Lauryl Sulphate Broth (Lauryl Tryptose Broth)

M080

Intended use

Recommended for the detection of coliforms in water, wastewater, dairy products,other food and clinical samples.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.6 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared.

Principle And Interpretation

Coliforms are considered to be members of *Enterobacteriaceae*, which grow in the presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C (4). These bacteria can also be defined as, members of *Enterobacteriaceae* capable of growing at 37°C, that normally possess β-galactosidase (2). Lauryl Sulphate Broth is used for the detection of coliforms in water, dairy products and other foods, as recommended by APHA (1,9,10). It can also be used for the presumptive detection of coliforms in water, effluent or sewage by the MPN test (3). Lauryl Sulphate Broth was developed by Mallmann and Darby (8). Cowls (3) demonstrated that inclusion of sodium lauryl sulphate makes the medium selective for coliform bacteria. It was later investigated that Lauryl Sulphate Broth gave a higher colon index than the confirmatory standard methods media and also that gas production in Lauryl Sulphate Broth not only acts as a presumptive test but also as a confirmatory test for the presence of coliforms, in the routine testing of water (8). Lauryl Sulphate Broth is also recommended by the ISO Committee for the detection of coliforms (5).

Lauryl Sulphate Broth is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms. Aerobic spore-bearers are completely inhibited in this medium. Tryptose provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. Sodium lauryl sulphate inhibits organisms other than coliforms.For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate these tubes at 37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovacs reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of *Escherichia coli*. If no fermentation occurs in the tube incubated at 37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms. Broth becomes cloudy if stored at 2-8°C, but it gets cleared at room temperature.Refer appropriate references for standard procedures (3, 4, 5).

Type of specimen

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

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1,4).
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. Due to poor nutritional variations, some strains may show poor growth.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

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

pH

6.60-7.00

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Gas Production	Indole production (44°C)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	positive reaction, red ring at the interface of the medium
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	negative reaction, no colour development / cloudy ring
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction	negative reaction, no colour development / cloudy ring
Staphylococcus aureus subsp aureus ATCC 25923 (00034*)	>=10^4	inhibited		

Key : (#) Formerly known as *Enterobacter aerogenes (**) 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 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

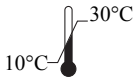
Reference

1. 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.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill, Livingstone
3. Cowls P. B., 1938, J. Am. Water Works Assoc., 30:979.
4. Department of Environment, Department of Health and Social Security, Public Health Laboratory Service, 1982, Methods for the Examination of Water and Associated Materials, The Bacteriological Examination of Drinking Water Supplies, 1982, Her Majestys Stationary Office, London.
5. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. Mallmann W. C. and Darby C. W., 1941, Am. J. Public Health, 31:127
9. 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.
10. 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 Marking



Storage temperature

Do not use if package is damaged

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Plate Count Agar (Standard Methods Agar)

M091

Intended use

Recommended for the determination of plate counts of microorganisms in food, water, waste water and also from clinical 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 (2) which is recommended by APHA (1,6,7) and FDA (3). 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

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

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4.5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1).
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. 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

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 casei 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. 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 (4,5).

Reference
1. 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.
2. Buchbinder L., Baris Y., Aldd E., Reynolds E., Dilon E., Pessin V., Pincas L. and Strauss A., 1951, Publ. Hlth. Rep., 66:327.
3. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
4. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. 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.
7. 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 : 02 / 2018

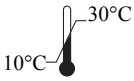
IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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Plate Count Agar

M091A

Intended Use:

Recommended for determining plate counts of microorganisms in milk and dairy products by pour plate technique.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	2.500
Dextrose (Glucose)	1.000
Agar	9.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17.5 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 equivalent to the medium recommended by APHA for the isolation of microorganisms in milk and other dairy products (1).

Tryptone provides amino acids and other complex nitrogenous substances. Yeast extract supplies Vitamin B complex. APHA recommends pour plate technique. The samples are diluted and appropriate dilutions are placed 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. Plate Count Agar is also used for the estimation of the number of live heterotrophic bacteria in water.

Type of specimen

Dairy samples

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow coloured homogeneous free flowing powder
Gelling
Firm, comparable with 0.9% Agar gel.
Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petriplates.

Reaction

Reaction of 1.75% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Bacillus subtilis ATCC 6633 (00003*)	50-100	luxuriant
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant
Lactobacillus acidophilus ATCC 4356 (00098*)	50-100	luxuriant
Lactobacillus casei ATCC 9595	50-100	luxuriant
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant
Streptococcus pyogenes ATCC 19615	50-100	luxuriant

Key :* - corresponding WDCM

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 (2,3).

Reference

1. American Public Health Association, 1978, Standard Methods for the Examination of Dairy Products, 14th ed., APHA Inc. Washington, D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. 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 :02 / 2019

Urea Agar Base, Christensen

M112I

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

a) adding glucose to the medium

b) decreasing the peptone concentration, and

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

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

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

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

Type of specimen

Pure isolate from clinical, food and water samples.

Specimen Collection and Handling

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

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

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

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance
Light yellow to light pink homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

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

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

pH
6.60-7.00

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

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

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

Storage and Shelf Life

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

Disposal

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

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Christensen W. B., 1946, J. Bacteriol., 52:461.
4. Farmer J. J. III, McWhorter A. C., Huntley G. A., Catignani J., J. Clin. Microbiol. 1975: 1 (1): 106-107.
5. International Organization for Standardization (ISO), ISO 6579-1:2017
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. MacFaddin J. F, 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williamsand Wilkins, Baltimore, Md.
9. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.Md.
10. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
11. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
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Brilliant Green Bile Broth

M121I

Intended Use:

Recommended for isolation and cultivation of coliform organismsfrom cream, yogurt and raw milk. The composition and performance criteria of this medium are as per the specifications laid down in ISO 4831:2006.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.51 grams (the equivalent weight of dehydrated medium per liter) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense the medium in quantities of 10ml in test tubes of approximately 16mm x 160mm containing Durham tubes. Sterilize in an autoclave set at 121°C for 15 minutes. Cool to 45-50°C.

Note: The Durham tube shall not contain air bubbles after sterilization.

Principle And Interpretation

Brilliant Green Bile Broth is formulated as per ISO 4831:2006(E) for confirmation of coliform bacteria (1) present in food samples or environmental samples in the area of food handling or food sampling.

Brilliant green and Dehydrated bile present in the medium inhibit gram-positive bacteria including lactose fermenting *Clostridia* (4). Production of gas from lactose fermentation is detected by incorporating inverted Durham's tube, indicates a positive evidence of faecal coliforms since nonfaecal coliforms growing in this medium do not produce gas. During examination of food samples or environmental samples, growth from presumptive positive tubes showing gas in Lauryl Tryptose Broth (M080) is inoculated in Brilliant Green Bile Broth wherein gas formation within 48 ± 2 hours confirms the presumptive test (1). Gram-positive spore-formers may produce gas if the bile or brilliant green inhibition is weakened by food material.

Type of specimen

Food samples

Specimen Collection and Handling:

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

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate.

Reaction

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

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Gas
Bacillus cereus ATCC 10876	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant	positive reaction
Enterobacter aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant	positive reaction
Citrobacter freundii ATCC 43864 (00006*)	50-100	good-luxuriant	positive reaction
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	negative reaction
Enterococcus faecalis ATCC 19433 (00009*)	50-100	none-poor	negative reaction
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. International Standard, ISO 4831:2006 (E). Microbiology of food and animal feeding stuff- Horizontal method for the detection and enumeration of coliforms- Most Probable number technique.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. McCrady and Langerin, 1932, J. Dairy Science, 15:321.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision :03 / 2019

EC Broth

M127

Intended Use:

Recommended for the selective enumeration of presumptive *Escherichia coli* by MPN technique from water samples and from clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Lactose	5.000
Bile salts mixture	1.500
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

EC Medium is used for detection of coliforms during bacteriological examination of water, milk and foods. It was originally described by Hajna and Perry (3). This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests (2). It is also used in MPN methods (1) and is often used for confirmation of coliforms. The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or non-fecal) when used as a confirmatory test (6). EC Broth should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of faecal coliforms is required. Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Lactose is the fermentable sugar. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal Streptococci. Phosphates control the pH during fermentation of lactose. Gas production in a fermentation tube within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish) or 45.5°C for foods.

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows:

Transfer a loopful of culture from all the tubes of Lauryl Sulphate Broth (M080) showing gas formation within 24 hours and from all the tubes showing xbacterial growth within 48 hours to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive.

Calculate the density of the faecal coliform organisms by using MPN tables. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (8).

Gas formation at 44.5°C or 45.5°C (and 37°C) *Escherichia coli*, possibly also other coliforms.

Gas formation at 37°C Coliform bacteria without *Escherichia coli*-

Type of specimen

Clinical - faeces ; Food samples; Water sample.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,9,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1)

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.For identification, organisms must be in pure culture.
2.Morphological, biochemical and/or serological tests should be performed for final identification.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

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

Reaction
Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 44.5°C ± 0.2 for 24 hours.

Organism	Inoculum (CFU)	Growth	Gas
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	positive reaction
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	fair to good	negative reaction
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
# Klebsiella aerogenes ATCC 13048 (00175*)	>=10^4	inhibited	

Key *- Corresponding WDCM Numbers ; # - Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 (4,5).

Reference

1. 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.
2. Fishbein M. and Surkiewicz B. F., 1964, Appl. Microbiol., 12:127.
3. Hajna A. A. and Perry C. A., 1943, Am. J. Public Health, 33:550.
4.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
7. Marshall, (Ed.), 1993, Standard Methods for the Examination of Dairy Products, 16th Ed., American Public Health Association, Washington, D.C.
8. Ray B., 1986, J. Food Prot., 49:651. 6. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
9. 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.
10.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 : 04 / 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

Disclaimer :

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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 (3) for the cultivation of certain anaerobes isolated from wounds. The present formulation is a modification, also called as Chopped M-Medium (7), 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 (6,7). FDA has recommended this medium for enumeration and identification of *Clostridium perfringens* from foods (10).

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* (3).

Type of specimen

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

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,9).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. 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

* - 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 (4,5).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Collins C. H., Lyne P. M., Grange J. M., 1985, 7th Ed., Microbiological Methods.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. MacFaddin J. F., 1985, Media for Isolation - Cultivation - Identification - Maintenance of Medical bacteria, Vol. I, Williams & Wilkins, Baltimore.
7. 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.
8. Robertson, 1916, J. Pathol. Bacteriol., 20:327.
9. 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.
10. U. S. Food and Drug Administration, 1984, Bacteriological Analytical Manual, 6th Ed., AOAC, Arlington, Va.

Revision :03 / 2018

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

Motility Test Medium

M260

Intended Use:

Recommended for detection of bacterial motility.

Composition**

Ingredients	Gms / Litre
Tryptose	10.000
Sodium chloride	5.000
Agar	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow tubed medium to cool to 45-50°C in an upright position.

Principle And Interpretation

Bacterial motility can be observed directly on microscopic slide or it can be visualized on motility media having agar concentration of 0.4% or less (4). Use of such semisolid media to observe or detect motility was reported by Tittsler and Sandholzer (6). Motility Test Medium is a modification of their formulation. Motility can be visualized as a diffused zone of growth flaring out from the line of inoculation (4). Hanging-drop technique in motility tests has practical difficulties, which is efficiently eliminated by use of culture-based methods using semi-solid media, as in semisolid media; the results obtained are macroscopic and cumulative.

Tryptose serve as a source of essential growth nutrients required for bacterial metabolism. Sodium chloride maintains the osmotic equilibrium of the medium. Small amount of agar helps to create a semisolid medium.

Bacterial motility can be observed directly by examination of the tubes following incubation. Inoculation is done by stabbing through the centre of the medium. Incubate at appropriate temperature for 18-40 hours. Non-motile organisms grow only along the line of inoculation whereas motile organisms grow away from the line of inoculation or may show growth even throughout the medium. All weak or equivocal motility results should be confirmed by flagellum stain or by direct wet microscopy (hanging drop) (1,5).

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

With inoculating needle, stab centre of medium to approximately one-half of depth.(5)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1.Growth from an 18-24 hr pure culture should be used. (5)

2. All weak or equivocal motility results should be confirmed by flagellum stain or by direct wet microscopy (hanging drop) (1,5).

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.5% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in tubes as butts

Reaction

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

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Motility
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive, growth away from stabline causing turbidity
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	positive, growth away from stabline causing turbidity
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	luxuriant	positive, growth away from stabline causing turbidity
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. DAmato R. F., and Tomfohrede K. M., 1981, J. Clin. Microbiol., 14 (3), 347-348.

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

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

4.Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., (Eds.), 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company.

5.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

6.Tittsler R. P. and Sandholzer L. A., 1936, J. Bacteriol., 31:575.

Revision :03/ 2019

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

M290

Intended use

Soyabean Casein Digest Agar is a general purpose medium used 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 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 (6, 3).

Tryptone Soya Agar conforms as per USP (6) and is used in microbial limit test and antimicrobial preservative - effective test. Gunn et al (2) 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 (1).

Type of specimen

Pharmaceutical samples, Clinical samples- blood and other body fluids

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For Pharmaceutical samples follow appropriate techniques for sample collection, handling and processing as per pharmacopeias. 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.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

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

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 perfringenes ATCC 13124 (00007)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

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 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 (4,5).

Reference

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

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

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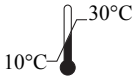
6. The United States Pharmacopoeia , 2019, The United States Pharmacopoeial Convention Inc., Rockville, MD.

Revision : 02/ 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature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Lysine Decarboxylase Broth without Peptone

M376I

Lysine Decarboxylase Broth w/o Peptone are used for differentiating *Salmonella* Arizonae from the Bethesda Ballerup group of *Enterobacteriaceae* .

Composition**

Ingredients	Gms / Litre
L-Lysine hydrochloride	5.000
Yeast extract	3.000
Dextrose	1.000
Bromocresol purple	0.015
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.01 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense 5 ml amount into screw-capped test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medum in an upright position and overlay with 2-3 ml of sterile mineral oil.

Principle And Interpretation

Decarboxylase media were first described by Moeller (1-3) for detecting lysine and ornithine decarboxylase and arginine dihydrolase. Falkow developed a lysine decarboxylase medium for the identification and differentiation of *Salmonella* and *Shigella* (4). Falkows Medium was further modified by Taylor (5) by deleting peptone from the formulation (M376I), thus eliminating false positives caused by *Citrobacter freundii* and its paracolons. Taylor's modification has same advantage of Falkow's formulation over Moeller; it does not require the special conditions of anaerobic culture and low pH.

During the initial stages of incubation, fermentation of dextrose by the organisms, with acid production results in a colour change of the indicator to yellow. On further incubation, if L-Lysine is decarboxylated to cadaverine, there will be an alkaline reaction and the indicator colour will then revert back to purple. If the colour remains yellow, the decarboxylase reaction is negative.

Yeast extract provide essential growth nutrients. Dextrose is the fermentable carbohydrate and bromo cresol purple is the pH indicator. Dextrose non-utilizers will not show any change in the medium colour. Use light inocula and do not read the tests under 24 hours incubation as some organisms require longer incubation time of upto 4 days.

Inoculate 25 grams of the test sample into Buffered Peptone Water (M614S). After incubation at 35-37°C for 16-20 hours, inoculate into RVS Broth (M1491) and Fluid Selenite Cystine Broth (M1533I) and incubate at 35-37°C for 24-48 hours. From the second enrichment, streak a loopful on Brilliant Green Agar Base w/ phosphates (M971S). Presumptive *Salmonella* so isolated on M971S are further confirmed by performing biochemical testing using the following media i.e. Nutrient Agar, pH 7.0 (M561A), Triple Sugar Iron Agar (M021S), Urea Agar Base, Christensen (M112I), Lysine Decarboxylase Broth w/o peptone (M376I), VP test, Indole test.

Quality Control

Appearance
Light yellow to greenish yellow homogeneous free flowing powder

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

Reaction
Reaction of 0.9% w/v aqueous soloution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response

M376I: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Lysine decarboxylation
Citrobacter freundii ATCC 8090	50-100	variable reaction
Escherichia coli ATCC 25922	50-100	variable reaction
Enterobacter aerogenes ATCC 13048	50-100	positive reaction, purple colour
Klebsiella pneumoniae ATCC 13883	50-100	positive reaction, purple colour
Proteus mirabilis ATCC 25933	50-100	negative reaction, yellow colour
Proteus vulgaris ATCC 13315	50-100	negative reaction, yellow colour
Salmonella Arizonae ATCC13314	50-100	Positive reaction, purple colour
Salmonella Paratyphi A ATCC 9150	50-100	negative reaction, yellow colour
Salmonella Typhi ATCC 6539	50-100	positive reaction, purple colour
Serratia marcescens ATCC 8100	50-100	positive reaction, purple colour
Shigella dysenteriae ATCC 13313	50-100	negative reaction, yellow colour

Storage and Shelf Life

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

Reference

1. Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:102.
2. Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:259.
3. Moeller V., 1955, Acta. Pathol. Microbiol. Scand., 36:158.
4. Falkow, 1958, Am. J. Clin. Pathol., 29:598.
Taylor W. I., 1961, Appl. Microbiol., 9:487.

Revision : 2 / 2015

Yeast extract Agar, Modified

M456I

Yeast extract Agar, Modified is recommended for enumeration of microorganisms from water.

Composition**

Ingredients	Gms / Litre
Tryptone	6.000
Yeast extract	3.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Yeast Extract Agar, Modified is a non selective medium formulated according to the ISO specification ISO 6222:1999 for enumeration of microorganisms from water.

Necessary growth nutrients are provided by tryptone and yeast extract. These serve as source of nitrogen, vitamins, growth factors as well as crude source of carbon. Agar acts as a solidifying agent.

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to very slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 2.4% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=70%
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%

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. ISO 6222:1999 water quality Enumeration of culturable microorganisms Colony count by incubation in a nutrient agar culture medium.

Revision : 02 / 2015

Bile Esculin Azide Agar

M493I

Bile Esculin Azide Agar is a selective medium used for isolation and presumptive identification of fecal Streptococci. The composition and performance criteria of this medium are as per the specifications laid down in ISO 7899-1:1984.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Peptic digest of animal tissue	3.000
Yeast extract	5.000
Oxgall	10.000
Sodium chloride	5.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.65 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

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

Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (6) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (7, 8) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (9) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar (6, 8) as per Isenberg (10). In this medium the bile concentration is reduced and additional sodium azide is incorporated. Bile Esculin Azide Agar, recommended by the ISO Committee (11) is a modification of Bile Esculin Azide Agar (M493), in the type of carbon sources used.

Casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall and sodium azide inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (12). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3).

Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate, preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation. All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (11). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

Quality Control

Appearance
Light yellow to brownish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Amber coloured, clear to slightly opalescent solution with a bluish tinge forms in Petri plates.

Reaction
Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Cultural Response				
Enterococcus faecalis ATCC 29212	50-100	luxuriant	>=50%	positive reaction,blackening of medium around the colony
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	50-100	good	40-50%	negative reaction
Proteus mirabilis ATCC 25933	50-100	good	40-50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction

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. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company
2. Meyer and Schonfeld, 1926, Zentralbl. Bakeriol, Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
4. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.
5. Facklam R., 1973, Appl. Microbiol., 26:138.
6. Swan, 1954, J. Clin. Pathol., 7:160.
7. Facklam R., 1972, Appl. Microbiol., 23:1131.
8. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.
9. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
10. Isenberg, 1970, Clin. Lab. Forum, July.
11. International Organization for Standardization (ISO), 2000, Draft, ISO/DIS 7899-2.
12. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

Revision : 2 / 2015

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Slanetz and Bartley Medium

M612I

Intended use

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

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Yeast extract	5.000
Dextrose	2.000
Dipotassium hydrogen phosphate	4.000
Sodium azide	0.400
2,3,5-Triphenyl tetrazolium chloride	0.100
Agar	15.000
Final pH (at 25°C)	7.2±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

Slanetz and Bartley Medium was originally devised by Slanetz and Bartley (9) for the detection and enumeration of Enterococci by membrane filtration technique. It can be also used as a direct plating medium (2,8). M612I differs from M612 in the type of buffering system used. This medium composition is as per specifications laid in ISO (5).

Tryptose and yeast extract serves as a source of essential nutrients along with B-complex vitamins and nitrogenous nutrients. The medium is highly selective for Enterococci. Sodium azide has inhibitory effect on gram-negative organisms. Triphenyl Tetrazolium Chloride is reduced to the insoluble formazan inside the bacterial cell forming dark red-coloured colonies. When the medium is incubated at higher temperature (44-45°C), all red or maroon colonies can be considered as presumptive Enterococci (7,10)

The Department of Health (3) has recommended this medium to be used for enumeration of Enterococci in water supplies. Water is filtered through a membrane filter which is then placed on the surface of the Slanetz and Bartley Medium plates and incubated at 35°C for 4 hours and then at 44-45°C for 44-48 hours. Red or maroon colonies are counted as Enterococci. The preliminary incubation at 35°C helps for the recovery of stressed organisms. Not all the species reduce TTC, hence pale colonies also should be considered. Food samples are homogenized and so diluted with physiological saline to give 15-150 colonies on each petri plate. Homogenates or dilutions are spread on agar surface and incubated at 35°C for 48 hours. Pink or dark red colonies with a narrow whitish border are counted (8).

Type of specimen

Water samples.

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.10 -7.30

Cultural Response

Cultural characteristics observed after an incubation at 44-45°C for 44-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecalis ATCC 19433 (00009*)	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecalis WDCM 00176	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecium ATCC 6057 (00177*)	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecium WDCM 00178	50-100	good-luxuriant	>=50%	red or maroon
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus ATCC* 6538 (00032*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^4	inhibited	0%	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,6).

Reference

1. 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.
2. Burkwall M.K. and Hartman P.A., 1964, Appl. Microbiol., 12:18.
3. Department of Health and Social Security, 1982, Report 71, HMSO, London.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. ISO 7899-2: 2000 Standard for Water Quality - Detection and enumeration of intestinal enterococci - Part 2 : Membrane filtration method.
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. Mead G.C., 1966, Proc. Soc. Wat. Treat. Exam., 15:207.
8. Nordic Committee on Food Analysis, 1968, Leaflet 68.
9. Slanetz L. W. and Bartley C.H., 1957, J. Bact., 74:591.
10. Taylor E.W. and Burman N.P., 1964, J. Appl. Bact., 27:294.

Revision : 05/ 2019

Lactobacillus MRS Agar

M641

Intended use

Recommended for cultivation of all *Lactobacillus* species from clinical and non- clinical samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM Peptone B #	10.000
Yeast extract	5.000
Dextrose (Glucose)	20.000
Tween 80 (Polysorbate 80)	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium hydrogen phosphate	2.000
Agar	12.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 67.15 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

Lactobacilli MRS medium is based on the formulation of deMan, Rogosa and Sharpe (2) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (2), dairy products (6), foods (8), faeces (7) and other sources (5).

Proteose peptone and HM peptone B supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms. Magnesium sulphate and manganese sulphate provide essential ions for multiplication of lactobacilli. Phosphates provide good buffering action in the media.

Lactobacilli are microaerophilic and generally require layer plates for aerobic cultivation on solid media. When the medium is set, another layer of un-inoculated MRS Agar is poured over the surface to produce a layer plate (5). Lactobacilli isolated on MRS Agar should be further confirmed biochemically.

Type of specimen

Clinical samples - Faeces; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,8,10).
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. Due to nutritional variation , some strains may show poor growth.

Performance and Evaluation

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

Quality Control

Appearance

Cream to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 6.71% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH

6.30-6.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer.(with 5% CO2)

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus casei ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	luxuriant	>=50%
Lactobacillus saki ATCC 15521(00015)*	50-100	luxuriant	>=70%
Lactobacillus lactis ATCC 19435(00016)*	50-100	luxuriant	>=70%
Pediococcus pentosaceas ATCC 33316(00158)*	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922(00013)*	>=10^4	Inhibition	0%
Bacillus cereus ATCC 11778(00001)*	>=10^4	Inhibition	0%

Key: * Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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

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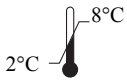
Revision : 04 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Dichloran Glycerol Medium Base

M1129

Intended use

Recommended for selective isolation of xerophilic moulds from food and clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.500
Dichloran	0.002
Chloramphenicol	0.100
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.8 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Add 110 grams of glycerol (Analytical Reagent Grade). Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Dichloran Glycerol Medium was formulated by Hocking and Pitt (2) and is recommended for isolation and enumeration of xerophilic moulds from dried and semidried foods. The glycerol at 18% (w/w) lowers the water activity (aw) from 0.999 to 0.95 (1) without causing any problem. This restrictive characteristic makes the medium especially suitable for foods. Peptone provides carbon, nitrogen, vitamins and minerals. Dextrose (Glucose) is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi.This medium can also be used for isolation of fungi from clinical samples. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing moulds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species.

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

Type of specimen

Clinical samples - skin scrapings, Food samples

Specimen Collection and Handling:

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

Warning and Precautions

In Vitro diagnostic Use. 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. Due to nutritional variations some strains may show poor growth.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.16% w/v aqueous solution 22 grams of glycerol at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed with added 22 grams of glycerol after an incubation at 25°C for upto 6 days.

Organism	Inoculum (CFU)	Growth	Recovery
Bacillus subtilis subsp spizizenii ATCC 6633 (00003)*	>=10^4	inhibited	0%
Candida albicans ATCC 10231 (00054)*	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	0%
Mucor racemosus ATCC 42647	-	good-luxuriant	
Saccharomyces cerevisiae ATCC 9763 (00058)*	50-100	good-luxuriant	>=50%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

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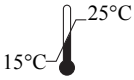

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