Spore Strips (Steam Sterilization Monitor Strips)

DD032

Steam Sterilization Monitor Strips are used for evaluating sterilization process. These indicators which are specified by the U.S. military specification MIL-S- 36586 are GMP requirements of U.S. FDA.

Directions

Place indicators in the areas of the pack or load least accessible to steam. Places such as the geometrical center, and the upper and lower regions of both front and rear of the load to be sterilized are considered suitable areas for placement of these indicators. A standard procedure should be established for the routine evaluation of each sterilizer. On completion of the sterilization cycle, remove the indicators from the test loads and deliver them to the laboratory for testing. All sterility tests should be performed in a clean dust free transfer area, preferably under positive air pressure, using rigid aseptic technique throughout the test procedure.

Using sterile scissors, cut open one end of the envelope. Thereafter remove the indicator with sterile tweezers and aseptically transfer it to a tube of sterile Soyabean Casein Digest Medium w/ Yeast Extract and Ferric pyrophosphate (M207) or Soyabean Casein Digest Medium (M011). Incubate the tubes for seven days at 55 - 60°C. Observe the tubes daily. If turbidity develops, failure of the sterilization process is indicated.

Precautions

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

Principle And Interpretation

Bacillus stearothermophilus is a thermophilic bacteria which can grow at 65°C and above. The spores are highly heat resistant and are used to monitor autoclave performance (1).

Sterilisation is the freeing of an article from all living organisms including viable spores(1). Sterilization quality control can only be achieved through the use of calibrated biological indicators (endospores). These indicators consist of *Bacillus stearothermophilus* spores impregnated on chromatography paper strips, individually placed into envelopes. Number of spores present per strip : 10^6. These organisms are difficult to destroy because they are more resistant to heat than other vegetative bacteria and viruses. Therefore, if they are destroyed during sterilization,it is assumed that all other life forms are also destroyed. This test is considered the most sensitive check of the autoclaves efficiency.

Precautions :

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

Quality Control

Appearance
Filter paper strip impregnated with spores of standard culture of B.stearothermophilus

Number of spores
1000000 spores/strip

Cultural response
Sterility checking of the autoclave was carried out using Spore strip. After autoclaving, strip was inoculated in 100ml of st. Soyabean Casein Digest Medium(M011) and incubated at 55°C upto 7 days. An unexposed spore strip was also inoculated separately in 100ml M011

Growth	Unexposed Spore Strip	Exposed Spore Strip	Positive control	Negative control
Growth in M011	Luxuriant	No growth	Luxuriant	No growth

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Reference

1.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee J. G., Fraser A. G., Marmion B, P., Simmons A (Eds.), Churchill Livingstone, Edinburgh.

Revision : 1 / 2011

HIMEDIA®

Technical Data

Egg Yolk Emulsion (50 ml/100 ml per vial)

FD045

Sterile stabilized emulsion of egg yolk recommended for use in various culture media.

Composition

Ingredients	Concentration	
	(100 ml per vial)	(50 ml per vial)
Egg yolk	30ml	15ml
Sterile saline	70ml	35ml

Directions:

Warm up the refrigerated egg yolk emulsion to room temperature. Shake well to attain uniform emulsion. (Since on refrigeration emulsion has a tendency to form layers or small lumps). Aseptically add 50 ml emulsion in 950 ml of sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043/ Baird Parker Agar Base M043S/ Baird Parker HiVeg™ Agar Base MV043/ Baird Parker HiCynth™ Agar MCD043/ Baird Parker Agar (Agar Medium O) ME043/ Baird Parker Agar (Agar Medium O) M043B/ Baird Parker Agar Base, Granulated GM043I/ Baird Parker Agar Base, Granulated GM043/ Baird Parker Agar Medium (In accordance with IP 1996) MM043/ Baird Parker Agar Medium MU043/ Baird Parker Agar Base M043I / Mannitol Salt Agar Base M118/Mannitol Salt Agar Base, Granulated GM118/Mannitol Salt HiCynth™ Agar Base MCD118 / Mannitol Salt HiVeg™ Agar Base MV118/ Baird Parker Agar Base w/Sulpha M1140.

Aseptically add in 475 ml of sterile, molten, cooled (45-50°C) Bacillus Cereus Agar Base M833/ Bacillus Cereus HiVeg™ Agar Base MV833/ Bacillus Cereus HiCynth™ Agar Base MCD833

OR

Aseptically add 100 ml emulsion in 900 ml of sterile, molten, cooled (45-50°C) McClung Toabe Agar Base M387/ McClung Toabe HiVeg™ Agar Base MV387/K.R.A.N.E.P. Agar Base M583/K.R.A.N.E.P. HiVeg™ Agar Base MV583 / MYP Agar Base (Phenol Red Egg Yolk Polymyxin Agar Base) M636/ M636S/ MYP HiVeg™ Agar Base (Phenol Red Egg Yolk Polymyxin HiVeg™ Agar Base MV636/ MYP Agar Base, Granulated (Phenol Red Egg Yolk Polymyxin Agar Base, Granulated) GM636 / MYP HiCynth™Agar Base (Phenol Red Egg Yolk Polymyxin HiCynth™ Agar Base) MCD636/ KG Agar Base M658/KG HiVeg™ Agar Base MV658/ L.D. Egg Yolk Agar Base M744/ Egg Yolk Agar Base M808 / Egg Yolk Agar Base, HiVeg™ MV808/ Egg Yolk Agar Base, Modified M1043 / Modified MYP Agar Base M1139/ Bacillus cereus Selective Agar Base (MYP) ISO 7932 M1139I /Modified MYP HiVeg™ Agar Base MV1139. Aseptically add in 890 ml of sterile, molten, cooled (45-50°C) TPEY Agar Base M402/ TPEY HiVeg™ Agar Base MV402.

Aseptically add 450 ml of sterile, molten, cooled (45-50°C) in C. botulinum Isolation Agar Base M911/ C. botulinum Isolation HiVeg™ Agar Base MV911

OR

Aseptically add 25 ml emulsion in 475 ml of sterile, molten, cooled (45-50°C) Perfringens Agar Base T.S.C./S.F.P.AgarBase) M837/ Perfringens Agar Base, Granulated (Tryptose Sulphite Cycloserine Agar Base, Granulated) (T.S.C./S.F.P. Agar Base, Granulated) GM837/ Perfringens HiCynth™ Agar Base (T.S.C/S.F.P HiCynth™ Agar Base) MCD837/ Perfringens HiVeg™ Agar Base (T.S.C. / S.F.P. HiVeg™ Agar Base) MV837/ S.F.P. Agar Base M1005/ S.F.P. HiVegTM Agar Base MV1005.

OR

Aseptically add 80 ml emulsion in 920 ml of sterile, molten, cooled (45-50°C) Anaerobic Egg Agar Base M902 / Anaerobic Egg HiVeg™ Agar Base MV902.

OR

Aseptically add 20 ml emulsion in 90 ml of sterile, molten, cooled (45-50°C) Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base (PEMBA) M1484.

OR

Aseptically add 15 ml emulsion in 420 ml of sterile, molten, cooled (45-50°C) Willis and Hobb's Medium Base M1375.

OR

Aseptically add 7ml of Emulsion in 93ml of sterile, molten, cooled (45-50°C) Lipovitellin Salt Mannitol Agar Base M627.

OR

Aseptically add 2 vials of Clostridium Difficile Supplement (FD010), 40 ml of Egg Yolk Emulsion (FD045) together with 10 ml lysed horse blood in 1000 ml of sterile, molten, cooled (45-50°C) Clostridium Brazier Agar Base M1803

OR

Aseptically add 50ml of concetrated Egg yolk emulsion (FD045) and rehydrated contents of 1 vial of LM Selective Supplement (FD330) in 950 ml of sterile, molten, cooled (45-50°C) L.mono Selective Agar Base (LM Selective Agar Base) M1994.

Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - faeces, urine etc. ; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For Food samples follow appropriate techniques for handling specimens as per established guidelines (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & 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.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

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 (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. 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.
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.

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Revision :02/2022

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

CE Marking

2°C 8°C **Storage temperature**

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PTe 1% Selective Supplement (1 ml per vial)

FD052

(Final concentration after addition of 8.9 ml sterile distilled water = 1%)

Recommended for the selective isolation of *Staphylococci* and *Corynebacteria*.

Composition

To achieve 1% solution dilute the contents in 8.9 ml sterile distilled water.

Ingredients	Concentration
Potassium tellurite Concentrate	1.100ml

Directions:

Warm up the refrigerated contents of one vial to room temperature. Add aseptically 8.9 ml sterile distilled water, mix well and add in sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043B/ MM043 / MU043/ ME043/ Vogel Johnson Agar Base w/o Tellurite M023/ MM023/MU023/ Vogel Johnson HiVeg™ Agar Base w/o Tellurite MV023/ Vogel Johnson Agar w/1.5% Agar M023F/ Vogel Johnson HiCynth™ Agar Base w/o Tellurite (V.J. HiCynth™ Agar) MCD023/ Mycoplasma Broth Base w/ CV M268/ Mycoplasma HiVeg™ Broth Base w/CV MV268/ TPEY Agar Base M402/ TPEY HiVeg™ Agar Base MV402/ Tellurite Glycine Agar Base M448/ Cholera Medium Base M558/ Cholera HiVeg™ Medium Base MV558/ Giolitti-Cantoni Broth Base M584I / Dextrose Proteose Peptone Agar Base M734/ Dextrose Proteose Peptone HiVeg™ Agar Base MV734/ Cystine Tellurite Agar Base M881 / Diphtheria Virulence Agar Base M882 / Diphtheria Virulence HiVeg™ Agar Base MV882 / Tryptone Tellurite Agar Base M1056/ Baird Staphylococcus Enrichment Broth Base M1091/ Baird Staphylococcus Enrichment Broth Base, Granulated GM1091/ Tellurite Blood Agar Base M1260/ Mitis Salivarius Agar Base M259/ Mitis Salivarius HiVeg™ Agar Base MV259/ Monsur Medium Base M474/ HiCrome™ ECO157:H7 Agar, Modified M1574A / as desired. Mix well and dispense in sterile Petri plates or tubes.

Type of specimen

Clinical samples- Throat swab, nasal swab, wound swab, pus, etc.; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For food samples follow appropriate techniques for handling specimens as per established guidelines (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & 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.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

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 (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. 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.
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.

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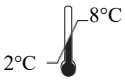

Storage temperature

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3951DB Maarn, NL
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Oxford Selective Supplement

FD071

An antimicrobial supplement recommended for selective isolation of *Listeria* species.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cycloheximide	200mg
Colistin sulphate	10mg
Acriflavin	2.500mg
Cefotetan	1mg
Fosfomycin	5mg

Directions:

Rehydrate the contents of 1 vial aseptically with 5 ml of 50% v/v aqueous ethanol. Mix gently to dissolve and aseptically add to 500 ml of sterile, molten, cooled (45-50ºC) Listeria Oxford Medium Base M1145/ Listeria Oxford Medium Base, Granulated GM1145/ Listeria Oxford Agar Base w/ 1.2% Agar M1145F/ Listeria Oxford HiVeg™ Medium Base MV1145/ Listeria Oxford HiCynth™ Medium Base MCD1145 / Listeria Oxford Medium Base, Modified M1781. Mix well and pour into sterile Petri plates.

Type of specimen

Food samples; Clinical samples - Blood, CSF samples

Specimen Collection and Handling

For food samples follow appropriate techniques for handling specimens as per established guidelines (1).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & 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.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

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 (2,3).

Reference

1. 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.
2. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
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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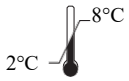
IVD ***In vitro*** **diagnostic medical device**

CE Marking



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CCDA Selective Supplement

FD135

An antibiotic supplement recommended for the selective cultivation of *Campylobacter* or *Arcobacter* species.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cefoperazone	16mg
Amphotericin B	5mg

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml of sterile distilled water. Mix well and aseptically add it to 500 ml of sterile, cooled (45-50°C) Blood Free Campylobacter Broth Base M1318 / Arcobacter Broth Base M1637 . Modified Charcoal Cefoperazone deoxycholate Agar Base M887I

Type of specimen

Clinical- stool, abscess; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). For food samples follow appropriate techniques for handling specimens as per established guidelines (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. For professional use. 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.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

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 (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. 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.
3. Salfinger Y. and Tortorello M. L., (Eds.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed.,APHA, Washington, D.C.
4. International Organization for Standardization (ISO), 110272-1:2017, Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method

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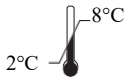

Storage temperature

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Glycerol, Hi-LR™ GRM081

Product Identifier

CAS No.	:	56-81-5
EC No.	:	200-289-5
Molecular Formula	:	$C_3H_8O_3$
Molecular Weight	:	92.09
HS Code	:	2905 45 00
Storage	:	Below 30°C
Shelf life	:	4 years

Technical Specification

Appearance	:	Colourless to faint yellow syrupy, very hygroscopic clear viscous liquid
Solubility	:	1 mL miscible in 1 mL of water
FTIR	:	Matches with the standard pattern
Refractive index (n 20/D)	:	1.4700 - 1.4750
Density (at 25°C)	:	1.245 - 1.255 g/mL
Sulphated ash	:	<= 0.01%
Sugars (Glucose)	:	<= 0.004%
Assay (NaOH Titration/GC)	:	98.00 - 102.00%

Safety Information

UN No.	:	Not dangerous goods
Class	:	-
Packing Group	:	-
RTECS	:	MA8050000
WGK	:	1

Soyabean Casein Digest Medium (Tryptone Soya Broth) M011

Intended Use:

Recommended as a general purpose medium used for cultivation of a wide variety of microorganisms and recommended for sterility testing of moulds and lower bacteria.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dextrose (Glucose)	2.500
Dipotassium hydrogen phosphate	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.0 grams in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

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

Soyabean Casein Digest Medium is recommended by various pharmacopeias as a sterility testing and as a microbial limit testing medium (1,2,3). This medium is a highly nutritious medium used for cultivation of a wide variety of organisms (4). The combination of Tryptone and soya peptone makes the medium nutritious by providing nitrogenous, carbonaceous substances, amino acids and long chain peptides for the growth of microorganisms. Dextrose/glucose serve as the carbohydrate source and dibasic potassium phosphate buffer the medium. Sodium chloride maintains the osmotic balance of the medium.

Type of specimen

Pharmaceutical samples, Clinical samples - urine, pus, wound samples.

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, processing as per pharmaceutical guidelines (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. Biochemical characterization is necessary to be performed on colonies from pure cultures for further identification.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.

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 (after sterilization). pH : 7.3±0.2

pH
7.10-7.50

Stability test
Light yellow coloured clear solution without any precipitation or sedimentation at room temperature for 7 days

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu (at 30-35°C for 18-24 hours for bacteria and 5days for fungal) Growth promotion is carried out as per USP/ EP/BP/JP/IP.

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Micrococcus luteus ATCC 9341	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant	20 -25 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Sterility Testing- Growth promotion+Validation				
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50 -100	luxuriant	20 -25 °C	<=3 d
# *Aspergillus brasiliensis* ATCC 16404 (00053*)	50 -100	luxuriant	20 -25 °C	<=5 d
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant	30 -35 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	20 -25 °C	<=3 d

Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant	20 -25 °C	<=3 d
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Abony NCTC 6017 *(*00029*)	50 -100	luxuriant	20 -25 °C	<=3 d
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli NCTC 9002	50 -100	luxuriant	20 -25 °C	<=3 d
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	20 -25 °C	<=3 d
Micrococcus luteus ATCC 9341	50 -100	luxuriant	20 -25 °C	<=3 d

Key : (#) Formerly known as *Aspergillus niger*, (*) 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. 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 (3,4).

Reference

1.Indian Pharmacopeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.
2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, M.d.
3.The United States Pharmacopeia, 2019, The United States Pharmacopeial Convention, Rockville, MD.
4.Forbes B. A., Sahm D. F. and Weissfeld A. S., 1998, Bailey & 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 : 04/2022

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**

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 :

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Bismuth Sulphite Agar (BS)

M027

Intended Use:

Recommended for selective isolation and enumeration of *Salmonella* species from food samples. The composition and performance criteria of this medium are as per specifications laid down in ISO 6579-1:2017.

Composition**

ISO 6579-1 Specification -Bismuth Sulphite Agar

Ingredients	Gms / Litre
Enzymatic digest of animal tissues Meat extract	10.000
	5.000
Dextrose	5.000
Disodium hydrogen phosphate, anhydrous	4.000
Ferrous sulphate, anhydrous	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.7±0.2

Bismuth Sulphite Agar

(BS) Ingredients	Gms / Litre
Peptone #	10.000
HM extract ##	5.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate, anhydrous	4.000
Ferrous sulphate, anhydrous	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.7±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Enzymatic digest of animal tissues ##-Equivalent to Meat extract

Directions

Suspend 52.33 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT STERILIZE IN AUTOCLAVE** or by fractional sterilization since overheating may destroy the selectivity of the medium. The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, which should be dispersed before pouring into sterile Petri plates.

Principle And Interpretation

The Salmonellae constitute the most taxonomically complex group of bacteria among*Enterobacteriaceae* (1). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Humans are the only reservoirs of *S.*Typhi (2). Of the various media employed for the isolation and preliminary identification of *Salmonellae*, particularly *Salmonella* Typhi; Bismuth Sulphite Agar is the most productive. Bismuth Sulphite Agar is a modification of original Wilson and Blair Medium (3-5). It is also recommended by various Associations (2,6-8) for the isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water, food and other products. Bismuth Sulphite Agar (M027I) is recommended for selective isolation and enumeration of *Salmonella* species in accordance with ISO Committee (8). *S.*Typhi, *S.*Enteritidis and *S.*Typhimurium typically grow as black colonies with or without a surrounding metallic sheen resulting from hydrogen sulphide production and reduction of sulphite to black ferric sulphide. *Salmonella* Paratyphi A grows as light green colonies. Bismuth Sulphite Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms. Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action towards gram-positive organisms and coliforms.

Peptone and HM extract serve as sources as carbon, nitrogen, long chain amino acids, vitamins and essential growth factors. Dextrose is the carbon source. Disodium phosphate maintains the osmotic equilibrium. Bismuth sulphite indicator along with brilliant green inhibits the intestinal gram-positive and gram-negative bacteria. Ferrous sulphate aids in detection of hydrogen sulphide production. In case of food samples, pre-enrichment of the sample is done prior to inoculation.

Type of specimen

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

Specimen Collection and Handling

Processesing : (8)

Pre-enrichment : Samples (25 grams in 225 ml) are pre-enriched 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 (M1428) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTT broth (M1496I) and incubated at 37± 1°C for 24 ± 3 hours. In-case of *Salmonella* Typhi and *Salmonella* Paratyphi A selective enrichment is carried out in Selenite Cystine broth and then incubated at 37± 1°C for 24 h ± 3 h and 48 h ± 3 h.

Isolation : The culture thus obtained is then plated on Bismuth Sulphite Agar (BS) (M027) and incubated at 37± 1°C for 24 ± 3 hours. An additional incubation of 24 ± 3 hours is recommended. Simultaneously plating on isolation agar XLD Agar, Modified (M031I) is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,10).

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. **DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM,** as it destroys the selectivity of the medium.
2. *S.*Typhi and *S.*Arizonae exhibit typical brown colonies , with or without metallic sheen.
3. This medium is highly selective and must be used in parallel with less selective media for isolation.
4. With certain *Salmonella* species, typical black colonies with metallic sheen is observed near heavy inoculation and isolated colonies may show green colonies.
5. Individual organisms differ in their growth requirement and may show variable growth patterns on 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 greenish yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish yellow coloured, opalescent with flocculent precipitate forms in Petri plates.

Reaction

Reaction of 5.23% w/v aqueous solution at 25°C. pH : 7.7±0.2.

pH

7.50-7.90

Cultural Response

Cultural response was observed after an incubation at 37 ± 1°C for 24 ± 3 hours. The plates are further incubated for an additional 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 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Selectivity & Specificity				
Escherichia coli ATCC 8739 (00012*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen
Escherichia coli ATCC 25922 (00013*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen

Selectivity				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0 %	-
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0 %	-
Additional testing				
Salmonella Typhi ATCC 6539	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Salmonella Paratyphi A ATCC 9150	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.

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 (7, 9).

Reference

1. Tindall B. J., Crimont P. A. D., Gorrity G. M., EUZESY B. P., 2005, Int. J. Sys. Evol. Microbiol., 55:521.
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.
3. Wilson and Blair, 1926, J. Pathol. Bateriol., 29:310.
4. Wilson and Blair, 1927, J. Hyg., 26:374.
5. Wilson and Blair, 1931, J. Hyg., 31:138.
6. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
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.
8. Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of Salmonella- Part I Detection of Salmonella . International Organization for Standardization (ISO), ISO/ DIS 6579-1:2017.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

Revision : 01/2022

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

10°C 30°C **Storage temperature**

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

CE **CE Marking**

Do not use if package is damaged

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, APHA.

Composition**

ISO 6579-1 Specification - XLD Agar

Ingredients	Gms / Litre
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	Gms / Litre
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 (7,9). 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 H2S 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 H2S 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

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 . Simultaneouslyy 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 (10,11).

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.
4.Taylor W. L. and Schelhart B., 1967, Am. J. Clin. Pathol., 48:356.
5.Taylor W. L. and Schelhart B., 1968, Am. J. Clin. Pathol., 16:1387.
6.Taylor W. L. and Schelhart B., 1969, Appl. Microbiol., 18.393-395.
7.Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of *Salmonella*- Part I Detection of Salmonella . International Organization for Standardization (ISO), ISO/DIS 6579-1:2017.
8.Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of *Salmonella*- Part I Detection of Salmonella . International Organization for Standardization (ISO), ISO/DIS 6579-1:2017/ Amd 1:2020.
9.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
10.Isenberg H. D., Kominos S., and Sigeal M., 1969, Appl Microbiol., 18, 656-659.
11.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 : 03/2022

Plate Count Agar (Standard Methods Agar)

M091

Intended use

Recommended for the determination of plate counts of microorganisms in food, water, waste water samples.

Composition**

Ingredients	Gms / Litre
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 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 2.35% 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 subtilis subsp. spizizenni 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.

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.
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.
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.
6. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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 : 04/2023

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	Gms / Litre
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.
9.9.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.

Revision :04/2022

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

30°C
10°C
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.

SIM Motility Medium, Modified

M181F

SIM Medium is recommended for determination of hydrogen sulphide production, indole formation and motility of enteric bacilli in accordance with FDA BAM.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	20.000
Peptic digest of animal tissue	6.100
Ferrous ammonium sulfate	0.200
Sodium thiosulfate	0.200
Agar	3.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 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

SIM Medium is recommended by FDA BAM, 1998 (1) to differentiate enteric bacilli particularly *Salmonella* and *Shigella* on the basis of sulphide production, indole formation and motility (2). Jordan and Victorson (3) reported that *Salmonella* Paratyphi A and Paratyphi B can be distinguished on the basis of H2S production using lead acetate. Sulkin and Willett (4) used Triple Sugar Iron Agar with 1% agar for motility along with H2S production and carbohydrate fermentation. Sosa (5) described a peptone medium with low agar for motility and indole determination.

Motility, indole and sulphide production tests are used to differentiate *Enterobacteriaceae* members. SIM Medium combines these three differential characteristics in a single medium. Peptonized iron and sodium thiosulphate are the indicators of H2S production. This H2S reacts with peptonized iron to form black precipitate of ferrous sulphide. *Salmonella* are H2S positive and *Shigella* are H2S negative. Motile organisms intensify the H2S reaction. Motile organisms grow away from line of inoculation showing diffused growth while non-motile organisms grow along the stabline. Motility detection is possible due to the semisolid nature of the medium. *Salmonella* is motile while *Shigella* are non motile. Tryptophan, from peptic digest of animal tissue, is degraded by specific bacteria to produce indole. The indole is detected by the addition of chemical reagents following the incubation period.

Inoculate fresh culture with a single stab using straight needle through the center of the medium. Following incubation, observe for motility (diffuse growth outward from the stabline or turbidity throughout the medium) and for H2S production (blackening of the medium). To detect indole production, add three or four drops of Kovacs reagent and observe for development of red color (positive reaction). Determine motility and H2S production prior to determination of indole production.

Quality Control

Appearance
Cream to beige homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium
Medium amber coloured slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 3.0% 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.

Cultural Response

Organism	Inoculum (CFU)	Growth	Motility	Indole production(on addition of Kovac's	H2S
Cultural Response					
Escherichia coli ATCC 25922	50-100	luxuriant	positive, growth away from stabline causing turbidity	positive reaction, red ring at the interface of the medium	negative reaction
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	positive, growth away from stabline causing turbidity	negative reaction	positive reaction, blackening of medium
Shigella flexneri ATCC 12022	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative reaction
Salmonella Paratyphi A ATCC 9150	50-100	luxuriant	positive, growth away from stabline causing turbidity	negative reaction	Negative reaction
Salmonella Paratyphi B ATCC 8739	50-100	luxuriant	positive, growth away from stabline causing turbidity	Negative reaction	Positive reaction, blackening of medium
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	Negative reaction	Negative reaction

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.MacFaddin, J. F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria vol. 1. Baltimore: Williams and Wilkins.
3.Jordan, E. O. and Victorson, R 1917. J. Inf. Dis, 21.
4.Sulkin, S. E. and Willett, J. C 1940. J. Lab. Clin. Med., 25.
5.Sosa, L 1943. Rev. Inst. Bacteriol, 11.

Revision : 2 / 2015

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	Gms / Litre
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 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. 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

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
Bacillus subtilis subsp. 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
Escherichia coli NCTC 9002	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Pseudomonas aeruginosa 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 %	-

Micrococcus luteus 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 : (#)- Formerly known as *Aspergillus niger*, (*) - 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.The United States Pharmacopoeia , 2020, The United States Pharmacopoeial Convention Inc., Rockville, MD.
2.Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi, 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.

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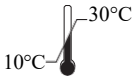
HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

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



Storage temperature

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

CE 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.

Modified Charcoal Cefoperazone Deoxycholate Agar Base (mCCD)M887I

Intended Use

Recommended for selective detection and enumeration of *Campylobacter* species from food chain. The composition and performance criteria of this medium are as per the specifications laid down in ISO 10272-2:2017.

Composition**

Ingredients	Gms / Litre
HM Extract #	10.000
Peptone ##	10.000
Tryptone ###	3.000
Sodium chloride	5.000
Sodium deoxycholate	1.000
Iron (II) sulfate, hydrate	0.250
Sodium pyruvate	0.250
Activated charcoal	4.000
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Meat extract

Enzymatic digest of animal tissues

Enzymatic digest of casein

Directions

Suspend 22.74 grams (the equivalent weight of dehydrated medium per litre) 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 CCDA Selective Supplement (FD135). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Campylobacters are carried in the intestinal tract of animal and therefore contaminate foods of animal origin (10). *Campylobacter* causes intestinal upset or abortion in animals. It is also one of the most important causes of human gastroenteritis, particularly in children. Initially blood was used in the isolation of *Campylobacter.* But, later it was reported by Bolton et al (3) that charcoal can be effectively used in place of blood. This rules out the variability obtained due to the use of blood.

Modified Charcoal Cefoperazone Deoxycholate Agar Base formulated as per APHA (10) and recommended by the ISO Committee (3) is used for selective isolation of *Campylobacter* species. Cephalothin in the original formulation was replaced by Cefoperazone as the selective agent since the latter gave better selectivity (4). *Campylobacter* species are highly resistant to cefoperazone, an antibiotic which effectively suppresses growth of *Pseudomonas* and *Enterobacteriaceae* (5, 6, 7). Addition of cefoperazone increases the selectivity of the medium. Due to this addition, the medium is also known as Campylobacter Charcoal Differential Agar (CCDA). Charcoal, sodium pyruvate and ferrous sulphate reduces the aero tolerance of medium by quenching photo chemically generated toxic oxygen derivatives (8).

Peptone, Tryptone and HM extract serve as sources of carbon, nitrogen, long chain amino acids and essential nutrients. Additional Amphotericin B suppresses the growth of yeast and mold contaminants. Colonies tend to swarm when initially isolated from clinical specimens.

Type of specimen

Food samples : meat and meat products.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,10,11).

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.
2. 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

Grey to black homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium

Black coloured, opaque gel forms in Petri plates

Reaction

Reaction of 4.55% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added CCDA SelectiveSupplement V(FD135),after an incubation at 41.5°C±1°C for 40 hours under microaerobic atmosphere.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Campylobacter coli ATCC 33559 (00004)*	50-100	good-luxuriant	>=50%	greyish,flat colonies, may have metallic sheen
Campylobacter jejuni ATCC 29428 (00005)*	50-100	good-luxuriant	>=50%	greyish,flat colonies, may have metallic sheen
Escherichia coli ATCC 25922 (00013)*	50-100	none-poor	<=10%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	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 (5,7).

Reference

1.Ahonkai V. I., et al, 1981, Antimicrob. Agents. Chemother.,20:850

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

3. Bolton F. J., Hutchinson D. N and Coates D., 1984, J. Clin. Microbiol., 19:169.

4. Hutchinson D. N and Bolton F.J., 1984, J. Clin. Pathol., 34:956.

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

6. Jones R. N., et al, 1980, Antimicrob. Agents. Chemother.,17:743

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.

8. Karmali M. A., et al, 1986, J. Clin. Microbiol., 23:456

9. Microbiology of food chain-Horizontal method for detection and enumeration of *Campylobacter spp*. International Organization for Standardization (ISO), 10272-2:2017.

10. Salfinger Y. and Tortorello M. L., (Eds.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed.,APHA, 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 : 01 / 2019

Listeria Oxford Medium Base

M1145

Intended use

Recommended for isolation of *Listeria* species from pathological specimens.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Lithium chloride	15.000
Sodium chloride	5.000
Corn starch	1.000
Esculin	1.000
Ammonium ferric citrate	0.500
Agar	10.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.75 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 the rehydrated contents of 1 vial of Oxford Listeria Supplement (FD071) or 1 vial of Listeria Moxalactam Supplement (FD126). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Listeria monocytogenes is the only species of the genus *Listeria* that is important as a human pathogen. *Listeria seeligeri, Listeria welshimeri* and *Listeria ivanovii* have been related with animal diseases. In any case, all the species are pathogenic between the ovine and bovine cattle. Positive diagnosis of listeriosis can be obtained only by the isolation and cultivation of the responsible bacteria from blood or CSF samples of the affected organisms. Listeria Oxford Medium Base is based on the formulation described by Curtis et al (2) for isolation of *L. monocytogenes* from clinical and food specimens. Peptone special serves as the source of essential nutrients to the organisms. Corn starch serves to neutralize the toxic metabolites formed. Lithium chloride and the antibiotics inhibit gram-negative bacteria and most gram-positive organisms but certain strains of Staphylococci may grow as esculin negative colonies. Cycloheximide is used to reduce fungal contamination; cefotetan and phosphomycin are inhibitors of bacterial overgrowth. Acriflavin, colistin sulphate and lithium chloride inhibit bacteria other than *Listeria* species. Alternatively moxalactam (FD126) can be added which inhibits both gram-positive and gram-negative bacteria. *L. monocytogenes* hydrolyzes esculin to esculetin and dextrose. Esculetin reacts with ferric ions and produces black zones around the colonies. Although the selectivity of the medium is enough to allow the isolation and differentiation by direct surface inoculation, a previous dilution of the inoculum is advisable or even more when the sample is highly polluted. The techniques for isolation vary with the material under examination (8). For all specimens selective and cold enrichment is recommended (3,4). For faecal and biological specimens, the sample is homogenized in 0.1% Peptone Water (M028) and 0.1 ml amount is either directly plated on Listeria Selective Medium or inoculated into the Selective Enrichment Broth and incubated at 30°C for 7 days and then further inoculated on Listeria Selective Medium. For food and environmental samples selective enrichment is generally used.

For isolation of Listeria from food (milk and milk products), add 25 ml or 25 grams of sample to 225 ml of Listeria Enrichment Broth, UVM (M890A). Homogenize and mix carefully. Incubate for 48 hours at 30°C. Streak the enriched cultures onto Listeria Oxford medium Base and incubate aerobically for 48 hours at 37°C. Take 5 typical colonies (esculin positive) and inoculate onto Soyabean Casein Digest Medium (M290). Incubate for 24 hours and then use these colonies for biochemical confirmation.

Type of specimen

Clinical samples - Body tissues or body fluids, 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 (1,7,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. 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 are needed for a final identification of the isolated organisms.

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 dark yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Dark amber coloured clear to slightly opalescent gel with a blue cast forms in Petri plates

Reaction
Reaction of 5.55% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Oxford Listeria Supplement (FD071) or Listeria Moxalactam supplement(FD126), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Bacillus subtilis ATCC 6633 (00003*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Enterococcus hirae ATCC 10541	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Listeria monocytogenes serovar 1 ATCC 19111 (00020*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Listeria monocytogenes ATCC 19112	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony

Listeria monocytogenes ATCC 19117	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good	40-50%	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 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. 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 (5,6).

References

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Curtis G. D. W., Mitchell R. G, King A. F., Griffin E. J., 1989,Lett. Appl. Microbiol.
3. Fernandez G. J. F., Dominguez R. L., Vazzuez B. J. A., Rodriguez F.E. F., Briones D. V., Blanco L. J. L., Suarez F. G., 1986, Can. J. Microbiol., 32:149.
4. Hayes P. S, Feeley J. L, Groves L. M, Ajello G. W. and Fleming D. W, 1986, Appl. Environ. Microbiol., 51:43
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. 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. Van Netten P., Peroles I., Van de Mosdik A., Curtis G. D. W., Mossel D. A. A, 1988, Int. J. Food Microbiol., 6:18
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 : 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

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