Laboratory Deodorizing Pearls **LA008A & LA008B**

Pleasant smelling deodorizers for masking the oppressive odours during autoclaving of exposed plates, bacterial growth and contaminated material.

These Deodorizers will remove unwanted odors from your facility by deodorizing techniques in autoclaves, incubators, refrigerators, and similar closed apparatus where tissue culture ware may be used or stored.

Application : Microbiology laboratory, Molecular biology laboratory ,Chemical laboratory , Plant Tissue culture work and various other laboratories.

Direction : Drop one or two pearls or as required, inside steam autoclave.

Product Name	Product Code	Description	Fragrance
Fresh Deordorising Pearls	**LA008A**	An autoclave deodorant. These pearls are a simple, convenient method of eliminating unpleasant odours associated with the disposal of laboratory waste.	Citrus Fragrance (pearls)
Fresh Deordorising Pearls	**LA008B**	An autoclave deodorant. These pearls are a simple, convenient method of eliminating unpleasant odours associated with the disposal of laboratory waste.	Rose Fragrance (pearls)

Product Features :

- Removes unwanted odor and Creates a natural, pleasant fragrance.
- Simple and easy to use.
- Available in two fragrances Rose and Citrus.
- Very effective.
- Pack Size : 5X50 and 10X50 Nos.

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Reason: MoldSign Signature
Location: Moldova

HiMedia Laboratories Pvt. Ltd. A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India.
Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com Website: www.himedialabs.com

Nutrient Agar

M001

Intended use

Nutrient Agar is used as a general purpose medium for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

Ingredients	g / L
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 28.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired ,the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (1,2). Nutrient Agar is ideal for demonstration and teaching purposes where a more prolonged survival of cultures at ambient temperature is often required without risk of overgrowth that can occur with more nutritious substrate. This relatively simple formula has been retained and is still widely used in the microbiological examination of variety of materials and is also recommended by standard methods. It is one of the several non-selective media useful in routine cultivation of microorganisms (3,4). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples - faeces, urine ; Food and dairy samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,7).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Productivity			
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=70%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good-luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=70%
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 9610 (00038*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 23715 (00160*)	50-100	good-luxuriant	>=70%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
2.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins,Baltimore.
3.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
4.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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8.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 07/2024

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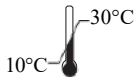
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Soyabean Casein Digest Medium Base w/o Polymyxin M011F

Intended use

Soyabean Casein Digest Medium Base with added Polymyxin is recommended for selective isolation and MPN method of *B.cereus* . The he composition and performance criteria are as per the specification laid down in FDA BAM,1998 , ISO 21871 and ISO 11133:2014 & Amd.2:2020 (E) .

Composition**

ISO 21871 : Tryptone Soya Polymyxin Broth

Ingredients	g / L
Tryptone #	17.000
Soya peptone ##	3.000
Sodium chloride	5.000
Dextrose	2.500
Dibasic potassium phosphate	2.500
Final pH (at 25°C)	7.3±0.2

M011F: Soyabean Casein Digest Medium Base w/o Polymyxin

Ingredients	g / L
Tryptone #	17.000
Soya peptone ##	3.000
Sodium chloride	5.000
Dextrose	2.500
Dibasic potassium phosphate	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein ## Papaic digest of soyabean meal

Directions

Suspend 30 gram in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Add one vial of sterile Polymixin B Sulphate (FD003) solution to a final concentration of 100 Units/ml.Mix well and dispense as desired.

Note: If any fibres are observed in the solution, it is recommended to filter the solution through a 0.22 micron filter to eliminate the possibility of presence of fibres.

Principle And Interpretation

Bacillus cereus is a large, 1 × 3-4 µm, Gram-positive, rod-shaped, endospore forming, and facultative aerobic bacterium. They are mesophilic and can grow in a wide range of environments and are commonly found in nature, vegetables and in several processed foods. Under favorable circumstances the microorganism grows to sufficient numbers and cause gastrointestinal illness. Outbreaks of food borne illness have been associated with boiled and cooked rice, cooked meat and vegetables (1). The infection mediates diarrhoeal illness that is attributed by a heat and acid labile enterotoxin. Soyabean Casein Digest Medium Base (SCDM) with polymyxin B (FD003) is recommended for the selective isolation and MPN method of *Bacillus cereus* in accordance with FDA BAM(2) and ISO (3,4) for selective enrichment of *B.cereus. B.cereus* in general is resistant to polymyxin B and the addition of it into the medium helps in the selective isolation of the organism. Without supplement, SCDM is a highly nutritious medium used for cultivation of a wide variety of organisms (3). The combination of tryptone and soya peptone makes the medium nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Dextrose and dibasic potassium phosphate serve as the carbohydrate source and the buffer, respectively in the medium. Sodium chloride maintains the osmotic balance of the medium. FDA BAM suggests two methods to check the presence of *B.cereus* that are 1) Serial dilution method and 2) MPN Method.

According to the serial dilution protocol, appropriate dilutions of the suspected samples are made in Butterfield's Phosphate Buffered Dilution Water (R094) and spread plate was done with 0.1 ml of respective dilutions in MYP Agar Base (M636F). According to the MPN method, 1 ml each of 10-1, 10-2 and 10-3 are inoculated into Soyabean Casein Digest Medium Base (M011F) with polymyxin (FD003) incubate for 48 ± 2 h at 30 ± 2°C. Observation of turbid growth after the incubation time is indicative of the presence of *B.cereus*. Positive cultures are further inoculated into MYP Agar Base (M636F) and incubated 18-24 h at 30°C. *B.cereus* appears as pink coloured colonies surrounded by a precipitate zone of lecithinase activity. Biochemical tests are performed to confirm the species.

According to ISO, selective enrichment is carried out in M011F and isolated on PEMBA Agar (M1484).

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (2-5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate.

Reaction

pH of 3.0% w/v aqueous solution at 25°C . pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Productivity : Cultural characteristics observed by adding Polymyxin B Selective Supplement (FD003), after an incubation at 30 ±1°C for 48± 4 hours.

Selectivity : Cultural characteristics observed by adding Polymyxin B Selective Supplement (FD003), after an incubation at 30 ±1°C for 48± 4 hours.

Organism	Inoculum (CFU)	Growth
Productivity		
Bacillus cereus ATCC 10876	50-100	luxuriant
Selectivity		
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.Hoffmaster, A., Hill, K., Gee, J., Marston, C., De, B., Popovic, T., Sue, D., Wilkins, P., Avashia, S., Drumgoole, R., Helma, C., Ticknor, L., Okinaka, R. and Jackson, J 2006. Journal of clinical microbiology, 44(9): 3352-3360.

2. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
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4. Microbiology of food and animal feeding stuffs Horizontal method for the determination of low numbers of presumptive Bacillus cereus Most probable number technique and detection method, ISO 21871:2006
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/ 2024

XLD Agar, Modified

M031I

Intended use

Recommended for selective isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species. The composition and performance criteria of this medium are as per specifications laid down in ISO 6579-1:2017./ Amd: 2020, ISO 19250:2010(E) and APHA.

Composition**

ISO 6579-1 Specification - XLD Agar

Ingredients	g/ L
Yeast extract	3.000
L-Lysine hydrochloride	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.750
Sodium chloride (NaCl)	5.000
Sodium deoxycholate	1.000
Sodium thiosulphate	6.800
Iron (III) ammonium citrate	0.800
Phenol red	0.080
Agar	9.00-18.00
Final pH (at 25°C)	7.4±0.2

M031I - XLD Agar, Modified

Ingredients	g/ L
Yeast extract	3.000
L-Lysine hydrochloride	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.750
Sodium chloride	5.000
Sodium deoxycholate	1.000
Sodium thiosulphate	6.800
Ferric ammonium citrate#	0.800
Phenol red	0.080
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Iron (III) ammonium citrate

Directions

Suspend 55.43 grams in 1000 ml purified/distilled water. Heat with frequent agitation until the medium boils. **DO NOT AUTOCLAVE OR OVERHEAT.** Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes which will require prolonged heating. *Note: Slight precipitation in the medium may occur, which is inheritant property of the medium, and does not affect the performance of the medium.*

Principle And Interpretation

XLD Agar was formulated by Taylor (1-6) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species. XLD Agar, Modified (M031I) is recommended for selective isolation and enumeration of *Salmonella* species in accordance with ISO Committee, APHA (7-11). The incubation conditions has been revised as per the amendment 1, 2020 (8). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* . The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by *Shigellae* but practically by all enterics. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. *Salmonellae* rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylated by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H_2S producers do not decarboxylate lysine therefore, the acid reaction produced by them prevents the blackening of the colonies . XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

Type of specimen

Food and meat samples. milk and milk products, animal feed, animal faeces, environmental samples, Water samples

Specimen Collection and Handling:

Processesing : (7)

Pre-enrichment : Samples (25 grams in 225 ml) are pre-enriched in Buffered Peptone Water (M1494I)/(GM1494I) and incubated at 34°C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre-enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTTn broth (M1496I) and incubated at 36± 2°C for 24 ± 3 hours .

Isolation : The culture thus obtained is then plated on XLD Agar, Modified (M031I) and incubated at 36± 2°C for 24 ± 3 hours . Simultaneously plating on second isolation agar is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Slight precipitation in the medium may occur, which is inheritant property of the medium,and does not affect the performance of the medium.
2. XLD Agar is based on fermentation reaction and H_2S production hence second medium should be selected so as to detect lactose positive and H_2S negative strains.
3. *S.*Paratyphi A, *S.*choleraesuis, *S.*pullorum and *S.*gallinarum may form red colonies without H_2S, thus resembling *Shigella* species.
4. Atypical *Salmonella* species which are lactose positive and/or H_2S negative should be confirmed by biochemical and serological tests.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.54% w/v aqueous solution at 25°C . pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural response was observed after an incubation at 34°C to 38°C for for 24 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Productivity				
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	good	>=50 %	red with black centres
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good	>=50 %	red with black centres
Selectivity				
Escherichia coli ATCC 8739 (00012*)	>=10^4	growth or partial inhibition		yellow

Escherichia coli ATCC 25922 (00013*)	>=10^4	growth or partial inhibition		yellow
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0 %	-
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0 %	-

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (12,13).

Reference

1.Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
2.Taylor W. L. and Harris B., 1965, Am. J. Clin. Pathol., 44:476.
3.Taylor W. L. and Harris B., 1967, Am. J. Clin. Pathol., 48:350.
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6.Taylor W. L. and Schelhart B., 1969, Appl. Microbiol., 18.393-395.
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9.Water Quality - Detection of *Salmonella* spp. International Organization for Standardization (ISO), ISO 19250:2010 (E).
10.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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12.Isenberg H. D., Kominos S., and Sigeal M., 1969, Appl Microbiol., 18, 656-659.
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Revision : 04/2024

Baird Parker Agar Base

M043I

Intended Use:

Recommended for the enumeration of coagulase positive Staphylococci from food and animal feeding stuffs. The composition and performance criteria are as per the specification laid down in ISO 6888-1:1999 / Amd :2018 and ISO 11133:2014 & Amd. 2 :2020 (E).

Composition**

ISO specification -Baird-Parker agar medium	
Ingredients	**g / L**
Pancreatic digest of casein	10.000
Meat extract	5.000
Yeast extract	1.000
L-Glycine	12.000
Sodium pyruvate	10.000
Lithium chloride	5.000
Agar	12 to 22
Final pH after sterilization (at 25°C)	7.2±0.2

Baird Parker Agar Base	**M043I**
Ingredients	**g / L**
Tryptone #	10.000
HM extract ##	5.000
Yeast extract	1.000
Glycine	12.000
Sodium pyruvate	10.000
Lithium chloride	5.000
Agar	15.000
Final pH after sterilization (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of casein, ## Equivalent to Meat extract

Supplements to be added after autoclaving per 1000ml of medium	
I Potassium tellurite solution	10ml
II Egg yolk emulsion	50 ml
III Sulfamezathine (sulfamethazine, sulfadimidine) solution (50mg)	25 ml

PTe 1% Selective Supplement (1 ml per vial) (FD052) for 1000ml medium	
Potassium tellurite Concentrate	10 ml
Egg Yolk Emulsion (FD045) per vial for 900ml medium	
Egg yolk emulsion	50 ml
BP S Selective Supplement (FD069) per vial for 1000ml medium	
Sulphamethazine (50 mg)	5ml

Directions

Suspend 58.0 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 50 ml concentrated Egg Yolk Emulsion (FD045) and 10 ml sterile PTe 1% Selective Supplement (1 ml per vial) (FD052). If desired add rehydrated contents of 1 vial of BP S Selective Supplement (FD069). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Baird Parker Agar was developed by Baird Parker (1,2) from the Tellurite-glycine formulation of Zebovitz et al (3) for isolation and enumeration of Staphylococci in food and other material since it allows a good differentiation. The composition laid down is as per ISO 6888-1 (4). A high correlation has been found between the coagulase test and the presence of clear zone of lypolysis in this medium, which is due to the lecithinase of Staphylococci that breakdown, the egg yolk. On the other hand, studies show that almost 100% of coagulase positive Staphylococci are capable of reducing tellurite, which produces black colonies, whereas other Staphylococci cannot always do so. The identity of *Staphylococcus aureus* isolated on Baird-Parker Agar must be confirmed with a coagulase reaction. Baird-Parker Agar can also be used to detect coagulase activity by adding fibrinogen plasma (5). Smith and Baird-Parker (6) found that the addition of 50 mg/l Sulphamethazine in the medium, suppresses the growth and swarming of *Proteus* species.

Tryptone, HM extract and yeast extract are sources of nitrogen, carbon, sulphur and vitamins. Sodium pyruvate not only protects injured cells and helps recovery but also stimulates *Staphylococcus aureus* growth destroying selectivity. Lithium chloride and potassium tellurite inhibit most of the contaminating microflora except *Staphylococcus aureus.* The tellurite additive is toxic to egg yolk-clearing strains other than *S.aureus* and imparts a grey to black colour to the colonies. Glycine, pyruvate enhances growth of *Staphylococcus.* With the addition of egg yolk, the medium becomes yellow,

opaque. The egg yolk additive, in addition to provide enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). A clear zone and grey-black colonies on this medium are diagnostic for coagulase positive Staphylococci. Upon further incubation, an opaque zone is developed around colonies, which can be due to lipolytic activity. Testing of medium is carried out as per ISO 11133:2014 (7)

Type of specimen

Food samples and animal feeding stuffs

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4,7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The identity of *Staphylococcus aureus* isolated on Baird-Parker Agar must be confirmed with a coagulase reaction.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% agar gel.
Colour and Clarity of prepared medium
Basal medium: Yellow coloured clear to slightly opalescent gel. After addition of Egg Yolk Emulsion and Tellurite solution: Yellow coloured opaque gel forms in Petri plates.
Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.2±0.2
pH
7.00-7.40
Cultural Response
Productivity :Cultural response was observed with added Egg Yolk Emulsion (FD045) and sterile PTe 1% Selective Supplement (FD052) after an incubation at 37±1°C for 24±2 to 48 ±2 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).
Specificity : Cultural response was observed with added Egg Yolk Emulsion (FD045) and sterile PTe 1% Selective Supplement (FD052), after an incubation at 37±1°C for 24-48±2 hours.
Selectivity : Cultural response was observed with added Egg Yolk Emulsion (FD045) and sterile PTe 1% Selective Supplement (FD052), after an incubation at 37±1°C for 48±2 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Characteristic reaction
Productivity				
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	>=50 %	Black or grey colonies with clear halo (egg yolk clearing reaction
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50 -100	luxuriant	>=50 %	Black or grey colonies with clear halo (egg yolk clearing reaction
Selectivity				
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited		
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited		
Specificity				
Staphylococcus epidermidis ATCC 12228 (00036*)	10^3 -10^4	growth		Black or grey colonies without egg yolk clearing reaction
Staphylococcus saprophyticus ATCC 15305 (00159*)	10^3 -10^4	growth		Black or grey colonies without egg yolk clearing reaction

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

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Revision : 05/2024

Phenol Red Broth Base

M054

Intended Use:

A basal medium to which carbohydrates are added for determination of fermentation reactions of pure cultures of microorganisms. The composition of this medium is in accordance with FDA BAM.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	5.000
Phenol red	0.018
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 16.02 grams in 1000 ml purified/distilled water,mix well. Heat if necessary to dissolve the medium completely. Mix well and dispense in fermentation tubes (tubes containing inverted Durham's tubes). Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Aseptically add filter sterilized or autoclave sterilized carbohydrate solution to sterile basal medium.

Principle And Interpretation

Phenol Red Broth Medium is formulated as per Vera (1) and is recommended to determine the fermentation reaction of carbohydrates for the differentiation of microorganisms (2-4). It is recommended by FDA BAM (5). Phenol Red Broth Medium with various added carbohydrates serves as a differential medium by aiding in differentiation of various species and genera by their ability to ferment the specific carbohydrate, with the production of acid or acid and gas (6). Phenol Red Broth Base is a complete medium without added carbohydrate, which can be used with the addition of 5-10 %, desired carbohydrate. It is used as a negative control for studying fermentations or as a base for the addition of carbohydrates. Proteose peptone and HM peptone B serve as sources for carbon and nitrogen. Sodium chloride is the osmotic stabilizer. Phenol red is the pH indicator, which turns yellow at acidic pH. Gas formation is seen in Durhams tubes. All of the *Enterobacteriaceae* grow well in this medium. In addition to producing a pH colour shift, the production of mixed acids, notably butyric acids, often results in a pungent, foul odour from the culture medium (7).

Type of specimen

Isolated Microorganisms from clinical and non clinical sample

Specimen Collection and Handling:

For isolated Microorganisms samples follow appropriate techniques for handling specimens as per established guidelines (8,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. In addition to producing a pH colour shift, the production of mixed acids, notably butyric acids, often results in a pungent, foul odour from the culture medium (2).

Performance and Evaluation

Performance of the medium is expected when used as per thedirection on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to pink coloured homogeneous free flowing powder

Colour and Clarity of prepared medium
Red coloured clear solution without any precipitate

Reaction
Reaction of 1.6% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.(longer if necessary)

Organism	Inoculum (CFU)	Growth	without carbohydrate, (Acid)	without carbohydrate, (Gas)	with dextrose, (Acid)	with dextrose, (Gas)
Citrobacter freundii ATCC 8090	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Proteus vulgaris ATCC 13315	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Salmonella Typhi ATCC 6539	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Serratia marcescens ATCC 8100	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Negative reaction

Key : (*) Corresponding WDCM numbers, (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

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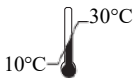

Storage temperature

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Blood Agar Base (Infusion Agar)

M073

Intended Use:

Recommended for isolation and cultivation of many fastidious pathogenic micro-organisms like *Neisseria, Streptococci* after addition of blood from clinical and non-clinical specimens.

Composition**

Ingredients	g / L
HM peptone B#	10.000
Tryptose	10.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef Heart peptone

Directions

Suspend 40.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. It can also be used as general-purpose media without the addition of blood.

Blood Agar Base media can be used with added phenolphthalein phosphate (1) for the detection of phosphate producing Staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass (2) and to determine salinity range of marine *Flavobacteria* (3). It can also be used for preparation of *Salmonella* Typhi antigens (4). Blood Agar Base is recommended by APHA (5) and Standard Methods (6,7) for testing of food samples.

HM peptone B and tryptose provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A Streptococci (8). But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* (9).

Type of specimen

Clinical material : Throat swabs, vaginal secretions and other pathological material; food samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Addition of sheep blood is recommended to detect haemolysis. This medium does not support the growth of *H.haemolyticus.*

2.Addition of Horse blood or rabbit blood to base medium supports growth of *H.haemolyticus* but resemble beta-haemolytic Streptococci and hence must be confirmed.
3.Haemolytic pattern varies with the source of blood used.
4.Other tests must be carried out in conjunction for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Basal medium : Light amber coloured clear to slightly opalescent gel. After addition of 5% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.
Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.3±0.2
pH
7.10-7.50
Cultural Response
Cultural characteristics observed with added 5% w/v sterile defibrinated blood,after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth w/o blood	Recovery w/o blood	Growth with blood	Recovery with blood	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	fair	40-50%	luxuriant	>=70%	none
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good	50-70%	luxuriant	>=70%	beta
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	good	50-70%	luxuriant	>=70%	none
Streptococcus pneumoniae ATCC 6303	50-100	fair-good	40-50%	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	fair-good	40-50%	luxuriant	>=70%	beta

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

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IVD ***In vitro*** **diagnostic medical device**

10°C–30°C **Storage temperature**

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Lauryl Sulphate Broth (Lauryl Tryptose Broth)

M080

Intended use

Recommended for detection and enumeration of coliform bacteria in water, waste water, dairy products ,other food samples. The composition and performance criteria are as per the specification laid down in ISO 11133:2014 & Amd :2018

Composition**

Ingredients	g / L
Tryptose	20.000
Lactose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.750
Potassium dihydrogen phosphate	2.750
Sodium lauryl sulphate (SLS)	0.100
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.60 gram in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared.

Principle And Interpretation

Coliforms are considered to be members of *Enterobacteriaceae*, which grow in the presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C (1). These bacteria can also be defined as, members of *Enterobacteriaceae* capable of growing at 37°C, that normally possess β-galactosidase (2). Lauryl Sulphate Broth is used for the detection of coliforms in water, dairy products and other foods, as recommended by APHA (3-5). It can also be used for the presumptive detection of coliforms in water, effluent or sewage by the MPN test (6). Lauryl Sulphate Broth was developed by Mallmann and Darby (7). Cowls (6) demonstrated that inclusion of sodium lauryl sulphate makes the medium selective for coliform bacteria. It was later investigated that Lauryl Sulphate Broth gave a higher colon index than the confirmatory standard methods media and also that gas production in Lauryl Sulphate Broth not only acts as a presumptive test but also as a confirmatory test for the presence of coliforms, in the routine testing of water (7). Lauryl Sulphate Broth is also recommended by the ISO Committee for the detection of coliforms (8).

Lauryl Sulphate Broth is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms. Aerobic spore-bearers are completely inhibited in this medium. Tryptose provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. Sodium lauryl sulphate inhibits organisms other than coliforms. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate these tubes at 37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovacs reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of Escherichia coli. If no fermentation occurs in the tube incubated at 37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms. Broth becomes cloudy if stored at 2-8°C, but it gets cleared at room temperature. Refer appropriate references for standard procedures (1,6,8).

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,8).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

Reaction of 3.56% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 30 ± 1°C for 24 ± 2 to 48 ± 2 hours.

Selectivity : Cultural characteristics observed after an incubation at 30 ± 1°C for 24 ± 2 to 48 ± 2 hours.

Organism	Inoculum (CFU)	Growth	Characteristic reaction	Indole production $ at 44°C
Productivity				
Escherichia coli ATCC 25922 (00013*)	50-100	good growth with gas in Durhams tube	Gas production and turbidity	positive reaction, red ring at the interface of the medium
Escherichia coli ATCC 8739 (00012*)	50-100	good growth with gas in Durhams tube	Gas production and turbidity	positive reaction, red ring at the interface of the medium
Citrobacter freundii ATCC 43864 (00006*)	50-100	good growth with gas in Durhams tube	Gas production and turbidity	negative reaction, no colour development / cloudy ring
Selectivity				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited		

Key : * Corresponding WDCM numbers $ on Addition of Kovacs reagent (R008)

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

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8. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05/2024

Pseudomonas Agar Base

M085

Intended use :

For selective isolation of *Pseudomonas* species.

Composition**

Ingredients	g / L
Tryptone	10.000
Gelatin peptone	16.000
Potassium sulphate	10.000
Magnesium chloride, anhydrous	1.400
Agar	11.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.2 grams in 500 ml purified/distilled water containing 5 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add sterile rehydrated contents of either CetriNix Supplement (FD029) or CFC Supplement (FD036) as desired. Mix well and pour into sterile Petri plates. *Note : Do not keep the molten agar for longer than 4 hours.*

Principle And Interpretation

Pseudomonas Agar Base is a modification of Kings A medium (1) which contains magnesium chloride and potassium sulphate to enhance pigment production. Goto and Enomoto (2) formulated CetriNix supplement for the selective isolation of *Pseudomonas aeruginosa* from clinical specimens. Lowbury and Collins (3) studied cetrimide as a selective agent. CetriNix supplement suppresses *Klebsiella, Proteus* and *Providencia* species.

Tryptone and gelatin peptone supplies nitrogenous and carbonaceous compounds, long chain amino acids, and other essential growth nutrients.

C-F-C Supplement was formulated by Mead and Adams (4) making the medium specific for isolation of *Pseudomonas* from chilled foods and processing plants, environmental samples and water. This medium is recommended for enumeration of *Pseudomonas* species from meat and meat products. It can also be used for clinical samples.

Examine inoculated plates after 24 hours and 48 hours using both white and UV light. The presence of blue-green or brown pigmentation may be considered as presumptive evidence of *Pseudomonas aeruginosa. Alteromonas* species may form brown or pink colonies on the medium.

Type of specimen

Clinical samples - pus, urine, body fluids, Food samples; Water samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Further biochemical and serological tests must be performed for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.1% Agar gel.

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.84% w/v aqueous solution containing 1% v/v glycerol at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation for 40-48 hours. Recovery rate is considered as 100% for growth on Soyabean Casein Digest Agar

Organisms	Inoculum (CFU)	Growth (at 34-38°C with FD029)	Recovery 34-38°C with FD029)	Growth (at 24-26°C with FD036)	Recovery (at 24-26°C with FD036)	Colour/ Fluorescence under uv
Proteus vulgaris ATCC 13315	>=10^4	inhibited	0%	-	-	-
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=50%	-	-	blue-green /positive
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	good-luxuriant	>=50%	-	-	blue-green /positive
Pseudomonas aeruginosa ATCC 10145 (00024*)	50-100	good-luxuriant	>=50%	-	-	blue-green /positive
Pseudomonas cepacia ATCC 10661	50-100	-	-	good-luxuriant	>=50%	
Pseudomonas fluorescens ATCC 13525 (00115*	50-100	-	-	good-luxuriant	>=50%	
Pseudomonas fragi ATCC 4973 (00116*	50-100	-	-	good-luxuriant	>=50%	
Enterococcus faecalis ATCC 29212 (00087*	>=10^4	inhibited	0%	-	-	-
Enterococcus faecalis ATCC 19433 (00009*	>=10^4	inhibited	0%	-	-	-
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%	inhibited	0%	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

References

1.King E.O., Ward M.K. and Raney D.E., 1954, J.Lab and Clin. Med., 44:301.

2.Goto S. and Entomoto S., 1970, Jap. J. Microbiol., 14:65.

3.Lowbury E.J. and Collins A.G., 1955, Clin. Path., 8:47.

4.Mead G.C. and Adams B.W., 1977, Br. Poult. Sci., 18:661.

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6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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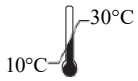
Revision : 08/2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD *In vitro* diagnostic medical device



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Plate Count Agar (Standard Methods Agar) M091

Intended use

Recommended for the determination of plate counts of microorganisms in food, water and wastewater.

Composition**

Ingredients	g / L
Tryptone	5.000
Yeast extract	2.500
Dextrose (Glucose)	1.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Plate Count Agar is formulated as described by Buchbinder et al (1) which is recommended by APHA (2,3,4) and FDA (5). Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids, and other essential nutrients. Yeast extract supplies Vitamin B complex. APHA recommends the use of pour plate technique. The samples are diluted and appropriate dilutions are added in Petri plates. Sterile molten agar is added to these plates and plates are rotated gently to ensure uniform mixing of the sample with agar. The poured plate count method is preferred to the surface inoculation method, since it gives higher results. Plate Count Agar is also suitable for enumerating bacterial count of sterile rooms.

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow granular media.

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.35% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
** *Bacillus spizizenii* ATCC 6633 (00003*)	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Lactobacillus rhamnosus ATCC 9595	50-100	luxuriant	>=70%
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%

Key : *Corresponding WDCM numbers. **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Buchbinder L., Baris Y., Aldd E., Reynolds E., Dilon E., Pessin V., Pincas L. and Strauss A., 1951, Publ. Hlth. Rep., 66:327.
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3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
5. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
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Revision : 05/2024

Urea Agar Base, Christensen

M112I

Intended Use:

Recommended for the detection of urease production, particularly by members of the genus *Proteus*. The composition and performance criteria are in accordance with ISO 6579-1 :2017.

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Potassium dihydrogen phosphate	2.000
Phenol red	0.012
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.01 grams in 950 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 45-50°C and aseptically add 50 ml of sterile 40% Urea Solution (FD048) and mix well. Dispense into sterile tubes and allow to set in a slanting position. Do not overheat or reheat the medium as urea decomposes very easily.

Principle And Interpretation

Urea Agar was described by Christensen (3,8) which detected urease activity by all rapidly urease-positive *Proteus* organisms and also by other members of *Enterobacteriaceae* (3) that exhibited a delayed urease reaction (9). This is accomplished by

a) adding glucose to the medium

b) decreasing the peptone concentration, and

c) decreasing the buffering system, as a less buffered medium detects even smaller amount of alkali (4).

ISO Committee has recommended Urea Agar Base, Christensen (M112I), with one phosphate, instead of two phosphates for detection of rapid urease activity (5).

Heavy inoculum of growth is inoculated on the surface of the slants. On incubation urea is utilized to form ammonia, which makes the medium alkaline, showing a pink-red colour by the change in the phenol red indicator. Prolonged incubation may cause alkaline reaction in the medium. Check using medium without urea as the negative control.

Peptone is the source of nitrogen and carbon, long chain amino acids, vitamins and other essential nutrients. Dextrose is the energy source. Sodium chloride maintains the osmotic equilibrium of the medium whereas phosphates serve to buffer the medium. Urea is hydrolyzed to liberate ammonia. Phenol red indicator detects the alkalinity generated by visible colour change from orange to pink.

Type of specimen

Pure isolate from clinical, food and water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines(6,7).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines(1,10,11).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(2).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Prolonged incubation may cause alkaline reaction in the medium.
2.Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (8).
3. The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids liberation results in false positive reaction.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to light pink homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellowish orange coloured clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.4% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed on addition of 40% Urea Solution (FD048) after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Urease
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	negative reaction, no change
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	negative reaction, no change
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	positive reaction, cerise colour
Proteus mirabilis ATCC 25933	50-100	luxuriant	positive reaction, cerise colour
Proteus vulgaris ATCC 13315	50-100	luxuriant	positive reaction, cerise colour
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction, no change

Key : *Corresponding WDCM numbers.
Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
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5. International Organization for Standardization (ISO), ISO 6579-1:2017
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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8. MacFaddin J. F, 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williamsand Wilkins, Baltimore, Md.
9. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.Md.
10. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
11. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, 23 Vadhani Industrial Estate, LBS Marg,Mumbai-86,MS,India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

Brilliant Green Bile Broth

M121I

Intended Use:

Recommended for isolation and cultivation of coliform organisms from cream, yogurt and raw milk. The composition and performance criteria of this medium are as per the specifications laid down in ISO 4831:2006, ISO 11133:2014 & Amd.2 :2020 (E).

Composition**

ISO Specifications : BGBLB

Ingredients	g / L
Enzymatic digest of casein	10.000
Lactose	10.000
Dehydrated Ox bile	20.000
Brilliant green	0.0133
Final pH (at 25°C)	7.2±0.2

Brilliant Green Bile Broth **M121I**

Ingredients	g / L
Tryptone$	10.000
Lactose monohydrate	10.000
Dehydrated bile	20.000
Brilliant green	0.0133
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters
$ Equivalent to Enzymatic digest of casein

Directions

Suspend 39.51 gram (the equivalent weight of dehydrated medium per liter) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense the medium in quantities of 10ml in test tubes of approximately 16mm x 160mm containing Durham tubes. Sterilize in an autoclave set at 121°C for 15 minutes. Cool to 45-50°C. *Note: The Durham tube shall not contain air bubbles after sterilization.*

Principle And Interpretation

Brilliant Green Bile Broth is formulated as per ISO for confirmation of coliform bacteria (1,2) present in food samples or environmental samples in the area of food handling or food sampling.

Brilliant green and dehydrated bile present in the medium inhibit gram-positive bacteria including lactose fermenting *Clostridia* (3). Production of gas from lactose fermentation is detected by incorporating inverted Durham's tube, indicates a positive evidence of faecal coliforms since nonfaecal coliforms growing in this medium do not produce gas.

During examination of samples, growth from presumptive positive tubes showing gas in Lauryl Tryptose Broth (M080) is inoculated in Brilliant Green Bile Broth wherein gas formation within 48 ± 2 hours confirms the presumptive test (1). Gram-positive spore-formers may produce gas if the bile or brilliant green inhibition is weakened by food material.

Type of specimen

Food samples

Specimen Collection and Handling:

ISO 4831:2006 (1,2)

Depending on the limit of detection that is required, x ml of the test sample if liquid, or x ml of the initial suspension in the case of other products, is transferred to a tube containing 10 ml of double-strength selective enrichment medium. Incubate at 30°C or 37°C (as agreed) for 24 h ± 2 h, continue incubation for another 24 h ± 2 h for gas formation. Gas formation is considered as positive.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further biochemical & serological identification is necessary for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate.

Reaction

Reaction of 3.95% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 30±1°C for 24±2h to 48±2h.

Selectivity : Cultural characteristics observed after an incubation at 30±1°C for 24±2h to 48±2h.

Organism	Inoculum (CFU)	Growth	Gas
Productivity			
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant	positive reaction
Citrobacter freundii ATCC 43864 (00006*)	50-100	good-luxuriant	positive reaction
Selectivity			
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	negative reaction
Enterococcus faecalis ATCC 19433 (00009*)	50-100	none-poor	negative reaction

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. International Standard, ISO 4831:2006 (E). Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique.

2. Microbiology of food, animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014(E) /Amd.: 2020 .

3. McCrady and Langerin, 1932, J. Dairy Science, 15:321.

4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision:04/2024

EC Broth

M127I

Intended Use:

Recommended for selective enumeration of presumptive *Escherichia coli* by MPN technique. The composition and performance criteria of this medium are as per the specifications laid down in ISO/DIS 7251:2005 Amd.1:2023 (E) and ISO 11133:2014 / Amd. 2 : 2020 (E) .

Composition**

ISO Specification - EC Broth (Selective Medium)		**M127I - EC Broth**	
Ingredients	**g / L**	**Ingredients**	**g / L**
Enzymatic digest of casein	20.000	Tryptone #	20.000
Lactose	5.000	Lactose	5.000
Bile salts No. 3	1.500	Bile salts mixture ##	1.500
Potassium monohydrogen phosphate (K2HPO4)	4.000	Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate (KH2PO4)	1.500	Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000	Sodium chloride	5.000
Final pH (at 25°C)	6.8±0.2	Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Key : # - Equivalent to Enzymatic digest of casein, ## - Equivalent to Bile salts No. 3

Directions

Suspend 37.0 gram in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Adjust the concentration of medium in accordance with sample size.

Principle And Interpretation

EC Medium is used for detection of coliforms during bacteriological examination of water, milk and foods. It was originally described by Hajna & Perry (1). This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests (2). It is also used in MPN methods (3) and is often used for confirmation of coliforms. The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or non-fecal) when used as a confirmatory test (4). It should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of fecal coliforms is required. The medium is as per specifications laid down in ISO (5,6)

Tryptose provides essential growth nutrients. Lactose is the fermentable sugar. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal Streptococci. Potassium phosphates control the pH during fermentation of lactose. Gas production in a fermentation tube within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish) or 45.5°C for foods

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows. Transfer a loopful of culture from all the tubes of Lauryl Sulphate Broth (M080) showing gas formation within 24 hours and from all the tubes showing xbacterial growth within 48 hours to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive. Calculate the density of the faecal coliform organisms by using MPN tables. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (7).

Gas formation at 44.5°C or 45.5°C (and 37°C) Gas formation at 37°C	*Escherichia coli*, possibly also other coliforms. Coliform bacteria without *Escherichia coli*

Type of specimen

Food samples - Food and animal feeding stuffs

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (6).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured, clear solution without any precipitate

Reaction

Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 44 ± 1°C for 24 ± 2 to 48 ± 2 hours.

Selectivity : Cultural characteristics observed after an incubation at 44 ± 1°C for 24 ± 2 to 48 ± 2 hours

Organism	Inoculum (CFU)	Growth	Gas production
Productivity			
Escherichia coli ATCC 25922 (00013*)	50-100	good	positive reaction
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	positive reaction
Selectivity			
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Hajna A. A. and Perry C. A., 1943, Am. J. Public Health, 33:550.
2. Fishbein M. and Surkiewicz B. F., 1964, Appl. Microbiol., 12:127.
3. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

4. Marshall, (Ed.), 1993, Standard Methods for the Examination of Dairy Products, 16th Ed., American Public Health Association, Washington, D.C.
5. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique. ISO/DIS 7251:2005 & Amd.1:2023(E)
6. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance culture media, ISO 11133:2014 /Amd. 2 : 2020 (E) .
7. Ray B., 1986, J. Food Prot., 49:651.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/2024

Cooked M Medium (R.C. Medium)

M149

Intended use

Recommended for cultivation of aerobes and anaerobes, especially pathogenic *Clostridia* from clinical, food and water samples. This can also be used as a maintenance medium for stock cultures.

Composition**

Ingredients	g / L
HMH peptone B #	98.000
Proteose peptone	20.000
Dextrose(Glucose)	2.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef heart, solids

Directions

Suspend 12.5 grams in 100 ml purified/distilled water (or suspend 1.25 grams in 10 ml distilled water in test tubes). Mix thoroughly and allow to stand for 15 minutes until all the particles are thoroughly wetted. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Clostridium is a large genus of gram-positive spore-bearing anaerobes. They are normally present in soil, some are responsible for human and animal diseases and others are associated with food spoilage. They may be saccharolytic, decomposing sugars to form butyric and acetic acids and alcohols. The HMH peptone in Robertson's Medium is reddened and gas is produced. Other proteolytic species attack the amino acids. HMH peptone B in Robertson's medium is blackened and decomposed by *Clostridium* species, giving the culture a foul odour. The mesophilic spore-forming anaerobes are of primary importance in the spoilage of low acid foods packed in sealed containers, because of their high heat resistance, their ability to grow in the absence of oxygen and a growth range which covers the temperature of normal storage of canned and other processed foods including the refrigerated storage of cured meats. Cooked M-Medium was originally developed by Robertson (1) for the cultivation of certain anaerobes isolated from wounds. The present formulation is a modification, also called as Chopped M-Medium (2), which supports the growth of many spore forming and non-spore forming strict anaerobes. It has the ability to initiate growth of bacteria from very small inocula and to maintain the viability of cultures over long period. Mixed cultures of bacteria survive in Cooked M-Medium without displacing the slower-growing organisms. The products of growth do not rapidly destroy the inoculated organisms and therefore it is an excellent medium for the storage of aerobic and anaerobic organisms. It is used for cultivation and maintenance of *Clostridia* and for determining proteolytic activity of anaerobes (2,3). FDA has recommended this medium for enumeration and identification of *Clostridium perfringens* from foods (4).

Cooked M-Medium contains HMH peptone B, which provide amino acids and other nutrients. It also contains glutathione, a reducing substance that permits the growth of obligate anaerobes. The sulfhydryl groups, which impart reducing effect, are more available in denatured protein and hence cooked meat is added in the medium. The addition of dextrose allows rapid and heavy growth of anaerobic bacteria in a short time and leads to more rapid identification of important anaerobes. Growth in this medium is indicated by turbidity or bubble formation by some organisms. Blackening and disintegration of the meat particles indicate proteolysis. For best results, medium should be used on the day it is prepared, otherwise it should be boiled or steamed for a few minutes and allowed to cool without agitation and then inoculated. Inoculation should be made near the bottom of the tube in the meat particles for anaerobic cultures. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium. For the isolation of *Clostridium* from food, use a stomacher to prepare 10% suspension of the food in Peptone Water (M028) diluent.

Make dilutions and plate, both suspensions and dilutions on Willis and Hobbs Medium Base (M1375), Tryptose Sulphite Cycloserine (T.C.S.) Agar Base (M837). Place a metronidazole disc on the inoculum. Incubate anaerobically at 37°C overnight. To count the clostridia, pour the plates with the dilutions on Perfringens Agar Base (O.P.S.P.) (M579). Incubate duplicate plates aerobically and anaerobically to distinguish between clostridia and other organisms. Add some of the suspension to two tubes of Cooked Medium. Heat one tube for 10 min at 80°C and incubate as above. Growth of clostridia is visualized as turbidity or gas bubbles. This medium can be further tested for presence of *Clostridium* (5).

Type of specimen

Clinical samples - Faeces, wounds, tissue, pus etc. ; Food and dairy samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1.Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Brown coloured granules
Colour and Clarity of prepared medium
Medium amber coloured, clear to slightly opalescent supernatant over insoluble granules.
Reaction
Reaction of 12.5% w/v aqueous suspension at 25°C. pH : 7.2±0.2
pH
7.00-7.40
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth
Clostridium botulinum ATCC 25763	50-100	luxuriant
Clostridium perfringens ATCC 12924	50-100	luxuriant
Clostridium sporogenes ATCC 11437	50-100	luxuriant
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant

Key :(*) - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.Robertson, 1916, J. Pathol. Bacteriol., 20:327.
2.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3.MacFaddin J. F., 1985, Media for Isolation - Cultivation - Identification - Maintenance of Medical bacteria, Vol. I, Williams & Wilkins, Baltimore.

4. U.S. Food and Drug Administration, 1984, Bacteriological Analytical Manual, 6th Ed., AOAC, Arlington, Va. Collins C. H., Lyne P. M., Grange J. M., 1985, 7th Ed., Microbiological Methods.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015)Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14thEd., Washington D.C.
8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
9.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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HiMedia Laboratories Pvt. Limited,
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MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro* diagnostic medical device**

30°C
10°C **Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

1 Identification of the substances/ mixture and of the company/ undertaking

1.1 Product Identifiers

Product Number	M290
Product Name	Soyabean Casein Digest Agar (Tryptone Soya Agar)(Casein Soyabean Digest Agar)
REACH Registration Number	This product is a mixture. Reach registration number is not available for this mixture.

1.2 Relevant identified uses of the substance or mixture and uses advised against

1.2.1 Relevant identified uses: Laboratory Chemicals, Analytical Purpose, Biochemical Analysis
For InVitro Diagnostic Use

1.3 Details of the supplier of the safety data sheet

Produced by	HiMedia Laboratories Private Limited		
Address	C - 40,Road No.21Y,MIDC, Wagle Industrial Area, Thane(W), - 400 604, India		
Tel. No.	+91-22- 6147 1919/6116 9797	Fax No.	: +91-22- 61471920
Mail Id	info@himedialabs.com	Website	: www.himedialabs.com

1.4 Emergency Tel. No.

Emergency Tel. No. Please contact the regional HiMedia representation in your country

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2 Hazards Identification

2.1 Classification of the substance or mixture

CLP Classification-Regulation (EC) No. 1272/2008[EU-GHS/CLP]

Not a hazardous substance or mixture according to Regulation (EC) No.1272/2008.

2.2 Label elements

Labeling according to Regulation (EC) No.1272/2008

The product does not need to be labelled in accordance with EC directives or respective national laws.

2.3 Other Hazards

None

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3 Composition/Information On Ingredients

3.2 Mixture

The components of this mixture need not be disclosed as per the regulations. All ingredients in this mixture are nonhazardous.

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4 First Aid Measures

4.1 Description of first aid measures

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Rinse immediately with plenty of water for at least 15 minutes. Consult a physician.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

No data available.

4.3 Indication of immediate medical attention and special treatment needed

No data available

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5 Fire Fighting Measures

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Unsuitable extinguishing media

No data available.

5.2 Special hazards arising from the substance or mixture

Carbon oxides, Sodium oxides, Hydrogen chloride gas

5.3 Precautions for fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary

5.4 Further information

No data available

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6 Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures

Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.

6.2 Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Soak up with inert adsorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections

For disposal see Section 13.

---

7 Handling and Storage

7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Normal measures for preventive fire protection.

7.2 Conditions for safe storage, including any incompatibilities

Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Recommended Storage Temperature : On receipt store between 10-30°C

7.3 Specific end uses

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated.

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8 Exposure Controls/Personal Protection

8.1 Control parameters

Components with workplace control parameters

8.2 Exposure controls

Appropriate engineering controls

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the products.

Personal protective equipment

Hygiene measure

Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with the product.

Eye/face protection

Tightly fitting saftey goggles; Faceshield (8-inch minimum). Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands. The selected protective gloves have to satisfy the specifications of EU Directive 2016/425/EEC and the standard EN ISO 374-1/2016 derived from it.

Body protection

Complete suit protecting against chemicals. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Environment exposure controls

Do not empty into drains.

---

9 Physical and chemical properties

9.1 Information on basic physical and chemical properties

Appearance	Cream to yellow coloured homogeneous free flowing powder
Odour	No data available
Odour Threshold	No data available
pH	7.10 - 7.50
Melting/freezing point	No data available

Initial boiling point and boiling range	No data available
Flash point	No data available
Flammability (Solid, gas)	No data available
Vapour pressure	No data available
Relative density	No data available
Water Solubility	No data available
Partition coefficient: n-octanol/water	No data available
Autoignition Temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidizing properties	No data available
Vapour density	No data available
Thermal decomposition	No data available

9.2 Other safety information

No data available

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10 Stability and Reactivity

10.1 Reactivity

No data available

10.2 Chemical stability

No data available

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

No data available

10.5 Incompatible materials

No data available

10.6 Hazardous decomposition products

Refer Section 5.2

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11 Toxicological Information

11.1 Information on toxicological effects

Acute toxicity

No data available

Skin corrosion/irritation

No data available

Serious eye damage/eye irritation

No data available

Respiratory or skin sensitisation

No data available

Germ cell mutagenicity

No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
No data available
Specific target organ toxicity- single exposure
No data available

Aspiration hazard
No data available
Potential Health Effects
Inhalation
REFER SECTION 2
Skin
REFER SECTION 2
Eyes
REFER SECTION 2
Ingestion
REFER SECTION 2
Additional Information
RTECS **:** No data available

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12 Ecological Information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 PBT and vPvB assessment
This substance or mixture contains no components considered to be persistent,bioaccumulating nor toxic (PBT) at levels of 0.1% or higher.

12.6 Other adverse effects
No data available

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13 Disposal Considerations

13.1 Waste treatments methods
Product
Offer surplus and non- recyclable solutions to a licenced company. Contact a licenced professional waste disposal service to dispose off this material.

13.2 Contaminated packaging
Dispose of as unused product.

---

14 Transport Information

14.1 UN-No

ADNR : ADR : IATA_C : IATA_P : IMDG : RID :

14.2 UN proper shipping name

ADNR : Not dangerous goods
ADR : Not dangerous goods
IATA_C : Not dangerous goods
IATA_P : Not dangerous goods
IMDG : Not dangerous goods
RID : Not dangerous goods

14.3 Transport hazard class(es)

ADNR : - ADR : - IATA_C : - IATA_P : - IMDG : - RID : -

14.4 Packaging group

ADNR : ADR : IATA_C : IATA_P : IMDG : RID :

14.5 Environmental hazards

ADNR : No ADR : No IMDG : Marine Pollutant No IATA_C : No IATA_P : No RID : No

14.6 Special precautions for use

No data available

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15 Regulatory Information

This safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006

15.1 Safety health and environment regulations/legislation specific for the substance or mixture

No data available

15.2 Chemical Safety Assessment

No data available

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16 Other information

Further Information

The information given in this safety data sheet is believed to be correct yet does not claim to be all inclusive. This document is intended only as a guide for appropriate precautionary handling of the material by properly trained individuals, information here being commensurate with the present state of our knowledge regarding the manner and conditions of use, handling, storage or disposal. The information provided herein shall not be considered as guarantee of the properties of the product. HiMedia Laboratories, shall not be held liable for any damage resulting from improper handling or contact with the above product. Unless explicitly stated on the product or in any of the documentation accompanying the product, it is intended for research or testing purpose only and is not to be used for any other purpose.

Soyabean Casein Digest Agar (Tryptone Soya Agar) (Casein Soyabean Digest Agar)

M290

Intended use

For cultivation of a wide variety of microorganisms from clinical and non-clinical samples and for sterility testing in pharmaceutical procedures.

Composition**

Ingredients	g/ L
Tryptone #	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Pancreatic digest of casein

Directions

Suspend 40.00 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, aseptically add 5% v/v defibrinated blood in previously cooled medium to 45-50°C for cultivation. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Soyabean Casein Digest Agar is a widely used medium, which supports the growth of wide variety of organisms even that of fastidious ones such as *Neisseria, Listeria*, and *Brucella* etc. The medium with addition of blood provides perfectly defined haemolysis zones, while preventing the lysis of erythrocytes due to its sodium chloride content. It has been frequently used in the health industry to produce antigens, toxins etc. It's simple and inhibitor-free composition makes it suitable for the detection of antimicrobial agents in the food and other products. Tryptone Soya Agar is recommended by various pharmacopoeias as sterility testing medium (1,2). Tryptone Soya Agar conforms as per USP (1) and is used in microbial limit test and antimicrobial preservative - effective test. Gunn et al (3) used this medium for the growth of fastidious organisms and study of haemolytic reaction after addition of 5%v/v blood. The combination of tryptone and soya peptone makes this media nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Sodium chloride maintains the osmotic balance. Soyabean Casein Digest Agar does not contains X and V growth factors. It can be conveniently used in determining the requirements of these growth factors by isolates of *Haemophilus* by the addition of X-factor (DD020), V-factor (DD021), and X+V factor discs (DD022) factor to inoculated TSA plates (4).

Type of specimen

Pharmaceutical samples, Clinical samples- urine, faeces, abscess etc.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For Pharmaceutical samples follow appropriate techniques for sample collection, handling and processing as per pharmacopoeias (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Further biochemical and serological tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of 5-7%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates

Reaction

pH of 4.0% w/v aqueous solution at 25°C .

pH

7.10-7.50

Cultural response

Productivity : Cultural characteristics was observed after an incubation for Bacterial at 30-35°C 18-24 hours and for Fungal at 30-35°C <=5days.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Observed Lot value (CFU) w/blood	Recovery w/ blood	Haemolysis
Productivity						
****Bacillus spizizenii* ATCC 6633 (00003)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	35 -100	>=70 %	35 -100	>=70%	beta
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	beta
Escherichia coli ATCC 25922 (00013)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli ATCC 8739 (00012)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli ATCC 11775 (00090)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli NCTC 13167 (00179)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
^ *Pseudomonas paraeruginosa* ATCC 9027 (00026)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Pseudomonas aeruginosa ATCC 10145 (00024)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

Salmonella Abony NCTC 6017 (00029)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
$ Kokuria rhizophila ATCC 9341	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Streptococcus pneumoniae ATCC 6305	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Salmonella Typhimurium ATCC 14028 (00031)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Enterococcus faecalis ATCC 29212 (00087)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Candida albicans ATCC 10231 (00054)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Candida albicans ATCC 2091 (00055)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
# Aspergillus brasiliensis ATCC 16404 (00053)*	50 -100	25 -70	50-70%			-
Clostridium sporogenes ATCC 19404 (00008)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none

Key : (*)- Corresponding WDCM numbers, (**) Formerly known as *Bacillus subtilis* subsp. *spizizenii* , (^) Formerly known as *Pseudomonas aeruginosa*, ($) Formerly known as *Micrococcus luteus* , (*) Formerly known as *Aspergillus niger*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.

2.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India

3.Gunn B. A., Ohashi D K., Gaydos C. A., Holt E. S., 1977, J. Clin. Microbiol., 5(6) : 650.

4.Forbes B. A., Sahm A. S. and Weissfeld D. F., 1998, Bailey and Scotts Diagnostic Microbiology, 10th Ed., Mosby Inc. St. Louis, Mo

5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/2024

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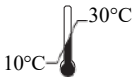

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Glucose OF Medium

M395I

Intended Use

Recommended for the determination of oxidative and fermentative metabolism of carbohydrates by gram-negative bacteria. The composition and performance criteria of this medium are as per the specifications laid down in ISO 21528-2:2017.

Composition**

Ingredients	Gms / Litre
Tryptone #	2.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	0.300
Glucose (Dextrose)	10.000
Bromo thymol blue	0.080
Agar	3.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Enzymatic digest of casein

Directions

Suspend 20.38 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes in duplicate for aerobic and anaerobic fermentation. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Hugh and Leifson developed OF Medium to study oxidative and fermentative metabolism of carbohydrates by gram-negative bacteria. This criterion is used during taxonomic studies of *Enterobacteriaceae* (1). Glucose is the most important carbohydrate for use in OF Basal Medium. Glucose OF Medium is recommended by ISO Committee (5).

However, certain organisms may metabolize other carbohydrates even if they are unable to utilize dextrose. Degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its colour to yellow. Oxidative utilization takes place when the medium is exposed to air while fermentative utilization occurs under exclusion of air. When a gram-negative organism is inoculated in this medium containing a carbohydrate in duplicate, of which one tube is covered with mineral oil to exclude oxygen and the second tube is uncovered; reactions of differential value can be observed. Fermentative organisms will produce an acid reaction in both the covered and uncovered medium. Oxidative organisms will produce an acid reaction in the uncovered medium and give slight growth without change in the covered medium. Organisms which are not classified either as oxidative or fermentative show no change in the covered medium and an alkaline reaction in the uncovered medium (4). The acidic reaction of oxidative organisms is more apparent at the surface of the medium that gradually spreads throughout the medium. If the oxidation reaction is weak or slow, an initial alkaline reaction at the surface of the uncovered tube may persist for several days and eventually convert to an acid reaction.

Tryptone in the medium provides the necessary carbon and nitrogen, vitamins etc required for bacterial growth. Phosphate buffers the medium and the low agar concentration determines motility and dispersion of the acid produced on the surface Bromothymol blue acts as the pH indicator. The low concentration of agar permits the determination of motility and aids in the even distribution of any acid produced at the surface of the medium. Motility is observed as diffused zone of flaring out from the line of inoculation. Non-motile organisms grow along the line of inoculation.

Type of specimen

Food samples : meat and meat products

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to variable nutritional requirements, some strains show poor growth on this medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of Prepared medium

Green coloured clear to slightly opalescent gel forms in tubes.

Reaction

Reaction of 2.04% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Aerobic	Anaerobic (overlayed with mineral oil)
Acinetobacter baumannii ATCC 19606	50-100	acidic reaction, yellowing of the medium	alkaline reaction,green colour of the medium
Alcaligenes faecalis ATCC 8750	50-100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium
Escherichia coli ATCC 25922 (00013*)	50-100	acidic reaction, yellowing of the medium with gas formation	acidic reaction, yellowing of the medium with gas formation
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	acidic reaction, yellowing of the medium with gas formation	acidic reaction, yellowing of the medium with gas formation
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	acidic reaction, yellowing of the medium	alkaline reaction, green colour of the medium
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	acidic reaction, yellowing of the medium with gas formation	acidic reaction, yellowing of the medium with gas formation

Shigella flexneri ATCC 12022 (00126*)	50-100	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Vibrio cholerae ATCC 15748	50-100	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

Key :- * *Corresponding WDCM Numbers*

\# Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Hugh R. and Leifson E., 1953, J. Bacteriol. 66:24.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. MacFaddin J. F., 1985, Media for the Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore

5. Microbiology of food chain-Horizontal method for detection and enumeration of *Enterobacteriaceae* International Organization for Standardization (ISO), 21528-2 .

6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 01 / 2018

Tryptone Water

M463I

Tryptone Water is used for the detection of indole production by coliforms.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Sodium chloride	5.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Dissolve 25 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Tryptone Water is recommended by APHA (1) and ISO Committee (2) for detection of indole production by coliforms, which is a key feature in differentiation of bacteria. A slight modification of Tryptone Water (M463I) is recommended by ISO committee (3) for the same purpose. This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium (4).

Casein enzymic hydrolysate is a good substrate for indole production because of its high tryptophan content. Certain organisms breakdown the amino acid tryptophan with the help of enzymes that mediate the production of indole by hydrolytic activity (5). The indole produced can be detected by either Kovacs or Ehrlichs reagent (6). Indole combines with the aldehyde present in the above reagent to give red colour in the alcoholic layer. The alcohol layer extracts and concentrates the red colour complex.

Tryptone Water is used in conjunction with Brilliant Green Bile Broth 2 % (M121) to determine the most probable number (MPN) of E. coli in food sample. Growth and gas production in M121 and indole production in Tryptone Water following incubation of both media at 44 ± 1°C is used as the basis for the presumptive E. coli test. For determination of indole, inoculate the medium with inoculum of an 18-24 hours pure culture. Incubate the tubes at 35 ± 2°C for 18-24 hours. Add 0.5 ml of indole reagent (R008) directly to the tube and agitate. Allow the tubes to stand for 5-10 minutes. Formation of red ring at the top of the tube indicates indole production.

Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. For complete identification of the organisms, further biochemical confirmation is necessary.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured clear solution without any precipitate

Reaction
Reaction of 2.5% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24 hours. Add 0.2-0.3ml kovac's indole reagent (R008) to each tube after incubation.

Cultural Response

Organism	Inoculum (CFU)	Growth	Indole reaction

Cultural Response

Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction, red ring at the interface of the medium
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	negative reaction, no colour development / cloudy ring

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington, D.C.
2. International Organization for Standardization (ISO), 1993, Draft ISO/DIS 9308-1.
3. International Organization for Standardization (ISO), 1990, Draft ISO/DIS 7251:1993.
4. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
5. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.
6. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

Revision : 02 / 2015

Bile Esculin Azide Agar

M493

Intended Use:

For selective isolation and presumptive identification of faecal Streptococci.

Composition**

Ingredients	g/ L
Tryptone	17.000
HM peptone B #	5.000
Proteose peptone	3.000
Bile ##	10.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.1±0.2

Equivalent to Beef extract ## - Equivalent to Oxgall

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.65 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: *Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.*

Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (5) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (6) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci.

Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (7) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (8).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar as per Isenberg (9). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

Tryptone, proteose peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and sodium azide inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (10). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3). Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation.

All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (10). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

Type of specimen

Clinical- Faeces, Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.
Reaction
Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good	40-50%	negative reaction
Proteus mirabilis ATCC 25933	50-100	good	40-50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
2. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
3. Facklam R., 1972, Appl. Microbiol., 23:1131.
4. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Swan, 1954, J. Clin. Pathol., 7:160.
6. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.
7. Meyer and Schonfeld, 1926, Zentralbl. Bakeriol, Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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Revision :06/2024

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HIMEDIA

Technical Data

Violet Red Bile Glucose Agar w/o Lactose

M581

Intended Use:

Recommended for enumeration of *Enterobacteriaceae* in raw food and clinical samples. The composition and performance criteria are in accordance with ISO 21528-1&2:2017.

Composition**

ISO Specifications-Violet Red Bile Glucose Agar w/o Lactose

Ingredients	g / L
Enzymatic digest of animal tissues	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts No.3	1.500
Glucose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	9.000-18.000
Final pH (at 25°C)	7.4±0.2

Violet Red Bile Glucose Agar w/o Lactose **M581**

Ingredients	g / L
Peptone $	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Glucose (Dextrose)	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

$ -Equivalent to Enzymatic digest of animal tissues

Directions

Suspend 38.53 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Violet Red Bile Agar, a modification of MacConkey original formulation (1) is used for the enumeration of coli-aerogenes bacterial group. Violet Red Bile Glucose Agar w/o Lactose, a modification of VRBA (M049), was designed for the enumeration of *Enterobacteriaceae* (2). It employs the selective inhibitory components crystals violet and bile salts and the indicator system glucose and neutral red. Sought bacteria will dissimilate glucose and produce purple zones around the colonies (3). ISO committee has also recommended this medium (4). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/or at elevated temperature, i.e. equal to or above 42°C (5-7).

Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (8).

Type of specimen

Clinical samples - faeces ; Food and dairy samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (11-13).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines & local standards (14).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

3. Over incubation may result in reverting of reaction.

4. Further biochemical tests must be carried out on colonies of pure culture for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Gelling

Firm,comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 3.85% w/v of aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Productivity : Cultural characteristics was observed after an incubation at 35±1°C for 24±2 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Selectivity: Cultural characteristics was observed after an incubation at 35±1°C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Productivity				
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=50 %	pink to red colonies with or without precipitation zone
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=50 %	pink to red colonies with or without precipitation zone
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	pink to red colonies with or without precipitation zone
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	>=50 %	pink to red colonies with or without precipitation zone
Selectivity				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited		

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

1. MacConkey A., 1905, J. Hyg., 5, 333-379.

2. Mossel D. A. A., Eclderink I., Koopmans M. and Van Rossem F., 1978, Lab. practice, 27 No. 12: 1049.
3. Corry J. E. L., Curtis G. D. W. and Baird R. M., (Ed.), 1995, Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, Elsevier, Amsterdam.
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HIMEDIA

Technical Data

Giolitti Cantoni Broth Base

M584I

Intended Use:

Recommended for selective enrichment of *Staphylococcus aureus* from suspected food stuffs. The composition and performance criteria are in accordance with ISO 6888-3:2003 and ISO 11133:2014 , Amd.2 : 2020 (E).

Composition**

ISO Specification Giolitti Cantoni Broth

Ingredients	g / L
Enzymatic digest of casein	10.000
HM extract #	5.000
Yeast extract	5.000
Mannitol	20.000
Sodium chloride	5.000
Lithium chloride	5.000
Glycine	1.200
Sodium pyruvate	3.000
Polysorbate 80 (Tween 80)	1.000
Final pH (after sterilization)	6.9±0.2

M584I - Giolitti Cantoni Broth Base

Ingredients	g / L
Tryptone$	10.000
HM extract #	5.000
Yeast extract	5.000
Mannitol	20.000
Sodium chloride	5.000
Lithium chloride	5.000
Glycine	1.200
Sodium pyruvate	3.000
Polysorbate 80 (Tween 80)	1.000
Final pH (after sterilization)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

$ Equivalent to Enzymatic digest of casein # Equivalent to Meat extract

Supplements to be added	g / L	Supplements to be added	
Potassium tellurite 0.1 ml of a filter-sterilized 1% aqueous solution of Potassium tellurite per 9 ml tube	0.050	Potassium tellurite (FD052)	0.1 ml / 9ml of tube

Directions

Suspend 55.20 gram in 1000 ml purified / distilled water. Warm gently to dissolve the medium completely. Dispense 19 ml amounts in 20mmx200mm test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool rapidly to room temperature and aseptically add 0.1 ml of 1% Potassium Tellurite Solution (FD052) to each tube. Add 0.03 ml for testing meat and meat products. Mix well before use.

Principle And Interpretation

Giolitti-Cantoni (1) formulated the broth base and Mossel et al (2) recommended it for detection of *Staphylococcus aureus* in dried baby milk and other weaning foods where the organism should be absent in 1 gram of sample. It is also recommended by ISO Committee (3,4) for the examination of meat and meat products.

Mannitol and sodium pyruvate present in the basal medium act as growth stimulants for *Staphylococcus aureus*, aiding in detection of small number of organisms (5). Lithium chloride inhibits gram-negative lactose fermenting bacilli (6). Potassium tellurite and glycine inhibit gram-positive bacilli. Addition of sterile paraffin wax to the inoculated medium inhibits *Micrococci* due to creation of anaerobic conditions. Potassium tellurite concentration must be reduced as per the weight of test sample (0.1 - 0.01 gram).

Inoculate 1 gram of sample or 1 ml of a suitable dilution of a sample into 19 ml of Giolitti-Cantoni Broth tubes in duplicate. Overlay the medium with 5 ml molten sterile paraffin wax and incubate at 37°C for 24-48 hours and examine daily. Blackening of the medium (usually at the bottom) within 48 hours indicates the presence of *Staphylococcus aureus*. The blackened medium, when streaked on Baird Parker Agar (M043I), shows black colonies surrounded by clear zones (7).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The medium should be inoculated as soon as it has been cooled after sterilization, otherwise absorbed oxygen should be expelled by placing the tubes in free-flowing steam for 15 - 20 minutes.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to brownish yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured clear solution without any precipitate.

Reaction

Reaction of 5.52% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Productivity : Cultural characteristics observed with addition of 1% Potassium Tellurite Solution (FD052) as directed after an incubation at 37±1°C for 24 ± 2h to 48 ± 2 hours. Recovery is carried out on Baird Parker Agar (M043I) or RPF Agar Base (M1736I).

Selectivity : Cultural characteristics observed with addition of 1% Potassium Tellurite Solution (FD052) as directed after an incubation at 37±1°C for 48 ± 2 hours. Recovery is carried out on Tryptone Soya Agar.

Organism	Inoculum (CFU)	Growth	Characteristic reaction on Baird Parker Agar (M043I)
Productivity			
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)+ *Escherichia coli* ATCC 25922 (00013*)	50-100 >=10^4	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)+ *Escherichia coli* ATCC 8739 (00012*)	50-100 >=10^4	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)+ *Escherichia coli* ATCC 25922 25922 (00013*)	50-100 >=10^4	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)+ *Escherichia coli* ATCC 8739 (00012*)	50-100 >=10^4	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
Selectivity			
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

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Slanetz and Bartley Medium

M612I

Intended use

Recommended for detection and enumeration of faecal Streptococci from water samples by membrane filtration technique. The composition and performance criteria of this medium are as per the specifications laid down in ISO/ DIS 7899 -2: 2000 (E) and APHA.

Composition**

ISO 7899-2:2000 (E), APHA	
Ingredients	**g / L**
Tryptose	20.000
Yeast extract	5.000
Glucose	2.000
Dipotassium hydrogen phosphate	4.000
Sodium azide	0.400
2,3,5-triphenyl tetrazolium chloride	10.00ml
Agar	8-18
Final pH (at 25°C)	7.2±0.1

Slanetz and Bartley Medium	**M612I**
Ingredients	**g / L**
Tryptose	20.000
Yeast extract	5.000
Dextrose (Glucose)	2.000
Dipotassium hydrogen phosphate	4.000
Sodium azide	0.400
2,3,5-Triphenyl tetrazolium chloride	0.100
Agar	15.000
Final pH (at 25°C)	7.2±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Excessive heating is detrimental. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Slanetz and Bartley Medium was originally devised by Slanetz and Bartley (1) for the detection and enumeration of Enterococci by membrane filtration technique. It can be also used as a direct plating medium (2,3). M612I differs from M612 in the type of buffering system used. This medium composition is as per specifications laid in ISO (4), APHA (5).

Tryptose and yeast extract serves as a source of essential nutrients along with B-complex vitamins and nitrogenous nutrients. The medium is highly selective for Enterococci. Sodium azide has inhibitory effect on gram-negative organisms. Triphenyl Tetrazolium Chloride is reduced to the insoluble formazan inside the bacterial cell forming dark red-coloured colonies. When the medium is incubated at higher temperature (44-45°C), all red or maroon colonies can be considered as presumptive Enterococci (6,7).

The Department of Health (8) has recommended this medium to be used for enumeration of Enterococci in water supplies. Water is filtered through a membrane filter which is then placed on the surface of the Slanetz and Bartley Medium plates and incubated at 35°C for 4 hours and then at 44-45°C for 44-48 hours. Red or maroon colonies are counted as Enterococci. If typical colonies are observed, a confirmation step is necessary, by transfer of the membrane, with all the colonies, onto bile-aesculin-azide agar, preheated at 44 °C. Intestinal enterococci hydrolyse aesculin on this medium in 2 h. The end-product, 6,7-dihydroxycoumarin, combines with iron(III) ions to give a tan-coloured to black compound which diffuses into the medium.The preliminary incubation at 35°C helps for the recovery of stressed organisms. Not all the species reduce TTC, hence pale colonies also should be considered. Food samples are homogenized and so diluted with physiological saline to give 15-150 colonies on each petri plate. Homogenates or dilutions are spread on agar surface and incubated at 35° C for 48 hours. Pink or dark red colonies with a narrow whitish border are counted (9).

Type of specimen

Water samples

Specimen Collection and Handling:

ISO 7899-2:2000:

Preparation of test sample: Prepare tenfold dilutions of water samples

Choice of technique:

a) Pour plate method
b) Spread plate method
c) Membrane filtration method

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical testing is required for identification of species.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.65% w/v aqueous solution at 25°C. pH : 7.2±0.1

pH

7.10 -7.30

Cultural Response

Productivity : Cultural response was observed after an incubation at 36±2°C for 44 ± 4 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

Selectivity : Cultural response was observed after an incubation at 36±2°C for 44 ± 4 hours.

Organism	Inoculum (CFU)	Growth	Recovery #	Colour of colony
Productivity				
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%	red or maroon or pink
Enterococcus faecalis ATCC 19433 (00009*)	50-100	good-luxuriant	>=50%	red or maroon or pink
Enterococcus faecalis WDCM 00176	50-100	good-luxuriant	>=50%	red or maroon or pink
Enterococcus faecium ATCC 6057 (00177*)	50-100	good-luxuriant	>=50%	red or maroon or pink
Enterococcus faecium WDCM 00178	50-100	good-luxuriant	>=50%	red or maroon or pink
Selectivity				
Escherichiacoli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%	

Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^4	inhibited	0%

Key : (*) - Corresponding WDCM numbers, # - Recovery obtained for productivity is >=70% when compared to a previously validated batch of Slanetz and Bartley Medium is used.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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HIMEDIA

Technical Data

Buffered Peptone Water

M614

Intended use

Recommended as a pre-enrichment medium used for increasing the recovery of injured *Salmonella* species from food prior to selective enrichment and isolation and also from samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate	3.500
Potassium hydrogen phosphate	1.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amounts into tubes or flasks or as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. If desired aseptically add rehydrated contents of one vial of CCV Supplement (FD247) to 1000 ml of medium for enrichment of *Escherichia coli* O157:H7.

Principle And Interpretation

Buffered Peptone Water is a pre-enrichment medium designed to help recovery of sub-lethally damaged *Salmonellae* before transfer to a selective medium. This pre-enrichment medium is free from inhibitors and is well buffered and provides conditions for resuscitation of the cells that have been injured by processes of food preservation. It was noted by Edel and Kampelmacher (1) that sub-lethal injury to *Salmonella* may occur due to food preservation techniques involving heat, desiccation, high osmotic pressure, preservatives or pH changes. Buffered Peptone Water during the pre-enrichment period helps in recovery of injured cells that may be sensitive to low pH (2). This is particularly important for vegetable specimens, which have low buffering capacity. This medium can be used for testing dry poultry feed (3). In a survey involving isolation of *Salmonellae* from meat that had been artificially contaminated with sub-lethally injured organisms, pre-enrichment in Buffered Peptone Water at 37°C for 18 hours before selection in Tetrathionate Brilliant Green Bile Broth (M1255) showed superior results compared with direct selection method. Lactose Broth is frequently used as a pre-enrichment medium but it may be detrimental to recovery of *Salmonellae* (4).

The media contain proteose peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance and phosphates buffer the medium. The broth is rich in nutrients and produces high resuscitation rates for sub lethally injured bacteria and supports intense growth. The phosphate buffer system prevents bacterial damage due to changes in the pH of the medium.

Inoculate 10 grams specimen in 50 ml of these media and incubate at 35-37°C for 18 hours. Transfer 10 ml from this medium to 100 ml of Tetrathionate Broth (M032) and incubate at 43°C for 24 - 48 hours and then subculture on selective plating media. Examine the plates for characteristic Salmonella colonies.

Type of specimen

Food and dairy samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further enrichment and isolation must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing media.

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

Reaction of 2.0% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.(Recovery is carried out using XLD Agar, (M031).

Organism	Inoculum (CFU)	Growth	Recovery
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50%
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	>=50%
Escherichia coli 0157:H7 NCTC 12900 (00014*)	50-100	good-luxuriant [Recovery on Tryptone soya Agar(M290)]	>=50%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

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5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

6.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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8.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

9.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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MYP Agar Base

M636F

MYP Agar Base is used for isolation and identification of *Bacillus cereus* in accordance with FDA BAM.

Composition**

Ingredients	Gms / Litre
HM Peptone B #	1.000
Peptone	10.000
Mannitol	10.000
Sodium chloride	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 23.01 grams in 450 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of sterile Polymyxin B Sulphate (FD003) solution to a final concentration of 100 units per ml and 25 ml sterile Egg Yolk Emulsion (FD045F). Mix well and pour into sterile Petri plates.

Principle And Interpretation

MYP Agar Base is used for isolation and identification of *Bacillus cereus* in accordance with FDA BAM(1). *B. cereus* is ubiquitously present in soil, on vegetables, and in many raw and processed foods, meat, cereals, pasteurized fresh milk and powdered milk (2-4) and other processed foods. Under favourable conditions, the organism multiplies and causes gastrointestinal illness (4). It is implicated in two different forms of food poisoning; an emetic illness and a diarrhoeal illness. The emetic illness is mediated by a highly stable toxin that survives high temperature, exposure to trypsin, pepsin and pH extremes. The diarrhoeal illness is mediated by a heat and acid labile enterotoxin. Lecithinase activity is the key reaction in the differential identification of *B.cereus*, the most commonly encountered and important species in clinical laboratories, from the majority of the other *Bacillus* species. If unknown isolate produces lecithinase, *Bacillus cereus* can be presumptively identified by also observing colony morphology, hemolytic reactivity and motility tests.

MYP Agar Base (M636F) is recommended by FDA BAM to isolate and enumerate *B.cereus* from foods (5, 4). This medium differentiates *B.cereus* from other bacteria on the basis of lecithinase activity, mannitol fermentation and resistance to polymyxin (FD003) (5, 6).

Peptone and HM Peptone B provide nitrogen and carbon source, long chain amino acids, vitamins and other essential nutrients. Mannitol acts as the carbon source that upon fermentation yields yellow colour to the colonies. Egg yolk emulsion aids in the differentiation of lecithinase producing colonies, which are surrounded by a zone of white precipitate. Polymixin B Sulphate acts as the inhibitor to restrict the growth of gram negative bacteria. These properties also help in the differentiation of B.cereus from other bacillus species (1).

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium :Red coloured clear to slightly opalescent gel. After Addition of Egg Yolk Emulsion (FD045) : Light orange coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed with added Egg Yolk Emulsion (FD045)and Polymyxin B Sulphate(FD003) after an incubation at 32°C for 18-40 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase activity
Cultural Response					
Bacillus cereus ATCC 10876	50-100	luxuriant	>=50%	red	positive, opaque zone around the colony
Bacillus subtilis ATCC 6633	50-100	luxuriant	>=50%	yellow	negative
Escherichia coli ATCC 25922	50-100	none-poor	<=10%		
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	red	negative
Pseudomonas aeruginosa ATCC 27853	50-100	none-poor	<=10%		
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=50%	yellow	positive, opaque zone around the colony

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
2.Bergdoll, M. S. 1981. Clin. Microbiol. Newsletter, 3: 85-87.
3.Centers for Disease Control: Bacillus cereus- Maine, MMWR, 35: 408-410, 1986.
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5.Downes, F.P. and Ito, K. 2001. Methods For The Microbiological Examination of Foods. APHA, Food 4 ed. Washington, D.C.
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Revision : 03 / 2017

B.C. Motility Test Medium

M906

Intended Use:

Recommended for testing motility of *Bacillus cereus*.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	2.500
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate	2.500
Agar	3.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in 2-3 ml amounts in screw capped tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in an upright position.

Principle And Interpretation

Bacillus cereus is widely distributed in nature and can be isolated from a variety of foods. *B. cereus* causes food poisoning due to the consumption of contaminated rice (4,6), eye infections (1) and a wide range of other clinical conditions like abscess formation, meningitis, septicemia and wound infection. *Bacillus cereus* is a known cause of disease mastitis, especially in ewes and heifers among the veterinarians. BC Motility Test Medium is formulated as per APHA (4) for the cultivation and examination of motility of *B. cereus* strains.

The medium contains tryptone, yeast extract and dextrose that provide nutrients while phosphate helps in maintaining the pH. Agar content of the medium is crucial for determining motility. 0.3% agar renders the medium semisolid in which motile bacteria produce diffused turbidity due to growth, while non-motile bacteria exhibit a line of growth only along the line of inoculation. This medium is inoculated by stabbing down the center with 3 mm loopful of culture and incubated at 18-24 hours at 30°C. Rhizoid strains of *B. cereus var mycoides* produce characteristic fuzzy growth in semisolid media due to expansion of the filamentous growth but they are not motile by means of flagella.

Type of specimen

Isolated microorganism

Specimen Collection and Handling

This medium is inoculated by stabbing down the center with 3 mm loopful of culture and incubated at 18-24 hours at 30°C. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. It is not a confirmatory test hence complete identification should include the morphology, gram reaction, biochemical and serological tests.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium
Yellow coloured, clear to very slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 2.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Motility
Bacillus anthracis ATCC 14578	50-100	good-luxuriant	negative reaction,growth along the stabline
Bacillus cereus ATCC 10876	50-100	good-luxuriant	positive reaction, growth away the stabline
Bacillus cereus var mycoides	50-100	good-luxuriant	negative reaction,growth along the stabline
Bacillus thuringiensis ATCC 10792	50-100	good-luxuriant	positive reaction, growth away from stabline

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Bouza E., Grant S., Jordan C., et al, 1979, Arch.Ophthalmol., 97:498
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Mortimer P.R. and McCann.G, 1974, Lancet, 104:3.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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Revision : 03/2020

Technical Data

Listeria Identification Agar Base (PALCAM)

M1064I

Intended use

Listeria Identification Agar Base (PALCAM) with added supplement is recommended for the detection and enumeration of *Listeria monocytogenes* from food and animal feeds. The composition and performance of this medium are as per the specification laid down in ISO 11290-2.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Yeast extract	3.000
D-Mannitol	10.000
Dextrose, anhydrous	0.500
Esculin	0.800
Ferric ammonium citrate	0.500
Lithium chloride	15.000
Phenol red	0.080
Agar	10.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.44 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Listeria Selective Supplement (FD061). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Listeria Identification Agar also known as Polymyxin Acriflavine Lithium Chloride Ceftazidime Esculin Mannitol (PALCAM) Agar was originally formulated by Van Netten et al (5). ISO Committee has recommended this medium with a slight modification for the detection and enumeration of *Listeria monocytogenes* from food and animal feeds (1).

Peptone special and yeast extract provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. This is highly selective medium due to the presence of lithium chloride, acriflavine hydrochloride, ceftazidime and polymyxin B. This medium employs two indicator systems, esculin-ferric ammonium citrate and mannitol-phenol red. *Listeria monocytogenes* hydrolyzes esculin to esculetin and dextrose. Esculetin reacts with ferric ammonium citrate to form a brown-black complex seen as a black halo around colonies. Dextrose and starch serve as energy source. Contaminants such as Staphylococci ferment mannitol and is indicated by colour change from red to yellow rendering easy differentiation. Incubation in microaerophilic condition inhibits strict aerobes such as *Bacillus* and *Pseudomonas* species.

Depending upon the sample type, selective enrichment is done prior to inoculation onto PALCAM Agar. Generally Listeria Selective Enrichment Medium is used for dairy products while Listeria Selective Enrichment Medium UVM (M890), Fraser Secondary Enrichment Broth (M1083) are used for meat and poultry products. After 24 hours incubation *Listeria* species show grey-greenish or olive coloured, 1.5-2 mm diameter colonies with black halo, usually with black center. After 48 hours grey-green coloured, about 2 mm diameter colonies are observed with black halo and black sunken center.

Type of specimen

Food and dairy samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,4).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical identification of organisms is required for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared Medium

Red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 6.9% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed under microaerophilic condition, with added Listeria Selective Supplement(FD061), after an incubation at 35-37°C for 48 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
*Enterococcus faecalis ATCC 29212 (00087)**	50-100	none - poor	<=10%	grey colonies with a brown-green halo
*Enterococcus faecalis ATCC 19433 (00009)**	50-100	none - poor	<=10%	grey colonies with a brown-green halo
*Listeria monocytogenes ATCC 19111 (00020)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19117	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19118	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
Staphylococcus aureus subsp. *aureus ATCC 25923 (00034)**	50-100	none - poor	<=10%	yellow colonies with yellow halo
Listeria monocytogenes ATCC 19112	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
*Listeria monocytogenes ATCC 13932 (00021)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo

*Listeria monocytogenes ATCC 35152 (00109)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
*Listeria innocua ATCC 33090 (00017)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
*Eschrichia coli ATCC 25922 (00013)**	50-100	none - poor	<=10%	
*Escherichia coli ATCC 8739 (00012)**	50-100	none - poor	<=10%	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. International Organisation for Standardization (ISO), ISO/DIS11290-2 : 2017. Microbiology of food and other animal feeding stuffs- Horizontal method for the detection and enumeration of *L. monocytogenes* and other *Listeria* species.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
5. Van Netten P. et al, 1989, Int. J. Food Microbiol., 8(4):299.

Revision : 03/ 2019

Sabouraud Chloramphenicol Agar

M1067

Intended use

For the selective cultivation of yeasts and moulds from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Tryptone	5.000
Peptone	5.000
Dextrose (Glucose)	40.000
Chloramphenicol	0.050
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.05 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: *Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet.*

Principle And Interpretation

Sabouraud Chloramphenicol Agar is cited as Medium C and recommended for cultivation of yeasts and moulds. This medium was described originally by Sabouraud (1) for the cultivation of fungi, particularly useful for the fungi associated with skin infections. The medium is often used with antibiotics such as Chloramphenicol (2) for the isolation of pathogenic fungi from materials containing large numbers of fungi or bacteria.

Tryptone and peptone provide nitrogenous and carbonaceous compounds, long chain amino acids, and other essential growth nutrients. Dextrose provides an energy source. Chloramphenicol inhibits a wide range of Gram-positive and Gram-negative bacteria which makes the medium selective for fungi (3). The low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (4).

Type of specimen

Clinical samples - skin scrapings, nail scrapings; Food and dairy samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Certain pathogenic fungi may show poor growth on this medium.
2. Presence of chloramphenicol may inhibit certain pathogenic fungi.
3. Overheating of the medium may result in low productivity and softening of gel.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.5% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed after an incubation at 20-25°C for 48-72 hours (Incubate for 7 days for Trichophyton species).

Organism	Inoculum (CFU)	Growth	Recovery
Aspergillus brasiliensis ATCC 16404 (00053*)	50-100	good-luxuriant	
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%
Lactobacillus casei ATCC 334	>=10^4	inhibited	0%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good-luxuriant	>=50%
Trichophyton rubrum ATCC 28191	50-100	good-luxuriant	
Escherichia coli NCTC 9002	>=10^4	inhibited	0%
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store the dehydrated powder and prepared medium on receipt between 15-25°C in a tightly closed container. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061.

2.Ajello L., 1957, J. Chron. Dis., 5:545.

3.Lorian (Ed.),1980, Antibiotics In Laboratory Medicine, Williams and Wilkins, Baltimore.

4.Murray, P. R 2005, In Manual of Clinical Microbiology, 7th ed., ASM, Washington, D.C.

5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

6.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

7.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
8.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
9.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 06/2024

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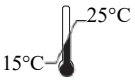
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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Do not use if package is damaged

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Deoxycholate Citrate Agar, Modified (Hynes) M1074

Intended Use:

Recommended selective medium for the isolation of *Salmonella* and *Shigella* species.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM peptone#	5.000
Lactose	10.000
Sodium citrate	8.500
Ferric citrate	1.000
Sodium deoxycholate	5.000
Sodium thiosulphate	5.400
Neutral red	0.020
Agar	12.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 51.92 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Excessive heating is detrimental.

Principle And Interpretation

Deoxycholate Citrate Agar, Modified (Hynes) is a selective medium used for isolation and identification of Salmonellae and Shigallae. Leifson (4) developed Deoxycholate Agar as a differential medium containing pure chemicals, citrates and deoxycholate as inhibitors. Leifsons medium has been modified by many authors by several ways. Deoxycholate Citrate Agar, Modified (Hynes) is a differential medium modified by Hynes (1) for the isolation of Salmonellae and Shigellae. Deoxycholate Citrate Agar, Modified consist of more concentrations of inhibitors and is used in food microbiology (6). Peptone and HM peptone provides carbon, nitrogen, long chain amino acids, vitamins and minerals. Coliform bacteria and gram-positive bacteria are inhibited or greatly suppressed due to sodium deoxycholate, sodium citrate and ferric citrate. Lactose helps in differentiating enteric bacilli, as lactose fermenters produce red colonies while lactose non-fermenters produce colourless colonies. Coliform bacteria, if present form pink colonies on this medium. The degradation of lactose causes acidification of the medium surrounding the relevant colonies causing the pH indicator neutral red to change its colour to red. These colonies usually are also surrounded by a turbid zone of precipitated deoxycholic acid due to acidification of the medium. Sodium deoxycholate combines with neutral red in an acidic environment, causing the dye to go out of the solution with the subsequent precipitation of deoxycholate (4). The reduction of sodium thiosulphate to sulfide is indicated by the formation of black iron sulfide. *Salmonella* and *Shigella* species do not ferment lactose but *Salmonella* may produce H_2S forming colorless colonies with or without black centers.

Citrate and iron (Fe) combination has a strong hydrolyzing effect on agar when the medium is heated, producing a soft and unelastic agar. If autoclaved the agar becomes soft and almost impossible to streak (4). Surface colonies of non-lactose fermenters often absorb a little colour (pinkish) from the medium and organisms may be mistaken for coliforms (4).

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.This medium is general purpose medium and may not support the growth of fastidious organisms.

2. Avoid excessive heating as it is pernicious to the medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Reddish orange coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.19% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	H2S
Bacillus cereus ATCC 10876	>=10^4	inhibited	0%		
Escherichia coli ATCC 25922 (00013*)	50-100	poor-fair	20-30%	red	negative reaction
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50%	colourless	positive reaction,black centered colonies
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant	>=50%	colourless	positive reaction, black centered colonies
Shigella flexneri ATCC 12022 (00126*)	50-100	good-luxuriant	>=50%	colourless	negative reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	>=50%	light pink	negative reaction
Shigella sonnei ATCC 25931	50-100	good-luxuriant	>=50%	pink with bile precipitate	negative reaction
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%		

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Hynes M., 1942, J. Path. Bacteriol., 54, 193-207.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. Leifson, 1935, J. Pathol. Bacteriol., 40:581.

5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

6. Speck M. (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.

Revision : 03/ 2019

Dichloran Glycerol Medium Base

M1129I

Intended Use

Recommended for selective isolation of xerophilic moulds from food samples. The composition & performance criteria are in accordance with ISO 21527-2 and 11133:2014 (E) /Amd. :2020

Composition**

ISO specification - Dichloran Glycerol Medium Base

Ingredients	g / L
Casein enzymatic digest	5.000
D-Glucose ($C_6H_{12}O_6$)	10.000
Potassium dihydrogen phosphate (KH_2PO_4)	1.000
Magnesium sulphate ($MgSO_4.H_2O$)	0.500
Dichloran (2,6-dichloro-4-nitroaniline)	0.002
Chloramphenicol	0.100
Agar	15.000
Final pH (at 25°C)	5.6±0.2
Glycerol anhydrous	220.000

Dichloran Glycerol Medium Base **M1129I**

Ingredients	g / L
Tryptone$	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.500
Dichloran	0.002
Chloramphenicol	0.100
Agar	15.000
Final pH (at 25°C)	5.6±0.2
Supplement to be added	**g / L**
Glycerol anhydrous	220.000

**Formula adjusted, standardized to suit performance parameters
$ - Equivalent to Casein enzymatic digest

Directions

Suspend 15.77 gram (the equivalent weight of dehydrated medium per litre) in 500 ml purified/ istilled water. Heat to boiling to dissolve the medium completely. Add 110 grams of glycerol (Analytical Reagent Grade). Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Dichloran Glycerol Medium was formulated by Hocking and Pitt (1) and is recommended for isolation and enumeration of xerophilic moulds from dried and semidried foods. The glycerol at 18% (w/w) lowers the water activity (aw) from 0.999 to 0.95 (1) without causing any problem. This restrictive characteristic makes the medium especially suitable for foods. This medium is also recommended by ISO (2, 3).

Tryptone provides carbon, nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing moulds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species.

Type of specimen

Food samples

Specimen Collection and Handling

Process 40 gm of food sample in a stomacher by adding 200 ml of 0.1% Peptone Water (M028). Shake periodically for 30 minutes with 0.1% Peptone Water for powdered products. Dilute the sample to 1:10 in 0.1% Peptone water and spread on plate. Count the number of Xerophilic colonies per gram of food. The medium can also be used as general medium for the isolation of yeasts and moulds from foodstuffs (2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.16% w/v aqueous solution 22 grams of glycerol after sterilization. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Productivity : Cultural characteristics observed with added 22 gram of glycerol after an incubation at 25±1°C for upto 5 days. Recovery rate is considered as 100% for fungal growth on Reference medium - Sabouraud Dextrose Agar

Selectivity : Cultural characteristics observed with added 22 gram of glycerol after an incubation at 25±1°C for upto 5 days.

Organism	Inoculum (CFU)	Growth	Recovery
Productivity			
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good-luxuriant	>=50%
Wallemia sebi ATCC 42694 (00182*)	50-100	good-luxuriant	>=50%
Aspergillus restrictus ATCC 42693 (00183*)	50-100	good-luxuriant	>=50%
Eurotium rubrum ATCC 42690 (00184*)	50-100	good-luxuriant	>=50%
Mucor racemosus ATCC 42647 (00181*)	50-100	good-luxuriant	>=50%
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%
Selectivity			
$Bacillus spizizenii ATCC 6633 (00003*)	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers. $ - Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Disposal

1. Hocking A.D. and Pitt J.I., 1980, J. Appl. Environ. Microbiol., 39:488.
2. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of yeasts and moulds-Part 2: Colony count technique in products with water activity less than or equal to 0.95 ISO 21527-2:2008
3. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd. :2020
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 02/2024

Rappaport Vassiliadis Soya Broth (RVS Broth)

M1491

Intended Use:

Selective enrichment medium for *Salmonellae* species from food and animal feeding stuffs and clinical specimens.

Composition**

Ingredients	g / L
Soya peptone	4.500
Sodium chloride	8.000
Potassium dihydrogen phosphate	0.600
Dipotassium hydrogen phosphate	0.400
Magnesium chloride hexahydrate	29.000
Malachite green	0.036
Final pH (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.11 grams (the equivalent weight of dehydrated medium per liter) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 115°C for 15 mins.

Principle And Interpretation

Rappaport Vassiliadis Soya Broth is designed according to the revised formulation by Van Schothorst et al (1) and is recommended for the selective enrichment of Salmonellae from pharmaceutical products. This medium can also be used in direct enrichment of samples containing low inoculum. Present medium is a modification of the Rappaport Vassiliadis Enrichment Broth described by Van Schothorst and Renauld (2). Addition of magnesium chloride to the medium was reported by Peterz et al (3). *Salmonella* species can be isolated from human faeces without pre-enrichment by using this medium.

Salmonella generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green compared to other bacteria. These characteristics are exploited in this medium for selective enrichment of *Salmonella*. Magnesium chloride present in the medium raises the osmotic pressure. Natural sugars of Papaic digest of soyabean meal provide essential growth nutrients and enhance the growth of *Salmonella* (4). Phosphate buffers the medium to maintain constant pH. Sodium chloride maintains the osmotic balance. Malachite green inhibits many gram-positive bacteria, while selectively enrich *Salmonella*. The relatively lower concentration of nutrition, also aids selective enrichment of Salmonella. This medium was reported to be superior to *Salmonella* selective medium like Tetrathionate Broth and Selenite enrichment broth and to Tetrathionate-Brilliant Green Broth for the detection of *Salmonella* in milk samples. The enriched culture of Rappaport Vasiliadis Soya Broth (M1491) can be further subcultured and isolated on Brilliant Green Agar (M016) or Deoxycholate Citrate Agar (M065), Xylose Lysine Deoxycholate Agar (M031).

Type of specimen

Clinical samples - faeces; Food samples and animal feeding stuffs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use . For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium contains inhibitory substances and may not support the growth of certain *Salmonella* species like *S.* Typhi.
2. Less selective enrichment broth must be used in conjunction.
3. After enrichment the organisms must be isolated on less selective media along with selective media.
4. Further biochemical and serological testing must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to light blue homogeneous free flowing powder.

Colour and Clarity of prepared medium

Greenish blue clear to slightly opalescent with a slight precipitate.

Reaction

Reaction of 2.77% w/v aqueous solution at 25°C. pH : 5.2±0.2

pH

5.00-5.40

Cultural Response

Cultural response was observed after an incubation at 30-35°C for 18-24 hours Recovery is carried out using Xylose Lysine Deoxycholate Agar (M031) after enrichment.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
***E.coli* +*S.*Typhimurium (mixed culture)**				
E.coli	50 -100	none-poor	<=10 %	yellow
*S.*Typhimurium	50 -100	luxuriant	>=50 %	red with black centers
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Salmonella Abony NCTC 6017 (00029*)	50-100	luxuriant	>=70 %	red with black centers
Salmonella Typhimurium *subsp. aureus* ATCC 14028 (00031*)	50-100	luxuriant	>=70 %	red with black centers
Staphylococcus aureus ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	50 -100	none-poor	0 -10	yellow
Salmonella Enteritidis ATCC *13076* (00030*)	50-100	luxuriant	>=70 %	red with black centre
Escherichia coli ATCC 8739 (00012*)	50-100	none-poor	<=10 %	yellow
Salmonella Paratyphi B ATCC 8759	50-100	luxuriant	>=70 %	red with black centre

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store below 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in-order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:11.
2. Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:209.
3. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
4. McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol. 1:171.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C.,Funke., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

Revision : 05/2024

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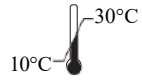
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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Mueller Kauffman Tetrathionate Novobiocin Broth Base M1496I

Intended Use:

Recommended for improved enrichment and isolation of *Salmonellae*. The composition and performance criteria of this media are as per the specification laid down in ISO 6579-1:2017 and ISO 11133:2014 (E) /Amd. : 2020.

ISO Specification - Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth

M1496I - Mueller Kauffman Tetrathionate Novobiocin Broth Base

Composition**

Ingredients	g / L
Meat extract	4.300
Enzymatic digest of casein	8.600
Ox bile for bacteriological use	4.780
Sodium chloride (NaCl)	2.600
Calcium carbonate ($CaCO_3$)	38.700
Sodium thiosulphate, pentahydrate ($Na_2S_2O_3$ $5H_2O$)	47.800
Brilliant green	0.0096
Final pH (at 25°C)	8.0±0.2
Supplements to be added after autoclaving	
Novobiocin sodium salt	0.040
Iodine-iodide solution	20.00ml
Iodine	4.000
Potassium iodide (KI)	5.000

Ingredients	g / L
HM extract#	4.300
Tryptone###	8.600
Bile##	4.780
Sodium chloride	2.600
Calcium carbonate	38.700
Sodium thiosulphate, pentahydrate	47.800
Brilliant green	0.0096
Final pH (at 25°C)	8.0±0.2
MKTT Supplement	**FD203**
Novobiocin	0.040
$ Iodine-iodide solution	20.000ml
Iodine	4.000
Potassium iodide (KI)	5.000

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract ## Equivalent to Ox bile ### Equivalent to Enzymatic digest of casein

$ To be added but not provided (To be freshly prepared)

Directions

Suspend 89.42 gram (equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat the medium just to boiling. DO NOT AUTOCLAVE. Cool to 45-50°C and just before use aseptically add rehydrated contents of 1 vial of MKTT Novobiocin Supplement (FD203) and 20 ml of iodine-iodide solution (20 gram iodine and 25 gram potassium iodide in 100 ml sterile distilled water). Mix well to disperse calcium carbonate uniformly before dispensing in sterile tubes.

Note: Due to presence of calcium carbonate, the prepared media forms opalescent solution with white precipitate.

Principle And Interpretation

The examination of various types of food products for *Salmonella* requires methods different from those used in clinical laboratories. The need for such method is due to the generally low numbers of Salmonellae in foods and the frequently poor physiological state of these pathogens following exposure to stressful conditions during food processing or storage. Injured *Salmonella* are resuscitated in non-selective broth medium, which facilitates detection of sublethally injured *Salmonella.* The ideal pre-enrichment broth should provide for the repair of cell damage, dilute toxic or inhibitory substances and nutritive enough to favour growth of *Salmonella.*

Mueller (1) recommended Tetrathionate Broth as a selective medium for the isolation of Salmonella. Kauffman (2) modified the formula to include ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* species. The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat, meat products, and from poultry and poultry products (3). ISO committee has also recommended this pre-enrichment medium for the detection of *Salmonella* species from from food stuffs and other materials (4). Selectivity is conferred by tetrathionate (from the reaction of thiosulphate and iodine). Using more than one selective broth increases the isolation of Salmonella from samples with multiple serotypes (1). This medium contains Tryptone and HM extract

as sources of carbon, nitrogen, vitamins and minerals. Bile and added brilliant green are selective agents, which inhibit gram-positive and other gram-negative organisms. Calcium carbonate is the buffer. Sodium chloride maintains osmotic equilibrium. Sodium thiosulphate is a source of sulfur. The tetrathionate (S_4O_6) anions constitute the principle selective agent in these enrichment media. Organisms other than Salmonellae, such as M*organella morganii* and some *Enterobacteriaceae* may grow in the medium. Therefore, confirmatory tests should be carried out on all presumptive Salmonella colonies that are recovered. Method (5).

Type of specimen

Food samples including milk and milk products, in animal feed, in animal faeces, and in environmental samples from the primary production stage.

Specimen Collection and Handling:

Processesing : ISO 6579-1:2017 (4)

Pre-enrichment : Samples (25 gram in 225 ml) are preenriched in Buffered Peptone Water (M1494I) and incubated at 34° C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428I) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTTn broth (M1496I) and incubated at 37± 1°C for 24 ± 3 hours .

Isolation : The culture thus obtained is then plated on XLD Agar, Modified (M031I) and incubated at 37± 1°C for 24 ± 3 hours . Simultaneously plating on second isolation agar is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

Limitations :

1.The complete medium is unstable and should be used immediately. After incubation, it is permissible to store the selective enrichment medium at 5°C for a maximum of 72 h.
2.Individual organisms differ in their growth requirement and may show variable growth patterns in the medium
3.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
4.Confirmatory tests should be carried out on all presumptive *Salmonella* colonies that are recovered.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light green coloured opalescent solution forms with heavy white precipitate

Reaction

Reaction of 8.93% w/v aqueous solution at 25°C. pH : 8.0±0.2

pH

7.80-8.20

Cultural Response

Productivity: Cultural response was observed after an incubation at 37 ±1°C for 24 ± 3 hours with added sterile 20ml iodine solution and MKTT Supplement (FD203). Further subculture is carried out on XLD Agar, Modified (M031I) and incubated at 37 ±1°C for 24 ± 3 hours.

Organism	Inoculum (CFU)	Recovery on XLD Agar (M031I)	Colour of colony on XLD Agar (M031I)
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	>10 colonies	red colonies w/ black centre
+*Escherichia coli* ATCC 8739 (00012*)	>=10^4		
+ *Pseudomonas aeruginosa* ATCC 27853 (00025*)	>=10^4		

Salmonella Typhimurium ATCC 14028 (00031*)	50-100	>10 colonies	red colonies w/ black centre
+ *Escherichia coli* ATCC 25922 (00013*)	>=10^4		
+ *Pseudomonas aeruginosa* ATCC 27853 (00025*)	>=10^4		

Selectivity : Cultural characteristics observed after an incubation at 37±1°C for 24±3 hours with added sterile 20ml iodine solution and MKTT Supplement (FD203). Further subculture is carried out on Tryptone Soya Agar (M290)and incubated at 37±1°C for 24 ± 3 hours.

Organism	Inoculum (CFU)	Growth	Recovery on Tryptone Soya Agar
Escherichia coli ATCC 8739 (00012*)	>=10^4	partial inhbition	<=100 colonies
Escherichia coli ATCC 25922 (00013*)	>=10^4	partial inhbition	<=100 colonies
Enterococcus faecalis ATCC 29212(00087*)	>=10^4	inhbition - partial inhibition	<10 colonies
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhbition - partial inhibition	<10 colonies

Key: (*) - Corresponding WDCM Numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1.Mueller L., 1923, C. R. Soc. Biol., (Paris) 89:434.Harvey R. W. S. and Price T. S., 1976, J. Hyg. Camb., 77:333.
2.Kauffman F., 1935, Ztschr. F. Hyg., 117:26.
3.Public Health Laboratory Service, 1974, Monograph Series No. 8, Public Health Laboratory Service, London, England.
4.Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Detection of *Salmonella* spp. ISO 6579-1:2017
5.Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd. :2020 .
6.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 06/2024

Tryptone Bile Glucuronic Agar (TBX Agar) M1591

Intended use

Selective agar for the detection and enumeration of *Escherichia coli* in foodstuffs, animal feed, water and clinical samples.

Composition**

Ingredients	Gms / Litre
Bile salt mixture	1.500
Tryptone	20.000
X-ß-D-glucoronic acid	0.075
Dimethyl sulfoxide	3.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.6 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour in sterile Petri plates.

Principle And Interpretation

The formulation of Tryptone Bile Glucuronic Agar is in accordance with ISO 16649-2 (1). Tryptone Bile Glucuronic Agar contains the enzyme ß -D- glucuronidase which differentiates most *E.coli* species from other coliforms.

E.coli absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (2).The enzyme ß-glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyl and the ß-D-glucuronide. *E.coli* colonies are blue green coloured (3,4). Growth of accompanying gram positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44°C.

Type of specimen

Clinical samples - urine, Food samples ; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,5).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Some species may show poor growth due to nutritional variations.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.66% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 44°C for 18- 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Citrobacter freundii ATCC 8090	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	blue-green
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,5).

Reference

1. International Standard ISO 16649-2: 2018. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of presumptive Escherichia coli; Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-ß-D-glucoronic acid.
2. Frampton E W, Restaino L, Blaszko L.1988. Eavaluation of ß-glucoridase substrate 5-bromo-4-chloro3-indolyl-B-D-glucuonide (X-GLUC) in a 24 hour direct plating method for Escherichia coli. J. Food Protection 51:402-404.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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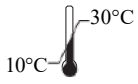
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**



Storage temperature

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www.cepartner4u.eu

CE **CE Marking**

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Disclaimer :

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HIMEDIA

Technical Data

Penicillin and Pimaricin Pseudomonas Agar Base (PP Pseudomonas Agar Base)

M1788

Intended Use:

Recommended for selective isolation of *Pseudomonas* species on addition of supplements. The composition and performance criteria are in accordance with ISO /TS 11059 .

Composition**

ISO specification - Penicillin and Pimaricin Pseudomonas Agar Base

Ingredients	g / L
Enzymatic digest of gelatine	/4,...
Enzymatic digest of casein	/.,...
Nmr_qqgs k qsjnf_rc (K_2SO_4)	/.,...
K_elcqgs k afjmpgbc($MgCl_2$)	/,2..
?e_p	12.000-/8,...
Dgl_j nF & _r 03ßA'	5,0Ú.,0

Supplements to be added

Penicillin solution

Nclgagjjgl E*nmr_qqgs k q_jr /.....GS

Pimaricin solution

Ngk_pgagl &N_r_kwagl' .,/..

((Dmp k sj_ _bhsqrcb* qr_lb_pbgxcb rm qsgr ncpdmp k _lac n_p_k crcpq

Penicillin and Pimaricin Pseudomonas Agar Base **M1788**

Ingredients	g / L
Ecj_rgl ncnrmlc	/4,...
Rpwnrmlc	/.,...
Nmr_qqgs k qsjnf_rc	/.,...
K_elcqgs k afjmpgbc	/,2..
?e_p	/3,...
Dgl_j nF & _r 03ßA'	5,0Ú.,0

*Ingredients	Concentration
PP Selective Supplement (FD264) - 1 vial	
Penicillin G, potassium salt	100000 IU
PP Selective Supplement II (FD265) - 1 vial	
Ngk_pgagl &L_r_kwagl'	.,./.e

Directions

Qsqnclb 30,2 ep_k gl /... kj nspgdgcb-bgqrgjjcb u_rcp, Fc_r rm `mgjgle rm bgqqmjtc rfc kcbgsk amknjcrcjw, Qrcpgjgxc `w _srmaj_tgle _r /3 j`q npcqqspc &/0/ßA' dmp /3 kglsrcq, Ammj rm 23+3.ßA _lb _qcnrga_jjw _bb qrcpgjc pcfwbp_rcb amlrclrq md NN Qcjcargtc Qsnnjckclr &DB042' _lb NN Qcjcargtc Qsnnjckclr GG &DB043', Kgv ucjj _lb nmsp glrm qrcpgjc Ncrpg nj_rcq,

Principle And Interpretation

Pseudomonas species are aerobic, non-spore forming, gram negative rods, found in water, soil and plants including fruits and vegetables. *Pseudomonas aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance especially in patients with compromised host defense mechanisms. Several different epidemiological studies have found its occurrence as a nosocomial pathogen (6). *Ps.aeruginosa* strains produces two types of soluble pigments, the fluorescent pigment pyoverdin and the blue pigment pyocyanin. Pyocyanin (from "pyocyaneus") refers to "blue pus", which is a characteristic of suppurative infections caused by *Ps.aeruginosa.* Penicillin and Pimaricin Pseudomonas Agar Base is formulated as recommended by ISO Committee (3).

The medium contains gelatin peptone and tryptone which serves provides essential nitrogenous nutrients and carbon, long chain amino acids and vitamins required for the growth of *Pseudomonas.* Potassium sulphate and magnesium chloride serves to enhance pigment production. Addition of PP Selective Supplement which contains Penicillin and PP Selective Supplement II which contains Pimaricin (natamycin) to the medium helps in the selective isolation of *Pseudomonas,* thereby inhibiting the accompanying flora.

Type of specimen

Fmmb _lb b_gpw q_k njcq

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium. 2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.24% w/v aqueous solution (containing 1% v/v glycerol) at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Productivity : Cultural characteristics observed with added 1% glycerol, PP Selective Supplement (FD264) and PP Selective Supplement II (FD265), after an incubation at 25° ± 1°C for 48 ± 2 hours. Recovery is considered as 100% on Soyabean Casein Digest Agar

Selectivity: Cultural characteristics observed with added 1% glycerol, PP Selective Supplement (FD264) and PP Selective Supplement II (FD265), after an incubation at 25° ± 1°C for 48 ± 2 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Productivity			
Pseudomonas fluorescens ATCC 13525	3.+/..	jsvspg_lr	<;3.#
Pseudomonas aeruginosa ATCC 27853 (00025*)	3.+/..	jsvspg_lr	<;3.#
Selectivity			
Escherichia coli ?RAA 25922 (00013*)	<;/.²	gl fg`grcb	
Escherichia coli ATCC 8739 (00012*)	<;/.²	gl fg`grcb	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5',

References

1. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
2. Milk and milk products - Method for the enumeration of Pseudomonas spp. International Organization for Standardization, 2009, Draft ISO/TS 11059:2009 (E).
3. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd. :2020 .
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03/ 2024

HiCrome™ Selective Salmonella Agar Base

M1842

Intended Use:

Recommended for the selective isolation of *Salmonella* species from food and clinical samples.

Composition**

Ingredients	g / L
HI powder #	12.000
Yeast hydrolysate	5.000
Tryptose	5.000
Sodium cholate	3.000
Sodium taurocholate	5.000
Sodium deoxycholate	1.000
Chromogenic mixture	8.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Heart Infusion powder

Directions

Suspend 54.00 gram in 1000 ml purified/ distilled water. Gently heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C. Aseptically add the rehydrated contents of one vial of NC Selective Supplement (FD274). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Salmonella species have been isolated from humans and almost all animals throughout the world. They cause many types of infections from mild, self-limiting gastroenteritis to life threatening typhoid fever. *Salmonella* Typhi and *Salmonella* Paratyphi A & B cause gastroenteritis, bacteremia and enteric fever, *Salmonella* Choleraesuis causes gastroenteritis and enteric fever, especially in children. *Salmonella* Typhimurium is the most frequently isolated serotype of *Salmonella*. *Salmonella* species are the major cause of food poisoning (1).

Various chromogenic media are available for the differentiation of Salmonella species. The original media formulated by Rambach (2) differentiates *Salmonella* based on propylene glycol utilization and presence of a chromogenic indicator. However HiCrome™ Selective Salmonella Agar Base uses chromogenic mixture for identification and differentiation of *Salmonella* species. Sodium cholate, Sodium taurocholate and Sodium deoxycholate in the medium helps to restrict the growth of other organisms. Besides the selective supplement added to the medium inhibits competing microorganisms.

HI powder, yeast hydrolysate and tryptose in the medium provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Due to the presence of chromogenic mix in the medium *Salmonella* are easily distinguishable and forms purple coloured colonies while some *Enterobacteriaceae* like *Klebsiella* and *Enterobacter* forms blue to dark blue coloured colonies.

Conventional method employes the H_2S production property for *Salmonella* detection which is also exhibited by other non *Salmonella* species such as *Citrobacter, Proteus* etc. Hence further biochemical confirmation is required for further identification. This medium is specially employed for food samples where the sample is initially enriched in *Salmonella* Selective Enrichment Broth (M1843) and then isolated on HiCrome™ Selective Salmonella Agar Base. *Salmonella* species give purple coloured colonies due to the enzyme specificity.

Type of specimen

Clinical samples- stool, urine, etc, Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4) .
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Being highly selective, some strains may show poor growth.
2. Most of the *Salmonella* strains shows purple colonies except few.
3. Final confirmation of suspected colonies must be carried out by serological and biochemical tests.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5 % Agar gel.

Colour and Clarity of prepared medium

Whitish cream coloured, opaque gel forms in Petri plates

Reaction

Reaction of 5.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added NC Selective Supplement (FD274), after an incubation at 35-37°C for 22-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^3	inhibited	0%	
Klebsiella pneumoniae ATCC 13883 (00097*)	50 -100	good	40 -50 %	blue
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	good-luxuriant	>=50 %	purple
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	>=50 %	purple
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0 -0 %	

Key: (*) Corresponding WDCM numbers

Storage and Shelf Life

Store dehydrated medium in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2.Rambach A., 1990, Appl. Environ. Microbiol., 56:301.
3.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 06/ 2024

HiMedia Laboratories Pvt. Limited,
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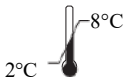
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD **_In vitro_ diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Iron Sulphite Agar Modified

M1852I

Intended Use:

Recommended for the enumeration of sulphite-reducing bacteria growing under anaerobic conditions. The composition and performance criteria of this media are as per the specifications laid down in ISO 15213-1:2023 and ISO 11133:2014 & Amd.;2020.

Composition**

ISO 15213-1:2023-Iron Sulphite Agar (ISA)

Ingredients	g / L
Peptone	15.000
Enzymatic digest of soya	5.000
Yeast extract	5.000
Sodium disulfite,anhydrous	0.500
Iron III ammonium citrate	1.000
Agar	9.0-18.0
Final pH (at 25°C)	7.6±0.2

Iron Sulphite Agar Modified **M1852I**

Ingredients	g / L
Peptone	15.000
Soya peptone	5.000
Yeast extract	5.000
Sodium disulfite	0.500
Iron III ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 41.50 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and dispense into sterile tubes or pour into sterile Petri plates.

Principle And Interpretation

Iron Sulphite Agar, Modified is recommended by ISO for the enumeration of sulphite reducing bacteria (1,2). Most *Clostridia* possess sulfite reductase in their cytoplasm but they are unable to expel them to the exterior. So when H_2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

Peptone and soya peptone provides carbon, nitrogen compounds, vitamins, minerals and amino acids necessary for the growth of organism. Yeast extract serves as a rich reservoir of vitamins especially B-complex vitamins. Ferric citrate ammonium citrate and Disodium sulfite serves as are H2S indicators, wherein Clostridium perfringens reduces the sulfite to sulfide which in turn reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies. Agar is the solidifying agent.

Enumeration with this medium can be performed using either tubes or plates. In case of Petri plates, Using a fresh sterile pipette, transfer to each dish of the first decimal dilution 10^{-1} of the test sample if the product is liquid, or of the first decimal dilution of the initial suspension 10^{-2} in the case of other products. Pour iron sulfite agar into each Petri dish. Carefully mix the inoculum with the medium by horizontal movements and allow the medium to solidify. After the medium has solidified, pour 5 to 10ml of the same medium into the dish as an overlay.

If tubes are used, inoculate a 1 ml volume from each dilution into each of two tubes of medium. Mix gently without forming bubbles, and leave the medium to solidify. After the medium has solidified, pour 2ml to 3ml of the same medium into each tube as an overlay. After solidification, incubate the medium at 36-38°C for 24-48 hours. If thermophilic bacteria are suspected, prepare a second set of Petri dishes. Incubate this set at 50°C ± 1°C. Black colonies, possibly surrounded by a black zone, are counted as sulfite-reducing bacteria.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling

For isolated microorganisms, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1,2).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to brownish yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured, slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH
7.40-7.80

Cultural Response

Productivity : Cultural characteristics observed under anaerobic atmosphere, after an incubation at 37±1°C for 24±3h to 48 ± 2h. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

Specificity : Cultural characteristics observed after an incubation at 37±1°C for 24±3h to 48± 2h.

Organism	Inoculum	Growth	Recovery	Characteristic reaction
Productivity				
Clostridium perfringens ATCC 13124 (00007)*	50-100	luxuriant	>=50%	black colonies
Clostridium perfringens ATCC 12916 (00080)*	50-100	luxuriant	>=50%	black colonies
Specificity				
Escherichia coli ATCC 25922 (00013)*	10^3-10^4	growth		no blackening
Escherichia coli ATCC 8739 (00012)*	10^3-10^4	growth		no blackening

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of sulphite reducing bacteria growing under anaerobic conditions, ISO 15213-1:2023(E).
2. Microbiology of food, animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, I.S. EN ISO 11133:2014 & A1:2018.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision :05/2024

Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) M1881

Intended Use:

Recommended for selective isolation of fungi-yeasts and moulds of significance in food spoilage. The composition and performance criteria are in accordance with ISO 21527-1 and ISO 11133:2014 (E) /Amd. :2020 .

Composition**

ISO specification - Dichloran Rose Bengal Chloramphenicol Agar medium

Ingredients	g / L
Enzymatic digest of animal & plant tissues	5.000
D-Glucose) ($C_6H_{12}O_6$	10.000
Potassium dihydrogen phosphate (KH_2PO_4)	1.000
Magnesium sulphate ($MgSO_4.H_2O$)	0.500
Rose Bengal	0.025
Chloramphenicol	0.100
Dichloran (2,6-dichloro-4-nitroaniline)	0.002
Agar	12.000-15.000
pH after sterilization (at 25°C)	5.6±0.2

Dichloran Rose Bengal Chloramphenicol Agar medium **M1881**

Ingredients	g / L
Peptone$	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.500
Rose Bengal	0.025
Chloramphenicol	0.100
Dichloran	0.002
Agar	15.000
pH after sterilization (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters $ - Equivalent to Enzymatic digest of animal & plant tissues

Directions

Suspend 31.63 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) is formulated by as described by King et.al (1) and is recommended for selective isolation of yeasts and moulds especially in food and animal feeding samples. It is recommended by ISO (2) This medium is a modification of Rose Bengal Chloramphenicol Agar which additionally contains dichloran.

Peptone provides nitrogeneous compounds, carbon, long chain amino acids, vitamins and other essential growth nutrients. Dextrose (Glucose) is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi. Rose Bengal exhibits an improved inhibitory activity at pH 5.6 and hence the final pH of the medium is maintained at 5.6 for the inhibition of spreading fungi (1) The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and colonies of the more rapidly growing moulds. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing moulds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. Additionally Rose Bengal is taken by yeast and moulds colonies, which allows these colonies to be easily recognized and enumerated. This medium should not be exposed to direct light as rose bengal undergoes photo-degradation leading to formation of toxic chemicals for fungi (3,4).

Type of specimen

Food sample : Eggs, Meat, Dairy products (except milk powder), Fruits, Vegetables,Fresh pastes, animal feeds, etc.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2, 3,5,6,7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Further biochemical identification is necessary for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Pink coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.16% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 25 ± 1°C for 5 days. Recovery is considered as 100% for fungi growth on Reference medium -Sabouraud Dextrose Agar.

Selectivity: Cultural characteristics observed after an incubation at 25 ± 1°C for 5 days.

Organism	Inoculum (CFU)	Growth	Recovery
Productivity			
Saccharomyces cerevisiae ATCC 9763 (00058)* #	50-100	good-luxuriant	>=50%
#*Aspergillus brasiliensis* ATCC 16404(00053)*	50-100	good-luxuriant	>=50%
Candida albicans ATCC 10231 (00054)*	50-100	good-luxuriant	>=50%
Mucor racemosus ATCC 42647 (00181)*		good-luxuriant	
Selectivity			
$ Bacillus spizizenii ATCC 6633 (00003)*	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	
Escherichia coli ATCC 8739 (00012)*	>=10^4	inhibited	

Key : (*) - Corresponding WDCM numbers

$ - Formerly known as *Bacillus subtilis* subsp. *spizizenii* # Formerly known as *Aspergillus niger*

Storage and Shelf Life

Store the dehydrated powder and the prepared medium between 15-25°C in a tightly closed container. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. King D.A. Jr., Hocking A.D. and Pitt J.I., 1979, J. Appl. Environ. Microbiol., 37:959.
2. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0,95, ISO 21527-1:2008
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
4. Sharp A.N. and Jackson A.K., 1972, J. Appl. Bact., 24:175.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
7. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd. 1:2020.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 06/2024

HiCrome™ Chromogenic Coliform agar (CCA Agar) M1991I

Intended Use

Recommended for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.

Composition**

ISO 9308-1:2014 Specification -Chromogenic Coliform agar (CCA Agar)

Ingredients	g / L
Enzymatic digest of casein	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, $2H_2O$	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt)	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.100
Agar	9.0 to 18.00
Final pH (at 25°C)	6.8±0.2

M1991I - HiCrome™ Chromogenic Coliform agar (CCA Agar)

Ingredients	g / L
Tryptone #	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, $2H_2O$	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt)	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside) Agar	0.100
Final pH (at 25°C)	15.000
	6.8±0.2

**Formula adjusted, standardized to suit performance parameters
Enzymatic digest of casein

Directions

Suspend 30.92 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE. DO NOT OVERHEAT.** Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ Chromogenic Agar is a selective medium recommended by ISO for enumeration of *Escherichia coli* and coliform bacteria (1). The medium contains three chromogenic substrates. The enzyme ß-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxy l-β-D-galactopyranoside to form pink to red coloured colonies (1). The enzyme ß-D glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxy l-β-D-glucuronic acid (1) Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Tryptone, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sub-lethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (1). The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli,* add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Type of specimen

Water samples.

Specimen Collection and Handling:

Processing (1)

Filration:

Filter 100ml of the sample using membrane filter. The minimum volume for filtration should be 10ml (or dilution) so that to ensure even distribution of the bacteria on the membrane filter.

Incubation and differentiation:

After filtration place the membrane filter on HiCrome™ Chromogenic Coliform agar (CCA Agar), ensuring that no air is trapped undereath, invert petri dish and incubate at 36°C ± 2 for 21 ± 3 hours. Examine the colony on membrane filters for color change.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

Read the label before opening the container. The media should be handled by trained personnel only. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical and serological test are necessary for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates

Reaction

Reaction of 3.09% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Productivity: Cultural response observed after an incubation at 36°C ± 2 for 21 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

Selectivity: Cultural response observed after an incubation at 36°C ± 2 for 21 ± 3 hours.

Specificity: Cultural response observed after an incubation at 36°C ± 2 for 21 ± 3 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony#
Productivity				
Escherichia coli ATCC 25922 (00013)*	50-100	luxuriant	>=70%	dark blue to violet
Escherichia coli ATCC 8739 (00012)*	50-100	luxuriant	>=70%	dark blue to violet
Citrobacter freundii ATCC 43864 (00006)*	50-100	luxuriant	>=70%	pink to red

##*Klebsiella aerogenes* ATCC 13048 (00175)*	50-100	luxuriant	>=70%	pink to red
Selectivity				
Enterococcus faecalis ATCC 19433 (00009)*	>=10^4	inhibited		
Specificity				
Pseudomonas aeruginosa ATCC 10145 (00024)*	10^3-10^4	growth	-	colourless

Key * : Corresponding WDCM numbers # : either on plate or membrane

Formerly known as *Enterobacter aerogens*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. International Organization for Standardization. Water quality: Enumeration of *E.coli* and coliform bacteria. Part I- Membrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 07/2024

Nessler's Reagent R010

Intended use

Nessler's Reagent is used to detect production of ammonia and ammonia salts.

Composition**

Ingredients	-
Mercuric chloride	10.0 g
Potassium iodide	7.0 g
Sodium hydroxide	16.0 g
Water (ammonia free)	100.0 ml
Final pH (at 25°C)	13.2 ± 0.05

**Formula adjusted, standardized to suit performance parameters

Directions

1. Emulsify a 24 hours old culture of organism to be tested for urease test in 0.5 ml substrate in a test tube containing 2% urea.
2. Place the tube in a water bath at 37°C for 3 hours.
3. Remove the tube and add 0.1 ml of Nessler's reagent and similar amount to the negative control and blank tubes.
4. Read the results after 3-5 minutes after adding the Nessler's Reagent. Both negative and control tubes must be absolutely colourless.
5. When isolated colonies are to be examined, the volume of substrate is reduced to 0.3 ml and only one drop of Nessler's reagent is added.
6. For detecting NH_3 production in L-arginine breakdown, Remove a loopful from a 4 day L-arginine culture and place into 0.5 ml of ammonia free distilled water.
7. Add 1 drop of Nessler's reagent. run the same check on the control.

Principle And Interpretation

Bacteria, particularly those growing naturally in an environment exposed to urine may decompose urea by means of the enzyme urease. The occurrence of this enzyme can be tested by growing the organism in the presence of urea and testing for alkali (NH_3) production by means of a suitable pH indicator. An alternative method is to test for the production of ammonia from urea by means of Nessler's reagent and/or to detect NH_3 production due to L-arginine breakdown.

Type of specimen

Used as biochemical reagent in diagnosis

Specimen Collection and Handling

1. For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
2. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
3. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The reaction time of ammonia with Nessler's reagent also affects the accurate quantification of NH_3.
2. The result of the Nessler's method becomes inaccurate if there are interfering substances such as Cl_2, Cl^-,hardness causing compounds (e.g., Mg^{2+}), and Fe^{2+} in target samples.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Pale yellow coloured solution.
- **Clarity :** Clear with no insoluble particles.

  Note : On storage of the reagent, precipitate may develop. This will not affect the performance criteria of the reagent.
- **Reaction :** Reaction of the solution at 25°C. pH :13.2 ± 0.05
- **Test :** Emulsify a 24 hour old culture of organism to be tested for urease test, in 0.5 ml substrate containing 2% urea. Place the tube in a water bath at 37°C for 3 hours. Remove tube and add 0.1 ml of Nessler's reagent. Read the results after 3-5 minutes.
- **Results :** A positive reaction for presence of ammonia is a colour ranging from a pale yellow to a dark brown precipitate.

Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

1. Mackie and MacCartney,1989, Practical Medical Microbiology, Collee J.G., Duguid J.p., Fraser A.G. and Marmion B.p (Eds.),13th ed., Churchill Livingstone, edinburgh.
2. Kauffmann F. and Moller U., 1955, Acta Pathol. Microbial. Scand.,36:173
3. MacFaddin J.,1980, Biochemical Tests for identification of Medical Bacteria,2nd ed. Williams and Wilkins, Baltimore.
4. Gaby, W. L., and L. Free. 1958. Differential diagnosis of pseudomonas-like microorganisms in the clinical laboratory.

J. Bateriol. 76:442–444.
5. Gaby, W. L., and C. Hadley. 1957. Practical laboratory test for the identification of Pseudomonas aeruginosa. J. Bacteriol. 74:356–358.
6. MacFaddin, J. 1972. Biochemical tests for the identification of medical bacteria. Williams and Wilkins Company, Baltimore, MD.
7. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
9. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
10. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.

Storage temperature


Do not use if package is damaged

In vitro diagnostic medical device


CE Marking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Revision 02/2023

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Sterile Mineral Oil

R045

Intended use

Sterile Mineral Oil is used as a sealant to create an anaerobic environment in biochemical tests . It is also recommended for preservation of microorganism

Composition**

Ingredients

Sterile Mineral Oil

Directions

1. Overlay each inoculated tube with sterile mineral oil (0.5-1cm).
2. Tighten the caps of inoculated, overlayed tubes and incubate at appropriate temperature.

Principle And Interpretation

Mineral oils are usually seen as a mixture of liquid hydrocarbons. It is derived from crude oil by distillation and refining. Sterile mineral oil is recommended to overlay in biochemical tests such as decarboxylase, oxidation and fermentation reactions. It is also used in preservation of microorganisms.

Type of specimen

Biological sample

Specimen Collection and Handling

Follow appropriate techniques for handling specimens as per established guidelines

Warning and Precautions

Non In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. It fails to prevent changes in the characteristics of a strain due to the development of variants and mutants
2. Once vial opened it has to be used or preserved carefully otherwise it will get slowly contaminated with microorganisms.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

→ **Appearance :** Colourless viscous solution.

- **Clarity :** Clear with no insoluble paricles.
- **Sterility Testing :** Sterility of the mineral oil was checked by inoculating 1 ml mineral oil in 100 ml sterile Soyabean Casein Digest Medium (M011) and Alternate Thioglycollate Medium (M010).Incubate at 30-35°C for 14 days.
- **Results :** Absence of turbidity (clear medium) after 14 days at 30-35°C.

Storage and Shelf Life

On receipt store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques .

Reference

1. Biochemical Tests for the Identification of Aerobic Bacteria. (2016). Clinical Microbiology Procedures Handbook, 3.17.1.1–3.17.48.3.
2. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.

Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt Limited
C-40,21/Y, MIDC, Wagle Ind Area,
Thane(W)–400604,Maharashtra,India

Revision : 01/2023

Disclaimer :

McFarland Standard set **R092**

McFarland standards are used to perform spectrophotometric comparisions of bacterial densities in water, saline or liquid growth medium. It provides laboratory guidance for the standardization of numbers of bacteria for susceptibility testing or other procedure requiring a standardization of the inoculum like growth promotion test (GPT).

Set Contains:

R092A (Standard 0.5)- 1 tube

R092B (Standard 1)-1 tube

R092C (Standard 2)- 1 tube

R092D (Standard 3)- 1 tube

R092E (Standard 4)- 1 tube

Directions

Prepare the inoculum of culture required for testing by using sterile saline. Match the density of the resultant suspension with the density of the desired standard. The standards must be thoroughly mixed on a vortex mixture at the time of use to obtain a uniform suspension. Adjust the density of cell suspension by adding saline if it is more turbid as compared to the desired standard or by adding culture if it is dilute. Check the density of the turbidity by determining the absorbance of 0.5 McFarland standard using a spectrophotometer with a 1 cm light path. The absorbance at 625 nm should be 0.08 to 0.10. The standards should be checked regularly to ensure the density accuracy.

Interpretation

McFarland standards are a set of tubes with increasing concentration of Barium Sulphate suspension. The turbidity of Barium Sulphate's white precipitation is used as a point of comparision of bacterial suspensions to known bacterial turbidity.

McFarland Standard	0.5	1	2	3	4
Approximate Corresponding suspension x 10^8 CFU/ml	1.5	3	6	9	12

Limitation of procedure

1. Coloured media may interfere with result interpretation and give incorrect results.

2. Bacterial suspensions of older cultures may not be comparable with expected bacterial counts.

Storage

Store the standards at 2-8°C, away from light after each use.

Reference

1. McFarland, J.1907. Nephelometer: JAMA 14:1176-1178

2. Murry,PR; Baron,EJ; Jorgensen,JH;Landry,ML;Pfaller,MA; Manual of Clinical Microbiology 9th edition ASM press, Washington DC.

Tryptone, ES, (Casitose, ES)

RM9111

Intended use

Tryptone, ES, (Casitose, ES) is obtained by refined processing of milk protein, manufactured under controlled enzymatic hydrolysis. It is rich in tryptophan content and is extra soluble as compared to other casitose. It can be used as a microbial nutrient in diagnostic and fermentation medias.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.It is biological origin product since variation in colour of powder and clarity may observed.
2.Each lot of the product has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's requirement.
3.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium prepared by the product.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Off white to light yellow homogenous free flowing powder characteristic odour but not putrescent
- **Solubility :** 10% Freely soluble in distilled/purified water, insoluble in alcohol and ether.
- **Clarity :** 1% w/v aqueous solution remains clear without haziness after autoclaving at 15 lbs pressure (121ºC) for 15 minutes.
- **pH :** pH of 2% w/v aqueous solution at 25ºC 6.3 - 7.3
- **Microbial Load :**

  Bacterial Count : <= 2000 CFU/gram by plate method, when incubated at 30-35ºC for not less than 3 days
  Yeast & mould Count : <= 100 CFU/gram by plate method, when incubated at 20-25ºC for not less than 5 days.
- **Test for pathogens :** 1. *Escherichia coli*- Absent/gram of sample 2. *Salmonella* species- Absent/10 gram of sample 3. *Pseudomonas aeruginosa*- Absent/gram of sample 4. *Staphylococcus aureus*- Absent/gram of sample 5. *Candida albicans*- Absent/gram of sample 6. *Clostridia*- Absent/gram of sample
- **Indole Test :** Tryptophan content: Passes

  Cultural response : Cultural response observed after an incubation for bacterial at 35-37ºC for 18-24 hours and for fungal at 20-25°C for not less than 5 days by preparing Soyabean Casein Digest Medium (M011) using Tryptone,ES, (Casitose, ES) as an ingredient.

Cultural Response

Organism	Growth
Staphylococcus aureus subsp. *aureus* ATCC 25923 (WDCM 00034)	Luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 6538 (WDCM 00032)	Luxuriant
Escherichia coli ATCC 25922 (WDCM 00013)	Luxuriant
Escherichia coli ATCC 8739 (WDCM 00012)	Luxuriant
Pseudomonas aeruginosa ATCC 27853 (WDCM 00025)	Luxuriant
Pseudomonas aeruginosa ATCC 9027 (WDCM 00026)	Luxuriant
Bacillus subtilis subsp. *Spizizenii* ATCC 6633 (WDCM 00003)	Luxuriant
Salmonella enterica subsp. *enterica* Typhimurium ATCC 14028(WDCM 00031)	Luxuriant
Salmonella enterica subsp.*enterica* serotype Abony NCTC 6017(WDCM 00029)	Luxuriant
Kocuria rhizophila ATCC 9341	Luxuriant
Streptococcus pneumoniae ATCC 6303	Luxuriant
Candida albicans ATCC 10231 (WDCM 00054)	Luxuriant
Aspergillus brasiliensis ATCC 16404 (WDCM 00053)	Luxuriant

Chemical Analysis :

Total Nitrogen : ≥13.00 %

Amino Nitrogen : ≥4.00 %

Sodium chloride : ≤5.00 %

Loss on drying : ≤5.00 %

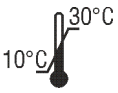
Residue on ignition : ≤15.00 %

Storage and Shelf Life

Store between 10- 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinicalsample must be decontaminated and disposed of in accordance with current laboratory techniques.



Storage temperature

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Revision **:** 13/2022

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CELLBIO™
in service of your CELL

Technical Datasheet

Cellulose Nitrate Filtration Membranes

Type: Gridded Cellulose Nitrate Membrane

Product code: SF97A-SF97F

Product description:

Gridded cellulose nitrate membrane are made up of cellulose nitrate material which assures effective retention with high flow rates. It has a fine uniform pore structure and high, non-specific adsorption. The grids do not interfere with microbial growth and allow typical colony morphology of bacteria on all culture media.

Features:

- High flow rate
- Uniform pore size distribution
- Low extractable levels
- Excellent for retention of yeasts and molds
- Printed grid facilitates easy counting of microbial colonies

Applications:

• Used for microbiological testing of water, beverages containing particles (such as fruit pulp), foods, pharmaceuticals cosmetics and air-borne bacteria.

Specifications:

*** Sterile**

Code	Diameter	Pore size	Water flow rate (ml/ min/cm^2) at ΔP=10psi, 20^{0}C	Maximum operating pressure (Kg/cm^2)	Sterile	Autoclavable	Packing
SF97A	47mm	0.22 µ	20	3	No	Yes	1x100 no
*SF97B		0.22 µ	20	3	Yes	No	100x1 no
SF97C		0.45 µ	46	3	No	Yes	1x100no
*SF97D		0.45 µ	46	3	Yes	No	100x1 no
SF97E		0.8 µ	259	3	No	Yes	1x100 no
*SF97F		0.8 µ	259	3	Yes	No	100x1 no

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Technical Datasheet

Cellulose Nitrate Filtration Membranes

Type: Gridded Cellulose Nitrate Membrane

Product code: SF97A-SF97F

Product description:

Gridded cellulose nitrate membrane are made up of cellulose nitrate material which assures effective retention with high flow rates. It has a fine uniform pore structure and high, non-specific adsorption. The grids do not interfere with microbial growth and allow typical colony morphology of bacteria on all culture media.

Features:

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- Uniform pore size distribution
- Low extractable levels
- Excellent for retention of yeasts and molds
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Applications:

• Used for microbiological testing of water, beverages containing particles (such as fruit pulp), foods, pharmaceuticals cosmetics and air-borne bacteria.

Specifications:

*** Sterile**

Code	Diameter	Pore size	Water flow rate (ml/ min/cm^2) at ΔP=10psi, 20^{0}C	Maximum operating pressure (Kg/cm^2)	Sterile	Autoclavable	Packing
SF97A	47mm	0.22 µ	20	3	No	Yes	1x100 no
*SF97B		0.22 µ	20	3	Yes	No	100x1 no
SF97C		0.45 µ	46	3	No	Yes	1x100no
*SF97D		0.45 µ	46	3	Yes	No	100x1 no
SF97E		0.8 µ	259	3	No	Yes	1x100 no
*SF97F		0.8 µ	259	3	Yes	No	100x1 no

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