteose peptone	07-625	Proteose Peptone No. 3
1.07232	BPLS Agar (USP) (Brilliant green Phenol red Lactose Sucrose Agar)	01-203	Brilliant Green Agar (BGA)
1.07284	Peptone from Gelatin (pancreatic)	07-153	Gelatin Pancreatic Peptone
1.07324	Tryptic Soy Agar (CASO) w. Polysorbate 80 and Lecithin	01-613	Microbial Content Test Agar
1.07620	Pseudomonas Selective Agar Base	01-609	CN Selective Agar Base
1.07661	Lactose Broth	02-105	Lactose Broth
1.07667	*Salmonella*-Shigella Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)
1.07680	Lactosa TTC Agar With TERGITOL R 7	01-053	Chapman TTC Agar (Tergitol® 7 Agar)
1.07700	Rappaport Vassiliadis Broth	02-379	Rappaport Vassiliadis Broth
1.07700	Rappaport Vassiliadis Broth	02-668	Rappaport Vassiliadis *Salmonella* Enrichment Broth
1.07709	Selenite Cystine Broth	02-602	Selenite Cystine Broth Base
1.07717	Selenite Enrichment Broth LEIFSON	02-598	Selenite Broth Base
1.07994	BAT medium	01-675	BAT Agar
1.08100	Thioglycollate Broth	02-186	Thioglycollate Broth
1.08190	Thioglycollate Broth	02-186	Thioglycollate Broth
1.08191	Fluid Thioglycollate Medium	03-187	Thioglycollate Fluid Medium
1.08339	Sabouraud 2% Dextrose Broth	02-165	Sabouraud Broth
1.08483	Urea Broth	02-202	Urea Broth Base
1.08492	Urea Agar Base acc CHRISTENSEN	01-261	Urea Agar Base
1.08960	Dermatophyte selective Agar (DTM) acc. To Taplin	01-442	Dermatophyte Selective Agar
1.08981	XLT4 Agar Supplement 4,6mL	06-709	XLT4 Agar Selective Supplement
1.09023	Griess-ilosvay´s Reagent	06-003	Nitrate Reagent A
1.09023	Griess-ilosvay´s Reagent	06-004	Nitrate Reagent B
1.09207	Sorbitol MacConkey (SMAC) Agar	01-541	MacConkey Sorbitol Agar
1.09293	Kovacs Indol Reagent	RE0007	Kovacs' Reagent
1.09875	*Bacillus cereus* Selective Supplement (Polimixine B 50000u)	06-021	Polymyxin B Sulfate Selective Supplement
1.09877	OGYE Selective Supplement	06-114	Sodium Disulfite (Sodium Meta-Bisulfite) for Bacteriology
1.09878	MSRV Medium, Base Modified	03-376	Rappaport Vassiliadis Modified Semisolid Medium Base
1.10128	TGE Agar	01-082	Tryptone Glucose Extract Agar (TGE Agar)
1.10130	Potato Dextrose Agar	01-483	Potato Dextrose Agar (Eur. Pharm.)
1.10213	Tryptose	07-197	Tryptose

Cross References

Cross-references: MERCK - SCHARLAU (numerical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.10235	SPS Agar	01-050	*Clostridium perfringens*, Selective Agar (SPS Agar)
1.10240	*Legionella* BCYE Growth Supplement	06-137	*Legionella* BCYE Growth Supplement
1.10241	*Legionella* GVPC Selective Supplement	06-138	*Legionella* GVPC Selective Supplement
1.10242	*Legionella* CYE Agar	01-687	*Legionella* BCYE Agar Base
1.10263	TCBS Agar (Vibrio Selective Agar)	01-567	TCBS Agar
1.10266	Lauryl Sulfate Broth	02-108	Tryptose Lauryl sulfate Broth
1.10275	VRBD (Violet Red Bile Dextrose) agar acc. to Mossel	01-295	Violet Red Bile Dextrose Agar (VRBD Agar)
1.10282	O/F Basal Medium acc. To Hugh & Leifson	03-037	Oxidation-Fermentation Fluid Medium Base (O/F Medium)
1.10283	LB-Agar (Miller)	01-385	LB Agar (Miller)
1.10285	LB-Broth (Miller)	02-385	LB Broth (Miller)
1.10293	Mueller Hinton Broth	02-136	Mueller-Hinton Broth
1.10328	Blood Agar Base N.2	01-505	Blood Agar Base No. 2
1.10398	Fraser *Listeria* Selective Enrichment broth Base	02-496	*Listeria* Enrichment Broth Base (Fraser)
1.10404	Letheen Agar Base Modified	01-237	Letheen Modified Agar
1.10405	Letheen Broth Modified	02-237	Letheen Modified Broth
1.10426	Chromocult Coliform Agar	01-695	Chromogenic Coliform Agar
1.10449	DNase Test Agar	01-346	DNase Agar
1.10453	APT Agar	01-026	APT Agar
1.10455	Columbia Agar Base	01-034	Blood Agar Base (Columbia)
1.10456	*Candida* Selective Agar acc. to Nickerson	01-137	Nickerson Agar (Biggy Agar)
1.10493	Brain Heart Broth	02-599	Brain Heart Infusion Broth (BHI Broth)
1.10549	*Listeria* Enrichment Broth (LEB) acc. To FDA/IDF-FIL	02-498	*Listeria* Enrichment Broth Base (Lovett)
1.10582	Sodium Chloride peptone Broth Buffered	02-494	Buffered Peptone Water (Eur. Pharm.)
1.10660	MRS Agar	01-135	MRS Agar
1.10661	MRS Broth	02-135	MRS Broth
1.10673	Orange Serum Agar	01-698	Orange Serum Agar
1.10675	Giolitti Cantoni Broth	02-230	Giolitti-Cantoni Broth
1.10707	KF Streptococcus Agar Base	01-294	Kenner Fecal Agar (KF Agar)
1.10747	BPLS Agar, mod	01-309	Brilliant Green Modified Agar (BGA Modified)
1.10756	GN Enrichment Broth acc. To HAJNA	02-093	Gram Negative Broth (GN Broth)
1.10765	EC Broth	02-060	*E. coli* Broth
1.10859	Tryptone Water	03-156	Tryptone Water (Peptone Water)
1.10860	Dextrose Tryptone Agar	01-556	Dextrose Tryptone Purple Bromcresol Agar
1.10866	WL Nutrient Agar	01-210	WL Nutrient Agar
1.10886	Blood Agar Base	01-352	Blood Agar Base
1.10987	Phenol-Red Broth Base	02-032	Phenol Red Broth Base
1.10988	Pseudomonas Agar P Base	01-001	King A Agar (P Agar)
1.10989	Pseudomonas Agar F Base	01-029	King B Agar (F Agar)
1.11640	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
1.11681	HEKTOEN Enteric Agar	01-216	Hektoen Enteric Agar
1.11723	TAT Broth Base	02-539	Casein Lecithin Polysorbate Broth Base
1.11755	Palcam *Listeria* Selective Agar Base acc. to Van Netten	01-470	Palcam Agar Base
1.11781	*Listeria* selective Enrichment Supplement acc. To FDA / BAM	06-107	*Listeria* Selective Supplement for Enrichment (FDA & IDF/FIL)
1.11885	Gram stainig set	RE0100	Barrit's Reagent (VP2)
1.11885	Gram stainig set	VI0027	Gram Stain
1.11885	Gram stainig set	LU0010	Gram Stain
1.11885	Gram stainig set	DE0010	Gram Stain
1.11885	Gram stainig set	DE0010	Gram Stain
1.11972	TSC Agar	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
1.12122	Palcam *Listeria* Selective Supplement acc. to Van Netten	06-110	Palcam Agar Selective Supplement
1.12523	Lactalbumin hydrolisate	07-455	Lactalbumin Hydrolysate
1.12535	Maximum recovery Diluent	02-510	Maximum Recovery Diluent (MRD)
1.13825	Brain Heart Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
1.13919	XLT4 Agar	01-708	XLT4 Agar

Cross References

Cross-references: MERCK - SCHARLAU (numerical)

Art. No. MERK	Description MERK	Art. No. SCHARLAU	Description SCHARLAU
1.15338	Plate Count Skim Milk Agar	01-412	Plate Count Skim Milk Agar
1.15363	Skim Milk Powder	06-019	Skimmed Milk Powder
1.16000	YGC Agar (Yeast Extract Glucose Chloramphenicol Agar FIL-IDF)	01-366	Chloramphenicol Glucose Agar (CGA)
1.16122	Chromocult TBX (tryptone Bile X-glucoronide) Agar	01-619	Tryptone Bile Glucuronic Agar (TBX Agar)
1.16434	*Yersinia* Selective agar acc. To Schiemann (CIN-Agar)	01-144	Nutrient Agar (APHA)
1.16466	*Yersinia* Selective Supplement (CIN)	06-143	*Yersinia* Selective Supplement
1.16761	Fluid Thioglycollate Medium G	03-187	Thioglycollate Fluid Medium
1037850	Egg-Yolk Tellurite Emulsion 20% Steril	06-026/064-BA1018	Sterile Egg Yolk Tellurite Emulsion

Cross References

Cross-references: OXOID - SCHARLAU (alphabetical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
LP0011B	Agar bacteriological (Agar n.1)	07-004	Agar Bacteriological
CM1028B	Alkaline Peptone Water	02-468	Alkaline Peptone Water
CM1117B	Alkaline Saline Peptone Water	02-697	Alkaline Saline Peptone Water
CM0327B	Antibiotic Medium n. 1	01-009	Antibiotic Medium A pH 6.6 (Eur. Pharm.)
CM0287B	Antibiotic Medium No. 3 (Assay Broth)	02-011	Antibiotic Medium C (Eur. Pharm.)
CM0868B	Azide Dextrose Broth (Rothe)	02-027	Azide Dextrose Broth (Rothe)
CM0617B	*Bacillus cereus* Selective Agar Base	01-487	*Bacillus cereus* Selective Agar
SR0099E	*Bacillus cereus* Selective Supplement	06-021	Polymyxin B Sulfate Selective Supplement
R21522	BactiDrop Indol, Kovacs	RE0007	Kovacs' Reagent
R21536	BactiDrop Nitrate A	06-003	Nitrate Reagent A
R21538	BactiDrop Nitrate B	06-004	Nitrate Reagent B
CM0275B	Baird Parker Agar Base	01-030	Baird Parker Agar Base
CM0589B	BiGGY Agar	01-137	Nickerson Agar (Biggy Agar)
CM0888B	Bile Aesculine agar	01-265	Bile Esculin Modified Agar
CM0055B	Blood Agar Base	01-352	Blood Agar Base
CM0271B	Blood Agar Base N.2	01-505	Blood Agar Base No. 2
SR0183E	Bolton Broth Selective Supplement	06-131	*Campylobacter* Bolton Selective Supplement
CM0983B	Bolton Selective Enrichment Broth	02-688	Bolton Enrichment Broth Base
CM1136B	Brain Heart Infusion Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
CM0225B	Brain Heart Infusion Broth	02-599	Brain Heart Infusion Broth (BHI Broth)
CM0263B	Brilliant Green Agar	01-203	Brilliant Green Agar (BGA)
CM0329B	Brilliant Green Agar (Modified)	01-309	Brilliant Green Modified Agar (BGA Modified)
CM0031B	Brilliant Green Bile (2%) Broth	02-041	Brilliant Green Bile 2% Broth
CM0509B	Buffered peptone Water	02-693	Tryptone Phosphate Water 09
CM1049B	Buffered Peptone Water (ISO)	02-277	Buffered Peptone Water
CM0982B	Buffered sodium Chloride Peptone	02-494	Buffered Peptone Water (Eur. Pharm.)
CM0739B	*Campylobacter* Blood-free Selective Agar Base	01-685	Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base
SR0232E	*Campylobacter* Growth Supplement	06-128	*Campylobacter* Growth Supplement
SR0069E	*Campylobacter* Selective Supplement Skirrow)	06-132	*Campylobacter* Skirrow Selective Supplement
CM0519B	Cary-Blair Medium	03-643	Cary-Blair Transport Medium
SR0155E	CCDA Selective Supplement	06-133	Campylobater Ccda Selective Supplement
CM0579B	Cetrimide Agar	01-160	Cetrimide Agar (Pseudomonas Selective Agar)
SR0078E	Chloramphenicol Selective Supplement	06-118	Chloramphenicol Selective Supplement
CM0333B	Cholera Medium TCBS	01-567	TCBS Agar
CM0301B	CLED Medium	01-047	CLED Agar
CM0331B	Columbia Blood Agar Base	01-034	Blood Agar Base (Columbia)
SR0222C	Cycloheximide	06-022	Cycloheximide Selective Supplement
SR0088E	Cycloserine (TSC) Supplement	06-116	Cycloserine Selective Supplement
CM0539B	Dermasel Medium	01-442	Dermatophyte Selective Agar
CM0163B	Desoxycholate Agar	01-057	Deoxycholate Lactose Agar
CM0729B	Dichloran Glycerol (DG18) Agar Base	01-485	Dichloran Glycerin Selective Agar (DG18 Agar)
CM0321B	DNase Agar	01-346	DNase Agar
CM0727B	DRBC Agar Base	01-657	Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)
CM0853B	EC Broth	02-060	*E. coli* Broth
CM0317B	EE Broth	02-064	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)
SR0054C	Egg Yolk tellurite Emulsion	06-026/064-BA1018	Sterile Egg Yolk Tellurite Emulsion
SR0047C	Egg-Yolk Emulsion	06-016	Sterile Egg Yolk Emulsion
CM0479B	Endo Agar Base	01-589	Endo Agar Base
CM0069B	Eosine Methylene Blue Agar , Modified Levine	01-068	Eosin Methylene Blue Agar (EMB Agar)
CM0523B	Giolitti Cantoni Broth	02-230	Giolitti-Cantoni Broth
R40080	Gram Stain Kit	RE0100	Barrit's Reagent (VP2)
R40080	Gram Stain Kit	VI0027	Gram Stain
R40080	Gram Stain Kit	LU0010	Gram Stain
R40080	Gram Stain Kit	DE0010	Gram Stain

Cross References

Cross-references: OXOID - SCHARLAU (alphabetical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
R40080	Gram Stain Kit	SA0042	Gram Stain
CM0898B	Haemophilus Test Medium (HTM)	01-706	HTM Agar Base (Haemophilus Test Medium Agar)
SR0166E	Half Fraser Supplement	06-136	*Listeria* Enrichment Half Fraser Selective Supplement (225 ml)
CM0419B	Hektoen Enteric Agar	01-216	Hektoen Enteric Agar
CM0591B	Kanamycin Aesculin Azide Agar Base	01-263	Kanamycin Esculin Azide Agar (KAA Agar)
CM0701B	KF Streptococcus Agar	01-294	Kenner Fecal Agar (KF Agar)
CM0033B	Kligler Iron Agar	01-103	Kligler Iron Agar (KIA)
CM0015B	Lab-Lembco Broth	02-144	Nutrient Broth (APHA)
CM0017B	Lab-Lemco Agar	01-144	Nutrient Agar (APHA)
LP0029B	Lab-Lemco powder	07-075	Meat Extract
LP0029B	Lab-Lemco powder	07-515	Beef Extract
LP0048B	Lactalbumin hydrolisate	07-455	Lactalbumin Hydrolysate
CM0137B	Lactose Broth	02-105	Lactose Broth
CM0451B	Lauryl Tryptose Broth	02-108	Tryptose Lauryl sulfate Broth
SR0110C	*Legionella* BCYE Growth Supplement	06-137	*Legionella* BCYE Growth Supplement
SR0175A	*Legionella* BCYE Growth Supplement without L-cysteine	06-134	*Legionella* BCYE without cysteine NO Growth Supplement
CM0655B	*Legionella* CYE Agar Base	01-687	*Legionella* BCYE Agar Base
SR0152E	*Legionella* GVPC Selective Supplement	06-138	*Legionella* GVPC Selective Supplement
CM0862B	*Listeria* enrichment Broth Base (FDA)	02-498	*Listeria* Enrichment Broth Base (Lovett)
CM0863B	*Listeria* Enrichment Broth Base (UVM formulatoin)	02-472	*Listeria* Enrichment Broth Base (UVM)
CM0895B	*Listeria* Enrichment Broth Base acc. to Fraser	02-496	*Listeria* Enrichment Broth Base (Fraser)
CM0856B	*Listeria* Selective Agar (OXFORD)	01-471	Oxford Agar Base
SR0141E	*Listeria* Selective Supplement for Enrichment FDA/IDF	06-107	*Listeria* Selective Supplement for Enrichment (FDA & IDF/FIL)
SR0142E	*Listeria* Selective Supplement for primary enrichment	06-102	MUG Supplement
SR0143E	*Listeria* Selective Supplement for secundary enrichment	06-111	*Listeria* Selective Supplement for Secondary Enrichment (UVM II/Fraser)
CM0381B	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
CM0308S	Lysine Decarboxylase Broth (taylor Modificaction)	02-336	Decarboxylase Lysine Broth (Taylor)
SR0188E	m-CP Selective Supplement	06-124	Nalidixic Acid Selective Supplement
CM0115B	MacCONKEY Agar No. 3	01-118	MacConkey Agar (Eur. Pharm.)
CM0109B	MacConkey Agar No. 2	01-682	MacConkey No. 2 Agar
CM0005B	MacCONKEY Broth	02-118	MacConkey Broth
CM0505B	MaCConkey Broth Purple	02-611	MacConkey Broth (Eur. Pharm.)
LP0039B	Malt Extract	07-080	Malt Extract
CM0059B	Malt Extract Agar	01-574	Malt Extract Agar No. 3
CM0057B	Malt Extract Broth	02-491	Malt Extract Broth No. 2
CM0085B	Mannitol Salt Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
CM0399B	Mannitol Selenite Broth Base	02-652	Mannitol Selenite Broth Base
CM0733B	Maximum recovery Diluent	02-510	Maximum Recovery Diluent (MRD)
CM0733B	Maximum recovery Diluent	02-631	Purple Maximum Recovery Diluent
CM0992B	Membrane *Clostridium perfringens* (m-CP) Medium	01-513	m-CP Agar Base
CM0681B	Milk Plate Count Agar	01-412	Plate Count Skim Milk Agar
CM0607G	Minerals Modified Medium Base	02-656	Mineral Modified Glutamate Medium Base
SR0204E	Modified Preston *Campylobacter* Selective Supplement	06-135	*Campylobacter* Preston Modified Selective Supplement
CM0910B	Modified Semi-Solid Rappaport-Vassiliadis (MSRV) Medium Base	03-376	Rappaport Vassiliadis Modified Semisolid Medium Base
CM0361B	MRS Agar	01-135	MRS Agar
CM0359B	MRS Broth	02-135	MRS Broth
CM0043B	MRVP Medium	02-207	Methyl Red Voges Proskauer Broth (MRVP)
CM0337B	Mueller hinton Agar	01-136	Mueller-Hinton Agar
CM0405B	Mueller Hinton Broth	02-136	Mueller-Hinton Broth
BR0071E	MUG Supplement	06-089	Potassium Tellurite Sterile Solution 1%
CM1048B	Muller KauffMann tetrathionate-Novobiocin Broth (MKTTn)	02-335	Muller-Kauffmann Tetrathionate Broth Base
CM0929B	MYP Agar	01-262	*Bacillus cereus* Agar
SR0181E	Novobiocin supplement	06-139	Novobiocin Selective Supplement (10 mg)
CM0003B	Nutrient Agar	01-140	Nutrient Agar

Cross References

Cross-references: OXOID - SCHARLAU (alphabetical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
CM0003B	Nutrient Agar	01-635	Nutrient Agar (ISO)
CM0001B	Nutrient Broth	02-140	Nutrient Broth
CM0067B	Nutrient Broth No.2 (Preston Selective Broth)	02-561	Preston *Campylobacter* Broth Base
SR0073A	OGYE Selective Supplement	06-114	Sodium Disulfite (Sodium Meta-Bisulfite) for Bacteriology
SR0140E	Oxford Agar Selective Supplement	06-109	Oxford Agar Selective Supplement
CM0545B	Oxytetracyclin Glucose Yeast Extract Agar (O.G.Y.E Agar)	01-275	Sabouraud Oxytetracycline Agar Base (OGYEA)
CM0877B	Palcam Agar Base	01-470	Palcam Agar Base
SR0150E	Palcam Selective Agar	06-110	Palcam Agar Selective Supplement
CM0009B	Peptone Water	03-156	Tryptone Water (Peptone Water)
CM0587B	Perfringens Agar Base (TSC & SFP)	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
CM0325B	Plate Count Agar (Tryptone Glucose Yeast Agar)	01-329	Plate Count Modified Agar
SR0030J	Potassium tellurite 3,5%	06-011	Potassium Tellurite Sterile Solution 3,5%
CM0139B	Potato Dextrose Agar	01-483	Potato Dextrose Agar (Eur. Pharm.)
SR0117E	Preston *Campylobacter* Selective Supplement	06-130	*Campylobacter* Preston Selective Supplement
CM0689B	Preston Selective Agar	01-451	Preston *Campylobacter* Agar Base
LP0085B	Proteose peptone	07-625	Proteose Peptone No. 3
CM0559B	Pseudomonas Agar Base	01-609	CN Selective Agar Base
CM0906B	R2A Agar	01-540	R2A Agar
CM0866B	Rappaport-Vasisliadissoya Peptone (RVS) Broth	02-379	Rappaport Vassiliadis Broth
CM0669B	Rappaport-Vassiliadis (RV) Enrichment Broth	02-668	Rappaport Vassiliadis *Salmonella* Enrichment Broth
CM0151B	Reinforced Clostridial Agar	01-289	Reinforced Clostridial Agar
CM0149B	Reinforced Clostridial Medium	03-289	Reinforced Clostridial Medium (Eur. Pharm.)
CM0549B	Rose Bengal Chloramphenicol Agar	01-301	Rose Bengal Agar
CM0041B	Sabouraud Dextrose Agar	01-165	Sabouraud Dextrose Agar
CM0147B	Sabouraud Liquid Medium	02-165	Sabouraud Broth
CM0533B	*Salmonella* Shigella Modificado Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)
CM0395B	Selenite Broth Base	02-598	Selenite Broth Base
CM0699B	Selenite Cystine Broth Base	02-602	Selenite Cystine Broth Base
CM0409B	Sensitest Agar	01-655	Sensitivity Test Agar (STA)
CM0435B	SIM Medium	03-176	SIM Medium
CM0155B	Simmons Citrate Agar	01-177	Simmons Citrate Agar
LP0031B	Skim Milk Powder	06-019	Skimmed Milk Powder
CM0377B	Slanetz & Bartley Medium	01-579	Slanetz Bartley Agar Base
LP0121A	Sodium bi-selenite	06-607	Basic Fuchsin (250) Selective Supplement
CM0813B	Sorbitol MaCConkey Agar	01-541	MacConkey Sorbitol Agar
CM0463B	Standard Plate Count Agar (APHA)	01-161	Plate Count Agar (PCA)
CM0111B	Stuart Transport Medium	03-454	Stuart Ringertz Transport Medium
CM0793B	Tergitol R 7 Agar	01-053	Chapman TTC Agar (Tergitol® 7 Agar)
CM0391B	Thioglycollate Broth USP-Alternative	02-186	Thioglycollate Broth
CM0173B	Thioglycollate Medium USP	03-187	Thioglycollate Fluid Medium
CM0189B	Todd-Hewitt Broth	02-191	Todd-Hewitt Broth
CM0277B	Triple Sugar Iron Agar	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
CM0989B	Tryptona Soya Broth Modified (mTSB)	02-691	Tryptic Soy Broth Modified
CM0595B	Tryptone Bile Agar	01-526	Tryptone Bile Agar
CM0945B	Tryptone Bile X-glucoronide Agar	01-619	Tryptone Bile Glucuronic Agar (TBX Agar)
CM0127B	Tryptone Glucose Extract Agar	01-082	Tryptone Glucose Extract Agar (TGE Agar)
CM0131B	Tryptone Soy Agar	01-200	Tryptic Soy Agar (TSA)
CM0129B	Tryptone Soya Broth	02-200	Tryptic Soy Broth (TSB)
LP0043B	Tryptone T	07-119	Casein Trypsic Peptone (Tryptone)
LP0043B	Tryptone T	07-489	Peptone From Casein (Tryptone)
LP0047B	Tryptose	07-197	Tryptose
SR0229K	TTC Solution (1%)	06-023	TTC Sterile Solution 1%
CM0053B	Urea Agar Base	01-261	Urea Agar Base
CM0071B	Urea Broth Base	02-202	Urea Broth Base

Cross References

Cross-references: OXOID - SCHARLAU (alphabetical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
SR0020K	Urea Steril Solution 40%	06-077	Vaseline Sterile
CM0485B	Violet Red Bile Glucose Agar	01-295	Violet Red Bile Dextrose Agar (VRBD Agar)
CM0107B	Violet Red Bile Lactose Agar	01-164	Violet Red Bile Agar (VRB Agar)
CM0641B	Vogel Johnson Agar	01-206	Vogel Johnson Agar (VJ Agar)
CM1012B	Water Plate Count Agar (ISO)	01-590	Tryptone Yeast Extract Agar
CM0309B	WL Nutrient Agar (Medium)	01-210	WL Nutrient Agar
CM0247B	Wort Agar	01-132	Wort Agar
CM0469B	XLD Medium	01-552	Xylose Lysine Deoxycholate Modified Agar
CM1061B	XLT-4 Agar	01-708	XLT4 Agar
SR0237E	XLT-4 Selective Supplement	06-709	XLT4 Agar Selective Supplement
LP0021B	Yeast Extract powder	07-079	Yeast Extract
CM0920B	Yeast Mold Agar (YM Agar)	01-219	Yeast Malt Agar
CM0653B	*Yersinia* Selective (CIN) Agar Base	01-144	Nutrient Agar (APHA)
SR0109E	*Yersinia* Selective Supplement	06-143	*Yersinia* Selective Supplement

Cross References

Cross-references: OXOID - SCHARLAU (numerical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
BR0071E	MUG Supplement	06-089	Potassium Tellurite Sterile Solution 1%
CM0001B	Nutrient Broth	02-140	Nutrient Broth
CM0003B	Nutrient Agar	01-140	Nutrient Agar
CM0003B	Nutrient Agar	01-635	Nutrient Agar (ISO)
CM0005B	MacConkey Broth	02-118	MacConkey Broth
CM0009B	Peptone Water	03-156	Tryptone Water (Peptone Water)
CM0015B	Lab-Lembco Broth	02-144	Nutrient Broth (APHA)
CM0017B	Lab-Lemco Agar	01-144	Nutrient Agar (APHA)
CM0031B	Brilliant Green Bile (2%) Broth	02-041	Brilliant Green Bile 2% Broth
CM0033B	Kligler Iron Agar	01-103	Kligler Iron Agar (KIA)
CM0041B	Sabouraud Dextrose Agar	01-165	Sabouraud Dextrose Agar
CM0043B	MRVP Medium	02-207	Methyl Red Voges Proskauer Broth (MRVP)
CM0053B	Urea Agar Base	01-261	Urea Agar Base
CM0055B	Blood Agar Base	01-352	Blood Agar Base
CM0057B	Malt Extract Broth	02-491	Malt Extract Broth No. 2
CM0059B	Malt Extract Agar	01-574	Malt Extract Agar No. 3
CM0067B	Nutrient Broth No.2 (Preston Selective Broth)	02-561	Preston *Campylobacter* Broth Base
CM0069B	Eosine Methylene Blue Agar , Modified Levine	01-068	Eosin Methylene Blue Agar (EMB Agar)
CM0071B	Urea Broth Base	02-202	Urea Broth Base
CM0085B	Mannitol Salt Agar	01-116	Mannitol Salt Agar (Eur. Pharm.)
CM0107B	Violet Red Bile Lactose Agar	01-164	Violet Red Bile Agar (VRB Agar)
CM0109B	MacConkey Agar No. 2	01-682	MacConkey No. 2 Agar
CM0111B	Stuart Transport Medium	03-454	Stuart Ringertz Transport Medium
CM0115B	MacCONKEY Agar n. 3	01-118	MacConkey Agar (Eur. Pharm.)
CM0127B	Tryptone Glucose Extract Agar	01-082	Tryptone Glucose Extract Agar (TGE Agar)
CM0129B	Tryptone Soya Broth	02-200	Tryptic Soy Broth (TSB)
CM0131B	Tryptone Soy Agar	01-200	Tryptic Soy Agar (TSA)
CM0137B	Lactose Broth	02-105	Lactose Broth
CM0139B	Potato Dextrose Agar	01-483	Potato Dextrose Agar (Eur. Pharm.)
CM0147B	Sabouraud Liquid Medium	02-165	Sabouraud Broth
CM0149B	Reinforced Clostridial Medium	03-289	Reinforced Clostridial Medium (Eur. Pharm.)
CM0151B	Reinforced Clostridial Agar	01-289	Reinforced Clostridial Agar
CM0155B	SIMMONS Citrate Agar	01-177	Simmons Citrate Agar
CM0163B	Desoxycholate Agar	01-057	Deoxycholate Lactose Agar
CM0173B	Thioglycollate Medium USP	03-187	Thioglycollate Fluid Medium
CM0189B	Todd-Hewitt Broth	02-191	Todd-Hewitt Broth
CM0225B	Brain Heart Infusion Broth	02-599	Brain Heart Infusion Broth (BHI Broth)
CM0247B	Wort Agar	01-132	Wort Agar
CM0263B	Brilliant Green Agar	01-203	Brilliant Green Agar (BGA)
CM0271B	Blood Agar Base N.2	01-505	Blood Agar Base No. 2
CM0275B	Baird Parker Agar Base	01-030	Baird Parker Agar Base
CM0277B	Triple Sugar Iron Agar	01-192	Triple Sugar Iron Agar (TSI Agar) (Eur. Pharm. Agar Medium M)
CM0287B	Antibiotic Medium No. 3 (Assay Broth)	02-011	Antibiotic Medium C (Eur. Pharm.)
CM0301B	CLED Medium	01-047	CLED Agar
CM0308S	Lysine Decarboxylase Broth (taylor Modificaction)	02-336	Decarboxylase Lysine Broth (Taylor)
CM0309B	WL Nutrient Agar (Medium)	01-210	WL Nutrient Agar
CM0317B	EE Broth	02-064	Enrichment Enterobacteriaceae Broth (EE Broth) (Eur. Pharm.)
CM0321B	DNase Agar	01-346	DNase Agar
CM0325B	Plate Count Agar (Tryptone Glucose Yeast Agar)	01-329	Plate Count Modified Agar
CM0327B	Antibiotic Medium n. 1	01-009	Antibiotic Medium A pH 6.6 (Eur. Pharm.)
CM0329B	Brilliant Green Agar (Modified)	01-309	Brilliant Green Modified Agar (BGA Modified)
CM0331B	Columbia Blood Agar Base	01-034	Blood Agar Base (Columbia)
CM0333B	Cholera Medium TCBS	01-567	TCBS Agar
CM0337B	Mueller hinton Agar	01-136	Mueller-Hinton Agar

Cross References

Cross-references: OXOID - SCHARLAU (numerical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
CM0359B	MRS Broth	02-135	MRS Broth
CM0361B	MRS Agar	01-135	MRS Agar
CM1136B	Brain Heart Infusion Agar	01-599	Brain Heart Infusion Agar (BHI Agar)
CM0377B	Slanetz & Bartley Medium	01-579	Slanetz Bartley Agar Base
CM0381B	Lysine Iron Agar	01-094	Lysine Iron Agar (LIA)
CM0391B	Thioglycollate Broth USP-Alternative	02-186	Thioglycollate Broth
CM0395B	Selenite Broth Base	02-598	Selenite Broth Base
CM0399B	Mannitol Selenite Broth Base	02-652	Mannitol Selenite Broth Base
CM0405B	Mueller Hinton Broth	02-136	Mueller-Hinton Broth
CM0409B	Sensitest Agar	01-655	Sensitivity Test Agar (STA)
CM0419B	Hektoen Enteric Agar	01-216	Hektoen Enteric Agar
CM0435B	SIM Medium	03-176	SIM Medium
CM0451B	Lauryl Tryptose Broth	02-108	Tryptose Lauryl sulfate Broth
CM0463B	Standard Plate Count Agar (APHA)	01-161	Plate Count Agar (PCA)
CM0469B	XLD Medium	01-552	Xylose Lysine Deoxycholate Modified Agar
CM0479B	Endo Agar Base	01-589	Endo Agar Base
CM0485B	Violet Red Bile Glucose Agar	01-295	Violet Red Bile Dextrose Agar (VRBD Agar)
CM0505B	MaCConkey Broth Purple	02-611	MacConkey Broth (Eur. Pharm.)
CM0509B	Buffered peptone Water	02-693	Tryptone Phosphate Water 09
CM0519B	Cary-Blair Medium	03-643	Cary-Blair Transport Medium
CM0523B	Giolitti Cantoni Broth	02-230	Giolitti-Cantoni Broth
CM0533B	*Salmonella* Shigella Modificado Agar	01-555	*Salmonella-Shigella* Agar (SS Agar)
CM0539B	Dermasel Medium	01-442	Dermatophyte Selective Agar
CM0545B	Oxytetracyclin Glucose Yeast Extract Agar (OGYE Agar)	01-275	Sabouraud Oxytetracycline Agar Base (OGYEA)
CM0549B	Rose Bengal Chloramphenicol Agar	01-301	Rose Bengal Agar
CM0559B	Pseudomonas Agar Base	01-609	CN Selective Agar Base
CM0579B	Cetrimide Agar	01-160	Cetrimide Agar (Pseudomonas Selective Agar)
CM0587B	Perfringens Agar Base (TSC & SFP)	01-278	Tryptose Sulfite Cycloserine Agar (TSC Agar)
CM0589B	BiGGY Agar	01-137	Nickerson Agar (Biggy Agar)
CM0591B	Kanamycin Aesculin Azide Agar Base	01-263	Kanamycin Esculin Azide Agar (KAA Agar)
CM0595B	Tryptone Bile Agar	01-526	Tryptone Bile Agar
CM0607G	Minerals Modified Medium Base	02-656	Mineral Modified Glutamate Medium Base
CM0617B	*Bacillus cereus* Selective Agar Base	01-487	*Bacillus cereus* Selective Agar
CM0641B	Vogel Johnson Agar	01-206	Vogel Johnson Agar (VJ Agar)
CM0653B	*Yersinia* Selective (CIN) Agar Base	01-144	Nutrient Agar (APHA)
CM0655B	*Legionella* CYE Agar Base	01-687	*Legionella* BCYE Agar Base
CM0669B	Rappaport-Vassiliadis (RV) Enrichment Broth	02-668	Rappaport Vassiliadis *Salmonella* Enrichment Broth
CM0681B	Milk Plate Count Agar	01-412	Plate Count Skim Milk Agar
CM0689B	Preston Selective Agar	01-451	Preston *Campylobacter* Agar Base
CM0699B	Selenite Cystine Broth Base	02-602	Selenite Cystine Broth Base
CM0701B	KF Streptococcus Agar	01-294	Kenner Fecal Agar (KF Agar)
CM0727B	DRBC Agar Base	01-657	Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar)
CM0729B	Dichloran Glycerol (DG18) Agar Base	01-485	Dichloran Glycerin Selective Agar (DG18 Agar)
CM0733B	Maximum recovery Diluent	02-510	Maximum Recovery Diluent (MRD)
CM0733B	Maximum recovery Diluent	02-631	Purple Maximum Recovery Diluent
CM0739B	*Campylobacter* Blood-free Selective Agar Base	01-685	Charcoal Cefoperazone Deoxycholate (CCD) Modified Agar Base
CM0793B	Tergitol R 7 Agar	01-053	Chapman TTC Agar (Tergitol® 7 Agar)
CM0813B	Sorbitol MaCConkey Agar	01-541	MacConkey Sorbitol Agar
CM0853B	EC Broth	02-060	*E. coli* Broth
CM0856B	*Listeria* Selective Agar (Oxford)	01-471	Oxford Agar Base
CM0862B	*Listeria* enrichment Broth Base (FDA)	02-498	*Listeria* Enrichment Broth Base (Lovett)
CM0863B	*Listeria* Enrichment Broth Base (UVM formulatoin)	02-472	*Listeria* Enrichment Broth Base (UVM)
CM0866B	Rappaport-Vasisliadissoya Peptone (RVS) Broth	02-379	Rappaport Vassiliadis Broth
CM0868B	Azide Dextrose Broth (Rothe)	02-027	Azide Dextrose Broth (Rothe)

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Cross References

Cross-references: OXOID - SCHARLAU (numerical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
CM0877B	Palcam Agar Base	01-470	Palcam Agar Base
CM0888B	Bile Aesculine agar	01-265	Bile Esculin Modified Agar
CM0895B	*Listeria* Enrichment Broth Base acc. to Fraser	02-496	*Listeria* Enrichment Broth Base (Fraser)
CM0898B	Haemophilus Test Medium (HTM)	01-706	HTM Agar Base (Haemophilus Test Medium Agar)
CM0906B	R2A Agar	01-540	R2A Agar
CM0910B	Modified Semi-Solid Rappaport-Vassiliadis (MSRV) Medium Base	03-376	Rappaport Vassiliadis Modified Semisolid Medium Base
CM0920B	Yeast Mold Agar (YM Agar)	01-219	Yeast Malt Agar
CM0929B	MYP Agar	01-262	*Bacillus cereus* Agar
CM0945B	Tryptone Bile X-glucoronide Agar	01-619	Tryptone Bile Glucuronic Agar (TBX Agar)
CM0982B	Buffered sodium Chloride Peptone	02-494	Buffered Peptone Water (Eur. Pharm.)
CM0983B	Bolton Selective Enrichment Broth	02-688	Bolton Enrichment Broth Base
CM0989B	Tryptona Soya Broth Modified (mTSB)	02-691	Tryptic Soy Broth Modified
CM0992B	Membrane *Clostridium perfringens* (m-CP) Medium	01-513	m-CP Agar Base
CM1012B	Water Plate Count Agar (ISO)	01-590	Tryptone Yeast Extract Agar
CM1028B	Alkaline Peptone Water	02-468	Alkaline Peptone Water
CM1048B	Muller KauffMann tetrathionate-Novobiocin Broth (MKTTn)	02-335	Muller-Kauffmann Tetrathionate Broth Base
CM1049B	Buffered Peptone Water (ISO)	02-277	Buffered Peptone Water
CM1061B	XLT-4 Agar	01-708	XLT4 Agar
CM1117B	Alkaline Saline Peptone Water	02-697	Alkaline Saline Peptone Water
LP0011B	Agar bacteriological (Agar n.1)	07-004	Agar Bacteriological
LP0021B	Yeast Extract powder	07-079	Yeast Extract
LP0029B	Lab-Lemco powder	07-075	Meat Extract
LP0029B	Lab-Lemco powder	07-515	Beef Extract
LP0031B	Skim Milk Powder	06-019	Skimmed Milk Powder
LP0039B	Malt Extract	07-080	Malt Extract
LP0043B	Tryptone T	07-119	Casein Trypsic Peptone (Tryptone)
LP0043B	Tryptone T	07-489	Peptone From Casein (Tryptone)
LP0047B	Tryptose	07-197	Tryptose
LP0048B	Lactalbumin hydrolisate	07-455	Lactalbumin Hydrolysate
LP0085B	Proteose peptone	07-625	Proteose Peptone No. 3
LP0121A	Sodium bi-selenite	06-607	Basic Fuchsin (250) Selective Supplement
R21522	BactiDrop Indol , Kovacs	RE0007	Kovacs' Reagent
R21536	BactiDrop Nitrate A	06-003	Nitrate Reagent A
R21538	BactiDrop Nitrate B	06-004	Nitrate Reagent B
R40080	Gram Stain Kit	RE0100	Barrit's Reagent (VP2)
R40080	Gram Stain Kit	VI0027	Gram Stain
R40080	Gram Stain Kit	LU0010	Gram Stain
R40080	Gram Stain Kit	DE0010	Gram Stain
R40080	Gram Stain Kit	SA0042	Gram Stain
SR0020K	Urea Steril Solution 40%	06-077	Vaseline Sterile
SR0030J	Potassium tellurite 3,5%	06-011	Potassium Tellurite Sterile Solution 3,5%
SR0047C	Egg-Yolk Emulsion	06-016	Sterile Egg Yolk Emulsion
SR0054C	Egg Yolk tellurite Emulsion	06-026/064-BA1018	Sterile Egg Yolk Tellurite Emulsion
SR0069E	*Campylobacter* Selective Supplement Skirrow)	06-132	*Campylobacter* Skirrow Selective Supplement
SR0073A	OGYE Selective Supplement	06-114	Sodium Disulfite (Sodium Meta-Bisulfite) for Bacteriology
SR0078E	Chloramphenicol Selective Supplement	06-118	Chloramphenicol Selective Supplement
SR0088E	Cycloserine (TSC) Supplement	06-116	Cycloserine Selective Supplement
SR0099E	*Bacillus cereus* Selective Supplement	06-021	Polymyxin B Sulfate Selective Supplement
SR0109E	*Yersinia* Selective Supplement	06-143	*Yersinia* Selective Supplement
SR0110C	*Legionella* BCYE Growth Supplement	06-137	*Legionella* BCYE Growth Supplement
SR0117E	Preston *Campylobacter* Selective Supplement	06-130	*Campylobacter* Preston Selective Supplement
SR0140E	Oxford Agar Selective Supplement	06-109	Oxford Agar Selective Supplement
SR0141E	*Listeria* Selective Supplement for Enrichment FDA/IDF	06-107	*Listeria* Selective Supplement for Enrichment (FDA & IDF/FIL)
SR0142E	*Listeria* Selective Supplement for primary enrichment	06-102	MUG Supplement

Cross References

Cross-references: OXOID - SCHARLAU (numerical)

Art. No. OXOID	Description OXOID	Art. No. SCHARLAU	Description SCHARLAU
SR0143E	*Listeria* Selective Supplement for secundary enrichment	06-111	*Listeria* Selective Supplement for Secondary Enrichment (UVM II/Fraser)
SR0150E	Palcam Selective Agar	06-110	Palcam Agar Selective Supplement
SR0152E	*Legionella* GVPC Selective Supplement	06-138	*Legionella* GVPC Selective Supplement
SR0155E	CCDA Selective Supplement	06-133	Campylobater CCDA Selective Supplement
SR0166E	Half Fraser Supplement	06-136	*Listeria* Enrichment Half Fraser Selective Supplement (225 ml)
SR0175A	*Legionella* BCYE Growth Supplement without L-cysteine	06-134	*Legionella* BCYE without Cysteine NO Growth Supplement
SR0181E	Novobiocin supplement	06-139	Novobiocin Selective Supplement (10 mg)
SR0183E	Bolton Broth Selective Supplement	06-131	*Campylobacter* Bolton Selective Supplement
SR0188E	m-CP Selective Supplement	06-124	Nalidixic Acid Selective Supplement
SR0204E	Modified Preston *Campylobacter* Selective Supplement	06-135	*Campylobacter* Preston Modified Selective Supplement
SR0222C	Cycloheximide	06-022	Cycloheximide Selective Supplement
SR0229K	TTC Solution (1%)	06-023	TTC Sterile Solution 1%
SR0232E	*Campylobacter* Growth Supplement	06-128	*Campylobacter* Growth Supplement
SR0237E	XLT-4 Selective Supplement	06-709	XLT4 Agar Selective Supplement

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