2.Eaton A.D, Clesceri L.S. Greenberg. A.W, 2005, Standard Methods for the Examination of Water and wastewater, 21st edn, APHA. Washington. DC.
3.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee, Duguid, Fraser and Marmion (Eds.), Churchill Livingstone, Edinburgh.

Revision : 1 / 2011

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Lysine Hydrochloride discs

DD049

Lysine Hydrochloride discs are used for lysine decarboxylation test.

Directions

To determine lysine decarboxylation, the Lysine disc (DD049) is added in the Decarboxyalse Broth Base, Moeller (M393) which is used as a negative control for studying decarboxylation or as a base for the addition of amino acids. The test organism is inoculated into the broth containing the Lysine disc (DD049). The inoculated tubes are overlaid with sterile mineral oil and incubated at 35-37°C for up to 4 days. A purple colour indicates the lysine decarboxylation.

Principle And Interpretation

Amino acid discs are used to differentiate the microorganisms on the basis of their ability to decarboxylate the amino acids. Lysine is an essential amino acid. Moeller introduced the Decarboxylase Broth for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase (1). Prior to Moellers work, bacterial amino acid decarboxylases were studied by Gale (2), Gale and Epps (3). Moeller Decarboxylase Broth Base (M393) contains dextrose which is the fermentable carbohydrate and pyridoxal is the co-factor for the decarboxylase enzyme. Bromo cresol purple and cresol red are the pH indicators in this medium. When the medium is inoculated with the dextrose fermenting bacteria, the pH is lowered due to acid production, which changes the colour of the indicator from purple to yellow. Acid produced stimulates decarboxylase enzyme. Decarboxylation of lysine yields cadaverine. Formation of this amine increases the pH of the medium, changing the colour of the indicator from yellow to purple. If the organisms do not produce the appropriate enzyme, the medium remains acidic, yellow in colour. Inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalinization at the surface of the medium which makes the test invalid.

Positive Test: Colour of the medium changes from yellow to purple

Negative Test: Colour of the medium changes to yellow or there is no change

Quality Control

Appearance

Filter paper discs of 10 mm diameter

Cultural Response

Cultural characteristics observed in Moeller Decarboxylase Broth Base (M393) with added Lysine Hydrochloride discs (DD049) after an incubation at 35-37°C upto 4 days (Inoculated tubes are overlaid with sterile mineral oil) .

Cultural Response

Organism	Inoculum (CFU)	Lysine decarboxylation
Cultural Response		
Citrobacter freundii ATCC 8090	50-100	negative reaction, yellow colour
Enterobacter aerogenes ATCC 13048	50-100	positive reaction, purple colour
Escherichia coli ATCC 25922	50-100	variable reaction
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
Pseudomonas aeruginosa ATCC 9027	50-100	negative reaction, yellow 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
Shigella flexneri ATCC 12022	50-100	negative reaction, yellow colour
Shigella sonnei ATCC 25931	50-100	negative reaction, yellow colour

Storage and Shelf Life

Store the discs at 10-30°C. Use before expiry date on the label.

Reference

1. Moeller V., 1955, Acta Pathol. Microbiol. Scand. 36:158.
2. Gale G. F., 1940, Biochem. J., 34:392.
3. Gale and Epps, 1943,Nature, 152:327.

Revision : 1 / 2011

Arginine Hydrochloride discs

DD050

Arginine hydrochloride discs are used for Arginine hyrolysation test.

Directions

To determine Arginine hydrolysation, the Arginine disc (DD050) is added in the Decarboxyalse Broth Base, Moeller (M393) which is used as a negative control for studying hydrolysation or as a base for the addition of amino acids. The test organism is inoculated into the broth containing the Arginine disc (DD050). The inoculated tubes are overlaid with sterile mineral oil and incubated at 35-37°C for up to 4 days. A purple colour indicates the Arginine hydrolysation.

Principle And Interpretation

Amino acid discs are used to differentiate the microorganisms on the basis of their ability to decarboxylate the amino acids. Arginine is an non essential amino acid. Moeller introduced the Decarboxylase Broth for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase (1). Prior to Moellers work, bacterial amino acid decarboxylases were studied by Gale (2), Gale and Epps (3). Moeller Decarboxylase Broth Base (M393) contains dextrose which is the fermentable carbohydrate and pyridoxal is the co-factor for the decarboxylase/ dihydrolase enzyme. Bromo cresol purple and cresol red are the pH indicators in this medium. When the medium is inoculated with the dextrose fermenting bacteria, the pH is lowered due to acid production, which changes the colour of the indicator from purple to yellow. Acid produced stimulates dihydrolase enzyme. Hydrolysation of arginine yields putrescine. Formation of this amine increases the pH of the medium, changing the colour of the indicator from yellow to purple. If the organisms do not produce the appropriate enzyme, the medium remains acidic, yellow in colour. Inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalinization at the surface of the medium which makes the test invalid.

Positive Test: Colour of the medium changes from yellow to purple.

Negative Test: Colour of the medium changes to yellow or there is no change

Quality Control

Appearance

Filter paper discs of 10 mm diameter

Cultural Response

Cultural characteristics observed in Moeller Decarboxylase Broth Base (M393) with added Arginine Hydrochloride discs (DD050) after an incubation at 35-37°C up to 4 days (Inoculated tubes are overlaid with sterile mineral oil) .

Cultural Response

Organism	Inoculum (CFU)	Arginine decarboxylation
Cultural Response		
Citrobacter freundii ATCC 8090	50-100	variable reaction
Enterobacter aerogenes ATCC 13048	50-100	negative reaction, yellow colour
Escherichia coli ATCC 25922	50-100	variable reaction
Klebsiella pneumoniae ATCC 13883	50-100	negative reaction, yellow colour
Proteus mirabilis ATCC 25933	50-100	negative reaction, yellow colour

Proteus vulgaris ATCC 13315	50-100	negative reaction, yellow colour
Pseudomonas aeruginosa ATCC 9027	50-100	positive reaction, purple colour
Salmonella Paratyphi A ATCC 9150	50-100	delayed positive reaction/ positive reaction, purple colour
Salmonella Typhi ATCC 6539	50-100	delayed positive reaction / negative reaction, yellow colour
Serratia marcescens ATCC 8100	50-100	negative reaction, yellow colour
Shigella dysenteriae ATCC 13313	50-100	delayed positive reaction/ negative reaction, yellow colour
Shigella flexneri ATCC 12022	50-100	delayed positive reaction/ negative reaction, yellow colour
Shigella sonnei ATCC 25931	50-100	variable reaction

Storage and Shelf Life

Store the discs at 10-30°C. Use before expiry date on the label.

Reference

1. Moeller V., 1955, Acta Pathol. Microbiol. Scand. 36:158.
2. Gale G. F., 1940, Biochem. J., 34:392.
3. Gale and Epps, 1943,Nature, 152:327.

Revision : 1 / 2011

Ornithine Hydrochloride Discs

DD051

Ornithine Hydrochloride discs are used for Ornithine decarboxylation test.

Directions

To determine ornithine decarboxylation, the Ornithine disc (DD051) is added in the Decarboxyalse Broth Base, Moeller (M393) which is used as a negative control for studying decarboxylation or as a base for the addition of amino acids. The test organism is inoculated into the broth containing the Ornithine disc (DD051). The inoculated tubes are overlaid with sterile mineral oil and incubated at 35-37°C for up to 4 days. A purple colour indicates the Ornithine decarboxylation.

Principle And Interpretation

Amino acid discs are used to differentiate the microorganisms on the basis of their ability to decarboxylate the amino acids. Ornithine is an essential amino acid. Moeller introduced the Decarboxylase Broth for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase (1). Prior to Moellers work, bacterial amino acid decarboxylases were studied by Gale (2), Gale and Epps (3). Moeller Decarboxylase Broth Base (M393) contains dextrose which is the fermentable carbohydrate and pyridoxal is the co-factor for the decarboxylase enzyme. Bromo cresol purple and cresol red are the pH indicators in this medium. When the medium is inoculated with the dextrose fermenting bacteria, the pH is lowered due to acid production, which changes the colour of the indicator from purple to yellow. Acid produced stimulates decarboxylase enzyme. Ornithine decarboxylation yields putrescine. Formation of this amine increases the pH of the medium, changing the colour of the indicator from yellow to purple. If the organisms do not produce the appropriate enzyme, the medium remains acidic, yellow in colour. Inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalinization at the surface of the medium which makes the test invalid.

Positive Test: Colour of the medium changes from yellow to purple

Negative Test: Colour of the medium changes to yellow or there is no change

Quality Control

Appearance

Filter paper discs of 10 mm diameter

Cultural Response

Cultural characteristics observed in Moeller Decarboxylase Broth Base (M393) with added Ornithine Hydrochloride discs (DD051) after an incubation at 35-37°C up to 4 days (Inoculated tubes are overlaid with sterile mineral oil) .

Cultural Response

Organism	Inoculum (CFU)	Ornithine decarboxylation
Cultural Response		
Citrobacter freundii ATCC 8090	50-100	variable reaction
Enterobacter aerogenes ATCC 13048	50-100	positive reaction, purple colour
Escherichia coli ATCC 25922	50-100	variable reaction
Klebsiella pneumoniae ATCC 13883	50-100	negative reaction, yellow colour
Proteus mirabilis ATCC 25933	50-100	positive reaction, purple colour

Proteus vulgaris ATCC 13315	50-100	negative reaction, yellow colour
Pseudomonas aeruginosa ATCC 9027	50-100	negative reaction, yellow colour
Salmonella Paratyphi A ATCC 9150	50-100	positive reaction, purple colour
Salmonella Typhi ATCC 6539	50-100	negative reaction, yellow colour
Serratia marcescens ATCC 8100	50-100	positive reaction,purple colour
Shigella dysenteriae ATCC 13313	50-100	negative reaction, yellow colour
Shigella flexneri ATCC 12022	50-100	negative reaction, yellow colour
Shigella sonnei ATCC 25931	50-100	positive reaction, purple colour

Storage and Shelf Life

Store the discs at 10-30°C. Use before expiry date on the label.

Reference

1. Moeller V., 1955, Acta Pathol. Microbiol. Scand. 36:158.
2. Gale G. F., 1940, Biochem. J., 34:392.
3. Gale and Epps, 1943,Nature, 152:327.

Revision : 1 / 2011

Technical Data

Bos Selective Supplement FD004

An antibiotic supplement for the selective isolation of *Bordetella pertussis*.

Composition

Per vial sufficient for 500 ml/ 1000 ml medium

Ingredients	Concentration
Cephalexin	20mg

Directions:

Rehydrate the content of 1 vial aseptically with 2 ml of sterile distilled water. Mix well and aseptically add it to 500 ml of sterile, molten, cooled (45-50°C) Bordet Gengou Agar Base M175/ M175A/ Bordet Gengou HiVeg™ Agar Base MV175 / MV175A or 1000 ml of sterile, molten Charcoal Agar Base w/Niacin M1053/ Charcoal HiVeg™ Agar Base w/Niacin MV1053 together with 10% v/v defibrinated horse blood. Mix well and pour into sterile petri plates. The vial content may be added to 500 ml of sterile half strength Charcoal Agar Base M344/ Charcoal HiVeg™ Agar Base MV344 with 10% v/ v defibrinated horse blood for use as a transport medium for *Bordetella pertussis*.

Type of specimen

Clinical samples -Pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

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CE **CE Marking**

2°C 8°C **Storage temperature**

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CC Selective Supplement I

FD010

An antibiotic supplement recommended for the selective isolation of *Clostridium difficile*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
D-Cycloserine	250mg
Cefoxitin	8mg

Directions:

Rehydrate the contents of one vial aseptically with 2 ml sterile distilled water. Mix well and aseptically add along with 7% v/v defibrinated horse blood to 500 ml sterile, molten, cooled (45-50°C) Clostridium Difficile Agar Base M836 / Clostridium Difficile HiVeg™ Agar Base MV836 / Clostridium Brazier Agar Base M1803. Mix well and pour into sterile petri plates. Sheep blood may be used in place of horse blood but some strains of the organism will show a slightly reduced growth.

Type of specimen

Clinical samples : stool, abscess, etc.; Food samples

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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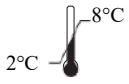

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Technical Data

T.S.C. Supplement

FD014

An antibiotic supplement, recommended for the selective isolation of *Clostridium perfringens*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
D-Cycloserine	200mg

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml sterile distilled water. Mix well and aseptically add to 475 ml of sterile, molten, cooled (45-50ºC) Perfringens Agar Base (T.S.C./S.F.P.)M837/Perfringens HiVeg™ Agar Base (T.S.C. / S.F.P.) MV837/Perfringens Agar Base, Granulated (Tryptose Sulphite Cycloserine Agar Base, Granulated) (T.S.C./S.F.P. Agar Base, Granulated)GM837/Perfringens HiCynth™ Agar Base (T.S.C/S.F.P HiCynth™ Agar Base) MCD837 alongwith 25 ml of Egg Yolk Emulsion FD045 or 500 ml of sterile, molten, cooled (45-50ºC) Perfringens Agar Base M837I or Tryptose Cycloserine Dextrose Agar Base M1233 /Tryptose Cycloserine Dextrose HiVeg™ Agar Base MV1233 or Tryptose Cycloserine Azide Agar Base M1279 or Tryptone Yeast Sodium sulphite Agar Base M2046I or S.F.P. Agar Base M1005F. Mix well and pour into sterile petri plates.

Type of specimen

Clinical- stool, abscess, etc.; Food samples

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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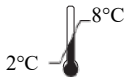

Storage temperature

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GC Selective Supplement

FD021

An antibiotic and enrichment supplement recommended for the selective isolation of pathogenic *Neisseria*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Yeast autolysate	5g
Colistin methane sulphonate	3.750mg
Dextrose	0.750g
Trimethoprim	2.500mg
Sodium bicarbonate	0.075g
Nystatin	6250Units
Vancomycin	1.500mg

Directions:

Rehydrate the contents of 1 vial aseptically with 15 ml of sterile distilled water. Mix well and add aseptically to 500 ml of sterile, molten, cooled (45-50°C) GC Agar Base M434 / GC HiVeg™ Agar Base MV434 / Thayer Martin Medium Base M413 / Thayer Martin HiVeg™ Medium MV413 along with separately prepared FO Growth Supplement FD022 Base. Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples : urine, respiratory exudates etc.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

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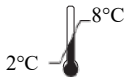

Storage temperature

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Technical Data

V.C.N. Supplement

FD023

An antibiotic supplement, recommended for the selective isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Vancomycin	1.500mg
Colistin methane sulphonate	3.750mg
Nystatin	6250Units

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml sterile distilled water. Mix well and aseptically add it to Thayer Martin Medium Base M413 / Thayer Martin HiVeg™ Medium Base MV413- for 440 ml of medium aseptically add 50ml sterile lysed blood and one vial of V.C.N. Supplement FD023 along with one vial of Vitamino Growth Supplement FD025. FO Growth Supplement (250ml) FD022 can be used instead of sterile lysed blood in 250ml of medium. In GC Agar Base M434/ GC HiVeg™ Agar Base MV434 for 250 ml of can be used instead of sterile lysed blood in 250 ml of FO Growth Supplement FD022 and GC Selective Supplement FD021, one vial of GC Selective Supplement FD021 for additional selectivity. If desired V.C.N. Supplement FD023 can be used along with GC Selective Supplement FD021 for additional selectivity.

In Transgrow Medium Base M1149 for 440 ml of medium aseptically add 50 ml of sterile FO Growth Supplement FD022 and one vial of V.C.N. Supplement FD023 along with one vial of Vitamino Growth Supplement FD025.

Type of specimen

Clinical samples - Stool, urine, respiratory exudates, etc.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

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

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

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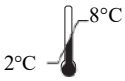

Storage temperature

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V.C.N.T. Supplement

FD024

An antibiotic supplement recommended for the selective isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Vancomycin	1.500mg
Colistin methane sulphonate	3.750mg
Trimethoprim	2.500mg
Nystatin	6250Units

Directions:

Rehydrate the contents of 1 vial aseptically with 5 ml of sterile distilled water. Mix well and aseptically add it to Thayer Martin Medium Base M413 / Thayer Martin HiVeg™ Medium Base MV413 - for 440 ml of medium aseptically add 50 ml sterile lysed blood and one vial of V.C.N.T. Supplement FD024 along with one vial of Vitamino Growth Supplement FD025.

FO Growth Supplement (250ml) FD022 can be used instead of sterile lysed blood in 250 ml of medium.

In GC Agar Base M434 / GC HiVeg™ Agar Base MV434 for 250 ml of medium aseptically add 250 ml of FO Growth Supplement FD022 and GC Selective Supplement FD021, one vial of Vitamino Growth Supplement FD025 or Yeast Autolysate Supplement FD027 . If desired V.C.N.T. Supplement FD024 can be used along with GC Selective Supplement FD021 for additional selectivity.

In Transgrow Medium Base M1149 for 440 ml of medium aseptically add 50 ml of sterile FO Growth Supplement FD022 and one vial of V.C.N.T. Supplement FD024 along with one vial of Vitamino Growth Supplement FD025 .

Type of specimen

Clinical samples - Stool, urine, respiratory exudates, etc.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

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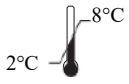

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Vitamino Growth Supplement (Twin Pack)

FD025

A chemically defined growth supplement recommended for cultivation of a wide variety of microorganisms.

Composition

Per vial sufficient for 500 ml medium

Ingredients	Concentration
Part I	"
Vitamin B12	0.100mg
L-Glutamine	100mg
Adenine sulphate	10mg
Guaninine hydrochloride	0.300mg
p-Aminobenzoic acid (PABA)	0.130mg
L-Cystine	11mg
NAD (Coenzyme I)	2.500mg
Cocarboxylase	1mg
Ferric nitrate	0.200mg
Thiamine hydrochloride	0.030mg
Cysteine hydrochloride	259mg
Part II (Rehydrating fluid)	"
Dextrose	1g
Distilled water	10ml

Directions:

Dissolve the contents of Part I in 10 ml of Part II Rehydrating fluid. Aseptically add this to 240 ml of sterile, molten, cooled (45-50ºC) G.C. Agar Base M434 / G.C. HiVeg™ Agar Base MV434 / Thayer Martin Medium Base M413 / Thayer Martin HiVeg™ Medium Base MV413 / Chocolate Agar Base M103 / Chocolate HiVeg™ Agar Base MV103 along with 250 ml of sterile 2% haemoglobin solution or to 500 ml of sterile, molten, cooled (45-50ºC) Modified Proteose Agar M1606 / Transgrow Medium Base M1149 / Mycoplasma Urogenital Broth Base M1374 Martin Lewis Agar Base M2085 or to 1000 ml sterile, molten, cooled (45-50ºC) Tellurite Blood Agar Base M1260 . Mix gently and pour into sterile petri plates.

Type of specimen

Clinical samples - Stool, urine, nasopharyngeal and oropharyngeal swabs, etc.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

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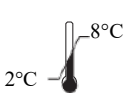

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Cetrinix Selective Supplement

FD029

An antibiotic supplement recommended for the selective isolation of *Pseudomonas* species.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cetrimide	100mg
Nalidixic acid	7.500mg

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml of sterile distilled water. Mix well and aseptically add it to 500 ml of sterile, molten, cooled (45-50°C) Pseudomonas Agar Base M085 / Pseudomonas HiVeg™ Agar Base MV085. Pseudomonas Agar Base, Granulated GM085. Mix well and pour into sterile petri plates.

Type of specimen

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

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

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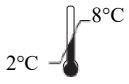

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CTN Selective Supplement

FD034

An antibiotic supplement recommended for the selective isolation of *Yersinia enterocolitica*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cefsulodin	7.500mg
Triclosan(Irgasan)	2mg
Novobiocin	1.250mg

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml of sterile distilled water and 1 ml of ethanol. Mix gently to dissolve the contents completely and aseptically add to 500 ml of sterile, molten, cooled (45-50ºC) Yersinia Selective Agar Base M843/Yersinia Selective HiVeg™ Agar Base MV843.Yersinia Selective Agar Base, w/1.2% Agar M843F. HiCrome™ Yersinia Agar Base M2025. Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - faeces, urine, etc.; Food samples

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.

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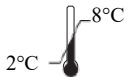

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Egg Yolk Tel Emulsion (50 ml per vial)

FD046L

Sterile stabilized tellurite emulsion of egg yolk recommended for identification of *Staphylococcus* species.

Composition

Ingredients	Concentration
Egg yolk	15ml
Sterile saline	32ml
Sterile 3.5%potassium tellurite solution	3ml

Directions:

Warm up the refrigerated Egg Yolk Tel Emulsion to 40-45°C. Shake well to attain uniform emulsion (since on refrigeration emulsion has a tendency to form layers or small lumps). Aseptically add 50 ml in 950 ml of sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043 /M043S/Baird Parker Agar Base, Granulated GM043 /Baird Parker HiCynth™ Agar Base MCD043 /Baird Parker HiVeg™ Agar Base MV043/ Baird Parker Agar Base w/Sulpha M1140/ HiCrome™ Aureus Agar Base M1468. Aseptically add 100 ml in 900 ml of sterile, molten, cooled (45-50°C) Clostridium Perfringens Agar Base M2070. Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - Skin scrapping, wounds, faeces, etc. ; Food samples

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.

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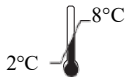

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U40 Supplement (5 ml per vial)

FD048

Filter sterilized urea solution recommended for detection of urease activity.

Composition

Per vial sufficient for 100 ml medium

Ingredients	Concentration
Urea	2g
Distilled water	5ml
Final pH (at 25°C)	8.0±0.2

Directions:

Warm up the refrigerated Urea Solution to room temperature and aseptically add 5 ml in 95 ml sterile, molten, cooled (45-50ºC) Urea Broth Base M111 / Urea Agar Base (Christensen) M112 / M112S / M112I / Urea HiVeg™ Agar Base (Christensen) MV112 / MIU Medium Base M1076 / Hemmes Medium Base M775 or 25 ml in 975 ml Kohn Two Tube Medium No. 1 Base M142 / Kohn Two Tube HiVeg™ Medium No.1 Base MV142 or to Yersinia Identification Broth Base M1221 as desired. Mix well and dispense in sterile tubes.

Type of specimen

Isolated microorganism from clinical, food and water samples.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

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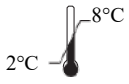

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PTe 1% Selective Supplement (1 ml per vial)

FD052

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

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

Composition

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

Ingredients	Concentration
Potassium tellurite Concentrate	1.100ml

Directions:

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

Type of specimen

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

Specimen Collection and Handling

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

Warning & Precautions

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Storage and Shelf Life

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

Disposal

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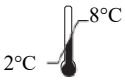

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Technical Data

Tinsdale Selective Supplement (Part A & Part B) FD073

A selective supplement recommended for the isolation and presumptive identification of *Corynebacterium diphtheriae*.

Composition

Per vial sufficient for 1000 ml medium

Ingredients	Concentration
Part A	
Horse serum	100ml
Part B	
Potassium tellurite	1ml

Directions:

Warm up the refrigerated contents of Part B vial and aseptically add 29 ml sterile distilled water. Mix thoroughly. Aseptically add warmed up (to 50°C) contents of Part A and B vials to sterile, molten, cooled (45-50°C) Tinsdale Agar Base M314 / Tinsdale HiVeg™ Agar Base MV314 as required. Mix well and pour into sterile petri plates.

For 10 ml of M314 : 1.0 ml of Part A and 0.3 ml of Part B, is recommended.

Type of specimen

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

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1.Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

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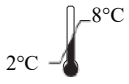
IVD ***In vitro* diagnostic medical device**

CE Marking



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OA Selective Supplement

FD212A

A selective supplement recommended by ISO Committee for the isolation of *Listeria* species.

Composition

Per vial sufficient for 500 ml medium

* Ingredients	Concentration
Polymyxin B sulphate	38350 IU
Ceftazidime	10 mg
Nalidixic acid, sodium salt	10 mg
Amphotericin B	5 mg

Directions

Rehydrate the contents of 1 vial aseptically with 2 ml of 0.2 N Sodium hydroxide, further add 8 ml of sterile distilled water. Mix well and aseptically add it to 465 ml of sterile, molten, cooled (45-50ºC) HiCrome™ Listeria Ottaviani-Agosti Agar BaseM1540I / HiCrome™ Listeria Ottaviani-Agosti HiVeg™ Agar Base MV1540A / HiCrome™ Listeria Ottaviani-Agosti HiCynth™ Agar Base MCD1540A along with sterile contents of one vial of LP Enrichment Supplement 1 FD214 or add in 475 ml of sterile, molten, cooled (45-50ºC) L. mono Confirmatory Agar Base M1552 / L. mono Confirmatory HiVeg™Agar Base MV1552 along with sterile contents of one vial of LM Enrichment Supplement II FD227. Mix well and pour into sterile petri plates.

Type of specimen

Food samples

Specimen Collection and Handling

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

Warning & 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

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

Disposal

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

Reference

1. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1 , Detection method ; ISO 11290-1:2017.
2. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of Listeria spp.- Part2, Enumeration method ; ISO 11290-2:2017.
3. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

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LP Enrichment Supplement 1 FD214

For selective differentiation of *Listeria monocytogenes* from other *Listeria* species, as per ISO Committee.

Composition

Per vial sufficient for 500 ml medium

Ingredients	Concentration
L – phosphatidylinositol	1g
Distilled water	25ml

Directions:

Thaw the contents of 1 vial of LP Enrichment Supplement I at room temperature. Aseptically add the sterile contents to 460 ml of sterile, molten, cooled (45-50ºC) HiCrome™ Listeria Ottaviani Agosti HiCynth™ Agar Base MCD1540A / HiCrome™ Listeria Ottaviani Agosti HiVeg™ Agar Base MV1540A / HiCrome™ Listeria Ottaviani Agosti Agar Base M1540I along with sterile rehydrated contents of 1 vial each of OA Selective Supplement FD212A and HiCrome™ L.mono Rapid Differential Agar Base M1924 / L. mono Confirmatory Agar Base M1552A / L. mono Confirmatory HiVeg™ Agar Base MV1552A. Mix well and pour into sterile Petri plates.

Type of specimen

Clinical samples - faecal and vaginal samples; Food and animal feeds, environmental samples in the area of food manufacturing and handling.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

On receipt product should be stored at -20°C. Use before the expiry date on the label.

Disposal

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

Reference:

1.Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria spp*. - Part 1 , Detection method ; ISO 11290-1:2017

2.Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria spp*. - Part 2 , Enumeration method ; ISO 11290-2:2017

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

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

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Ch250 Selective Supplement

FD283R

An antibiotic supplement recommended for the selective isolation of *Candida* species.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Chloramphenicol	250mg

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml of 95% queoua ethanol. Mix well and aseptically add to 500 ml of sterile, molten cooled (45-50ºC) HiCrome™ Candida Differential Agar Base M1297AR. Mix well and pour into sterile Petri plates.

Type of specimen

Clinical samples - Blood; Food and dairy samples

Specimen Collection and Handling

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

Warning & Precautions

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Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

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

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

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

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

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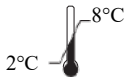

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ZN Acid Fast Stains - Kit K005

Intended use

ZN Acid Fast Stains-Kit is used for staining of acid fast bacteria

Kit Contents

S005 Carbol Fuchsin (ZN,Strong)	125ml
S033 Acid Fast Decolourizer	125ml
S022 Methylene Blue (Loeffler's)	125ml

Composition**

Ingredients	
Carbol Fuchsin (ZN,Strong) (S005)	-
Basic Fuchsin	0.30 gm
Ethyl alcohol, 95%	10.0 ml
Phenol	5.0 gm
Distilled Water	95.0 ml
Acid Fast Decolourizer (S033)	-
Hydrochloric acid,concentrated	3.0 ml
Ethyl alcohol,95%	97.0 ml
Methylene Blue (Loeffler's) (S022)	-
Methylene Blue	0.30 gm
Ethyl alcohol,95%	30.0 ml
Distilled Water	100.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a smear on a clear, dry glass slide.
2. Allow it to air dry and fix with gentle heat.
3. Flood the smear with Carbol Fuchsin stain (S005). Heat to steaming for 5 minutes with a low flame; do not boil the stain and do not permit drying of the smear.
4. Allow it to stand for 5 minutes without further heating.
5. Wash in running tap water.
6. Decolourize with Acid Fast Decolourizer (S033) for 2 minutes or until no more stain comes off in the washings. (If washing is not thorough, you may get false positive results).
7. Wash with tap water.
8. Counterstain for 30 seconds with Methylene Blue (S022).
9. Wash with tap water, dry in air, then examine under oil immersion objective.

Principle And Interpretation

Mycobacteria (AFB/Acid Fast Bacteria) are difficult to stain due to high lipid and wax content in their cell walls. This differential staining technique is useful for identification of the tubercle bacillus, other Mycobacteria, and

Nocardia, which depends on the chemical composition of the bacterial cell wall. Because of the difficulty in staining these organisms with ordinary dyes, basic dyes in the presence of controlled amounts of acid are used. Generally, heat must be applied during the staining procedure, or wetting agents must be used, to aid dye penetration. Organisms exhibiting the property of acid fastness, once stained, are not easily decolourized by alcohol. Non-acid fast organisms are decolourized by acid fast decolourizer and take up the counter stain.

Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens.
Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc.

Specimen Collection and Handling

All testing for acid-fast bacilli is sent to the reference laboratory in an effort to meet the 24 hrs. TAT time for smear results. Use sterile, leak proof disposable plastic containers for collection. Do not use wax containers as these can cause false positive smear results. Do not use any fixative or preservatives. Swabs are not recommended as a collection device for the isolation of mycobacteria. They are acceptable only if the specimen can not be obtained by any other means. A negative result from a swab specimen is not reliable. In general, the number of acid fast bacilli in a specimen is small. Early morning specimens are the specimens of choice for urine and sputum because the mycobacteria have had a chance to pool and concentrate, and so increase the chances of recovery. Always collect and submit the maximum volume possible of specimens normally considered sterile. Do not submit 24 hour collections, as they are likely to be diluted and contaminated. Collect specimens before antimicrobial therapy is started. Even a few days of therapy may kill or inhibit sufficient numbers of mycobacteria to prevent recovery on culture and so leave confirmation of disease in doubt. If a specimen is submitted after therapy has been initiated, note on the request. Avoid contamination of the specimen with tap water, as environmental mycobacteria exist and their recovery by smear or culture can cause confusion for the patient diagnosis

Warning and Precautions

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

Limitations

1. Smears that too thick may flake. during staining and may be difficult decolourizer.
2. Excessive washing following the carbol fuchsin may cause a heightens decolourization effect.
3. Excessive washing after the counterstain lightens the blue colour of the non acid fast material.

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

- **Microscopic examination :** Acid fast staining is carried and staining characteristics of organisms is observed under microscope by using oil immersion lens
- **Results :** Bright red - Acid fast organisms
  Blue - Other organisms and cellular material.

Storage and Shelf Life

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

Disposal

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

Reference

1. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
2. 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.
3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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. Shanhooltzer, C.J., P. Schaper, and L.R. Peterson. 1982. Concentrated Gram stain smear prepared with a cytospin centrifuge.
7. Collee J. G./Fraser A.G., Marmion B.P./Simmons A., 1996, 14th ed., MACKIE McCARTNEY, PRACTICAL MEDICAL
MICROBIOLOGY: 45(800-802).
8. George Clark et al, 1981, 4th ed., Staining procedures: 17(380-382).
9. Godkar B. P., 1996, Textbook of medical laboratory technology: 23(313-315).

Storage temperature


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HiIndicator™ pH papers **LA310, LA312, LA315, LA318, LA321, LA323, LA334, LA335.**

The convenience of using HiIndicator papers for the rapid determination of pH values has led to many applications in laboratories and industry. These pH papers are made with special indicator dyes that change color at specified pH value.

Somewhat uneven colour of the strips is of no consequence. The colour obtained on use is indicative of the correct pH.

Application : Analytical chemistry, biology & various laboratories and industries etc.

Product Name	Product Code	Description	pH Range
HiIndicator™ pH papers.	**LA310**	HiIndicator pH paper	2.00 – 10.50
	LA312	HiIndicator pH paper	3.50 – 6.00
	LA315	HiIndicator pH paper	3.80 – 5.30
	LA318	HiIndicator pH paper	5.00 – 7.50
	LA321	HiIndicator pH paper	6.50 – 9.00
	LA323	HiIndicator pH paper	8.00 – 10.50
	LA334	HiIndicator pH paper	2.00 - 4.50
	LA335	HiIndicator pH paper	1.00 - 14.00

Direction for use : Tear off strip of indicator paper and insert it for a few seconds into the solution to be tested. With highly viscous or stained liquids and with suspensions, drip the substance onto the indicator paper. Compare the wet paper with the colour scale. For papers where liquids are dripped, compare the reverse side. Possible discolouration of the dry new papers may be caused by their high sensitivity. This does not impair the efficacy of the Indicator papers for pH determinations.

The so-called indicator error may occur with very weakly buffered or unbuffered solution and can be compensated for up to a point in the following manner. : - The strip can be made to adhere to the inner wall of the a test tube, which can then filled to the upper edge of the paper with the fluid to be tested. After 1/2 to 1 minute, the colour of the paper may be compared with the scale through the glass of test tube.

Please refer disclaimer Overleaf

Product Features:

- Instant pH readings.
- Accurate for a wide range of routine pH testing.
- Convenient and portable for field use.
- Pack Size : 1 pack-200 Nos.

Nutrient Broth

M002

Intended use

Nutrient Broth is used for the general cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 13.0 grams in 1000 ml purified / distilled water. Heat, if necessary, to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (1,2). Nutrient Broth has the formula originally designed for use in the Standard Method for Examination of Water and Waste water. It is one of the several non-selective media useful in routine cultivation of microorganisms (3,4). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

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

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent solution

Reaction
Reaction of 1.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

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

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant
Salmonella Typhi ATCC *6539*	50-100	good-luxuriant
Staphylococcus aureus aubsp.aureus ATCC 25923 (00034*)	50-100	good-luxuriant
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
2. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C
5. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
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. 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 : 11/2024

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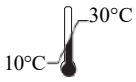

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Fluid Thioglycollate medium (Thioglycollate medium Fluid) M009

Intended use

Recommended for sterility testing of biologicals and for cultivation of anaerobes, aerobes and microaerophiles from pharmaceutical and clinical samples.

Composition**

Ingredients	g / L
Tryptone	15.000
Yeast extract	5.000
Dextrose (Glucose)	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500
Resazurin sodium	0.001
Agar	0.750
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.75 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 25°C and store in a cool dark place preferably below 25°C. Note : If more than the upper one-third of the medium has acquired a pink-purple colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink-purple colour disappears.

Principle And Interpretation

Brewer (1) formulated Fluid Thioglycollate Medium for rapid cultivation of aerobes as well as anaerobes including microaerophiles by adding a reducing agent and small amount of agar. The BP (2), EP (3), USP (4), and AOAC (5) have recommended the media for sterility testing of antibiotics, biologicals and foods and for determining the phenol coefficient and sporicidal effect of disinfectants. However, it is intended for the examination of clear liquid or water-soluble materials. Fluid Thioglycollate Medium is also routinely used to check the sterility of stored blood in blood banks (6). Dextrose, tryptone, yeast extract, L-cystine provide the growth factors necessary for bacterial multiplication. L-cystine and sodium thioglycollate allows Clostridium to grow in this medium even under aerobic conditions (7). Also the small amount of agar used in the medium favors the growth of aerobes as well as anaerobes in the medium, even if sodium thioglycollate is deleted from the medium(1). Sodium thioglycollate act as a reducing agent and neutralizes the toxic effects of mercurial preservatives and peroxides formed in the medium, thereby promoting anaerobiosis, and making the medium suitable to test materials containing heavy metals. (8,9). Any increase in the oxygen content is indicated by a colour change of redox indicator, resazurin to red (6,10,11). The small amount of agar helps in maintaining low redox potential for stabilizing the medium (9).

Type of specimen

Pharmaceutical samples for sterility testing, clinical samples- pus, wounds

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (12,13).
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (2,3,4) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations :

1. It is intended for the examination of clear liquid or water-soluble materials.

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 straw coloured, clear to slightly opalescent solution with upper 10% or less medium pink-purple on standing.

Reaction

Reaction of 2.97% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Growth Promotion Test

As per USP/EP/BP/IP

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu at 30-35°C for or not more than 3 days for aerobes and anaerobes.

Sterility Testing + Validation

The medium is tested with suitable strains of microrganisms inoculating <=100cfu and incubating at 20-25°C for not more than 3 days in case of bacteria and not more than 5 days in case of fungi.

Testing in accordance with EN ISO 11133:2014/Amd.1:2018(E) (10)

Cultural characteristics observed after an incubation at 36-38°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Incubation at
Growth promoting			
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	30-35°C
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*) ^	50 -100	luxuriant	30-35°C
$ *Bacillus spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant	30-35°C
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	30-35°C
##*Kocuria rhizophila* ATCC 9341	50 -100	luxuriant	30-35°C
Clostridium sporogenes ATCC 19404 (00008*)	50 -100	luxuriant	30-35°C
Clostridium sporogenes ATCC 11437	50 -100	luxuriant	30-35°C
$$*Phocaeicola vulgatus* ATCC 8482	50 -100	luxuriant	30-35°C
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	30-35°C
Salmonella Typhimurium ATCC *14028 (00031*)*	50 -100	luxuriant	30-35°C
Salmonella Abony NCTC 6017	50 -100	luxuriant	30-35°C
Sterility Testing- Growth promotion + Validation			
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	20-25°C
$ *Bacillus spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant	20-25°C

^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	20-25°C
##*Kocuria rhizophila* ATCC 9341	50 -100	luxuriant	20-25°C
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant	20-25°C
#*Aspergillus brasiliensis* ATCC 16404 (00053*)	50 -100	luxuriant	20-25°C

Testing in accordance with EN ISO 11133:2014/Amd.1:2018(E) (10)

Cultural characteristics observed after an incubation at 36-38°C for 18-24 hours

Clostridium perfringens ATCC 13124 (00007*)	50 -100	luxuriant	36-38°C

Key : * Corresponding WDCM numbers, # Formerly known as *Aspergillus niger,*
Formerly known as *Micrococcus luteus* $ Formerly known as *Bacillus subtilis* subsp. *spizizenii*
$$ Formerly known as *Bacteroides vulgatus* ^ Formerly known as *Pseudomonas aeruginosa*

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 (12,13).

Reference

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Revision : 07 /2024

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IVD *In vitro* diagnostic medical device

10°C–30°C Storage temperature

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Soyabean Casein Digest Medium (Tryptone Soya Broth) M011

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

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

Principle And Interpretation

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

Type of specimen

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

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

1. Biochemical characterization is necessary to be performed on colonies from pure cultures for further identification.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate.

Reaction
pH of 3.0% w/v aqueous solution at 25°C (after sterilization). pH : 7.3±0.2

pH
7.10-7.50

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

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

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

^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	20 -25 °C	<=3 d
** *Bacillus spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Abony NCTC 6017 *(*00029*)	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	20 -25 °C	<=3 d
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	20 -25 °C	<=3 d
$ *Kokuria rhizophila* ATCC 9341	50 -100	luxuriant	20 -25 °C	<=3 d

Key : (*) Corresponding WDCM numbers

^ Formerly known as *Pseudomonas aeruginosa*

**Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Formerly known as *Aspergillus niger*

$ Formerly known as *Micrococcus luteus*

Storage and Shelf Life

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

Disposal

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

Reference

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

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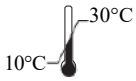
IVD ***In vitro* diagnostic medical device**



Storage temperature

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EMB Agar, Levine

M022

Intended Use:

Recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* from clinical and non clinical samples.

Composition**

Ingredients	g / L
Peptone	10.000
Dipotassium hydrogen phosphate	2.000
Lactose	10.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.46 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. **AVOID OVERHEATING**. Cool to 50°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour) and to suspend the precipitate, which is an essential part of the medium.

Precaution : Store the medium away from light to avoid photo-oxidation.

Principle And Interpretation

Levine EMB Agar was developed by Levine (1,2) and is used for the differentiation of *Escherichia coli* and *Klebsiella* aerogenes and also for the rapid identification of *Candida albicans.* This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association (3,4,5). Weld (6,7) proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. A positive identification of *Candida albicans* can be made after 24-48 hours incubation at 35-37°C in 10% carbon dioxide atmosphere, from specimens such as faeces, oral and vaginal secretions and nail or skin scraping etc. However, the typical appearance is variable.

Eosin Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes serve as differential indicators in response to the fermentation of carbohydrates. This helps to differentiate between lactose-fermenters and non-fermenters in EMB Agar, Levine. The ratio of eosin-methylene blue is adjusted to approximately 6:1. Coliforms produce purplish black colonies due to uptake of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Non-fermenters probably raise the pH of surrounding medium by oxidative de-amination of protein, which solubilizes the methylene blue-eosin complex resulting in formation of colourless colonies . Peptone serves as source of carbon, nitrogen, long chain amino acids, vitamins and other essential growth nutrients. Lactose serves as the source of energy by being the fermentable carbohydrate. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium.

The test sample can be directly streaked on the medium plates. Inoculated plates should be incubated, protected from light. However standard procedures should be followed to obtain isolated colonies. A non-selective medium should be inoculated in conjunction with EMB Agar. Confirmatory tests should be further carried out for identification of isolated colonies.

Type of specimen

Clinical samples - urine, faeces, oral and vaginal secretions and nail or skin scraping , Foodstuffs; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).

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

Warning and Precautions

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

Limitations

1. A non-selective medium should be inoculated in conjunction with EMB Agar.
2. Confirmatory tests should be further carried out for identification of isolated colonies.
3. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.

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 pink to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates

Reaction

Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans ATCC 10231 (00054*)	50-100	luxuriant (incubated in 10% carbon dioxide)	>=50%	colourless
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good	40-50%	pink-red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	blue-black with metallic sheen
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	colourless
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	none-poor	<=10%	colourless
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	colourless
Saccharomyces cerevisiae ATCC 9763	50-100	none-poor	<=10%	cream
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00058*)	50-100	none-poor	<=10%	colourless
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	>=50%	blue-black with green metallic sheen

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

Storage and Shelf Life

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

Disposal

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

Reference

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Revision : 06/2024

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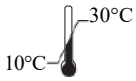
IVD ***In vitro* diagnostic medical device**



Storage temperature

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Peptone Water

M028

Intended Use:

Peptone Water is used as a growth medium and as a base for carbohydrate fermentation media.

Composition**

Ingredients	g / L
Peptone	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.0 grams in 1000 ml purified/distilled water. Add the test carbohydrate in desired quantity and dissolve completely. Dispense in tubes with or without inverted Durhams tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Peptone Water can be utilized as a base for carbohydrate fermentation studies with the addition of sugar and indicators such as bromocresol purple, phenol red or bromothymol blue. Peptone Water is also suitable as a substrate in the study of indole production. Peptone used in Peptone Water is rich in tryptophan content. Presence of indole can be demonstrated using either Kovacs or Ehlrich reagent.

Peptone Water is recommended (1,2,3) for studying the ability of an organism to ferment a specific carbohydrate which aid in differentiation of genera and species. Peptone water is formulated as per Shread, Donovan and Lee (4). Peptone Water with pH adjusted to 8.4 is suitable for the cultivation and enrichment of *Vibrio* species. Peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins provides essential nutrients. Sodium chloride maintains the osmotic balance of the medium. To study the fermentation ability of carbohydrates, saccharose, rhamnose, salicin are generally added in 0.5% amount separately to the basal medium before or after sterilization. The acidity formed during fermentation can be detected by addition of phenol red indicator, which shows a colour change of the medium from red to yellow under acidic conditions. If desired, Durham's tube may be used to detect the gas production if produced.

Type of specimen

Isolated microorganism from clinical specimen, food, dairy and water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (10). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling clinical 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.
2. Further serological and biochemical tests should be carried out on pure colony for complete 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

Light amber coloured clear solution without any precipitate

Reaction

Reaction of 1.5% 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.

Organism	Inoculum (CFU)	Growth	Indole test
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	negative reaction, no red ring at the interface of the medium on addition of Kovac's reagent (R008)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction, red ring at the interface of the medium on addition of Kovac's reagent (R008)
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	negative reaction, no red ring atthe interface of the medium on addition of Kovac's reagent (R008)

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

References

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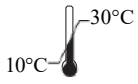
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

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Endo Agar, Special

M029R

Endo Agar, Special is recommended for the detection of coliform and other enteric organisms.

Composition**

Ingredients	Gms / Litre
Peptone, special	11.500
Lactose	12.900
Dipotassium phosphate	0.480
Monopotassium phosphate	0.220
Sodium chloride	3.600
Sodium sulphite	0.860
Sodium lauryl sulphate	0.010
Basic fuchsin	0.830
Agar	9.600
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 grams in 1000 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: Basic Fuchsin is a potential Carcinogen and care should be taken to avoid inhalation of the powdered dye and contamination of the skin.

Principle And Interpretation

Endo (1) had first developed a culture medium for differentiation of lactose fermentors and non-fermenters and further developed as todays Endo Agar (2). Endo agar is used for microbiological examination of potable water and waste water, dairy products and food (3,4,5).

Sodium sulphite and basic fuchsin has inhibitory effect on gram-positive microorganisms. Sodium Lauryl sulphate inhibits many organisms other than coliforms. Lactose fermenting coliforms produce aldehyde and acid. The aldehyde in turn liberates fuchsin from the fuchsin-sulphite complex, giving rise to a red colouration of colonies. With *Escherichia coli* this reaction is very pronounced that the fuchsin crystallises, exhibiting to the colonies a permanent greenish metallic lustre (fuchsin lustre). The phosphates buffer the medium. Peptone special provides essential nutrients especially nitrogenous for the coliforms.

Quality Control

Appearance
Light pink to purple homogeneous free flowing powder

Gelling
Firm,comparable with 0.96% Agar gel.

Colour and Clarity of prepared medium
Pink Clear to slightly opalescent gel with a slight precipitate forms in Petri plates.

Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

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

Cultural Response

Organism	Growth	Inoculum (CFU)	Recovery	Colour of Colony
Cultural Response				
Bacillus subtilis ATCC 6633	inhibited	>=10^3	0%	
Enterobacter aerogenes ATCC 13048	good-luxuriant	50-100	>=50%	pink
Enterococcus faecalis ATCC 29212	none-poor	50-100	<=10%	pink, small
Escherichia coli ATCC 25922	good-luxuriant	50-100	>=50%	pink to rose red with metallic sheen
Klebsiella pneumoniae ATCC 13883	good-luxuriant	50-100	>=50%	pink, mucoid
Salmonella Typhi ATCC 6539	good-luxuriant	50-100	>=50%	colourless to pale pink
Staphylococcus aureus ATCC 25923	inhibited	>=10^3	0%	
Pseudomonas aeruginosa ATCC 27853	good-luxuriant	50-100	>=50%	colourless, irregular
Proteus vulgaris ATCC 13315	good-luxuriant	50-100	>=50%	colourless to pale pink
Shigella sonnei ATCC 25931	good-luxuriant	50-100	>=50%	colourless to pale pink

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2 – 8°C away from light to avoid photo-oxidation. Use before expiry date on the label.

Reference

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Revision : 01 / 2014

Xylose-Lysine Deoxycholate Agar (XLD Agar)

M031

Intended use

Recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.68 grams in 1000 ml purified / distilled water. Heat with frequent agitation until the medium boils. **DO NOT AUTOCLAVE OR OVERHEAT**. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate. ***Note :*** *Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.*

Principle And Interpretation

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (1) and for the microbiological testing. XLD Agar was formulated by Taylor (2-6) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species of foods, water and dairy products (7-11). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar (M108), EMB Agar (M022) and Bismuth Sulphite Agar (M027) (3,5,7,12-15). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (16). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (M1482) (17). It is also recommended by FDA (18).

The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by Shigellae but practically by all enterics. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. Salmonellae rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylate by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H_2S producers do not decarboxylase lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies (2).

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

Type of specimen

Clinical samples - Faeces; Food and dairy samples; Water samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (19,20). 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 (8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.
3. Some *Proteus* strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions.
4. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies.
5. *S.* Paratyphi A, *S.*Choleraesuis, *S.* Pullorum and *S.* Gallinarum may form red colonies without H_2S, thus resembling *Shigella* 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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.67% w/v aqueous solution at 25°C . pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural response was observed after an incubation at 35-37°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation period
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Escherichia coli ATCC 8739 (00012*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922 (00013*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs

$ Proteus hauseri ATCC 13315	50 -100	good-luxuriant	25 -100	>=50 %	grey with black centres	18 -72 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Typhi ATCC 6539	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Shigella dysenteriae ATCC 13313	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
Shigella sonnei ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Enterobacter cloacae ATCC 13047 (00083*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	$>=10^4$	inhibited	0	0%		>=72 hrs
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	$>=10^4$	inhibited	0	0%		>=72 hrs
Enterococcus faecalis ATCC 29212 (00087*)	$>=10^4$	inhibited	0	0%		>=72 hrs

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

Storage and Shelf Life

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

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (19,20).

Reference

1.Chadwick P., Delisle G. H and Byer M., 1974, Can. J. Microbiol., 20, 1653-1664.
2.Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
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19. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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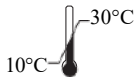
IVD ***In vitro* diagnostic medical device**



Storage temperature

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Baird Parker Agar Base

M043I

Intended Use:

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

Composition**

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

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

**Formula adjusted, standardized to suit performance parameters

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

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

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

Directions

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

Principle And Interpretation

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

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

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

Type of specimen

Food samples and animal feeding stuffs

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

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

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

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

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

Reference

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Revision : 05/2024

Violet Red Bile Agar

M049

Intended use

Recommended for selective isolation, detection and enumeration of coli-aerogenes bacteria in water, milk other dairy, food products and clinical samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 4832 : 2010 and ISO 11133 :2014, Amd. 2 : 2020 (E)

Composition**

ISO specification :

Crystal violet neutral red bile lactose (VRBL) agar

Ingredients	g / L
Enzymatic digest of animal tissues	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Lactose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH (at 25°C)	7.4±0.2

M049- Violet Red Bile Agar

Ingredients	g / L
Peptone $	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Lactose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

$ - Equivalent to Enzymatic digest of animal tissues

Directions

Suspend 41.53 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Cool to 45-50°C and immediately pour into sterile Petri plates containing the inoculum. If desired, the medium can be sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. The historical definition of this group has been based on the method used for detection i.e. lactose fermentation. This group is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming rod shaped bacteria that ferment lactose with gas and acid formation within 48 hour at 35°C (1,2). Examination of foods, ingredients and raw materials, for the presence of marker groups such as coliforms is the one of the common tests.

Violet Red Bile Agar, a modification of MacConkey's original formulation (1) is used for the enumeration of coli-aerogenes bacterial group. It relies on the use of the selective inhibitory components crystals violet and bile salts and the indicator system lactose, and neutral red. Thus, the growth of many unwanted organisms is suppressed, while tentative identification of sought bacteria can be made. Organisms, which rapidly attack lactose, produce purple colonies surrounded by purple halos. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones (3). VRBA is recommended by APHA (4,5). Selectivity of VRBA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (6-8). It is also recommended by ISO (9,10).

Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Lactose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Violet Red Bile Agar is not completely specific for enteric; other accompanying bacteria may give the same reaction. Further biochemical tests are necessary for positive identification (5).

Type of specimen

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

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3-5,9,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines & local standards (11).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (12,13).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 30 ± 1°C for 24 ± 2 hours. Recovery rate is considered as 100% for bacteria growth on Reference Medium - Tryptone Soya Agar

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

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Productivity				
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	purplish red colonies with or without precipitation
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	>=50%	purplish red colonies with or without precipitation
Selectivity				
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited		
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Selectivity				
Pseudomonas aeruginosa ATCC 27853 (00025*)	10^3-10^4	good		colourless to beige colonies

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

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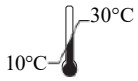
IVD *In vitro* diagnostic medical device



Storage temperature

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Bile Broth Base

M071

Intended Use:

Recommended for cultivation of members of the *Enterobacteriaceae* and in culture of blood clots from patients with suspected enteric fever.

Composition**

Ingredients	g / L
Peptone	20.000
Sodium taurocholate	5.000
Sodium chloride	5.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.0 grams in 1000 ml distilled/purified water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add 1 ml of Streptokinase solution (100000 units/ml). Mix well and dispense into tubes or flasks as desired.

Principle And Interpretation

Enterobacteriaceae inhabit a wide variety of niches that include the human gastrointestinal tract and various environmental niches. When blood samples from a patient with suspected enteric fever is submitted for the widal test, it is useful as a routine to culture the clot after separation of serum (1). If it is known that the blood has been withdrawn with strict aseptic precautions, the clot may be placed in a wide tube half-filled with broth, or in a wide mouth screw-capped bottle containing 80 ml of broth. When there is any doubt regarding the presence of contaminating organisms, and this is always a possibility when blood specimens are sent to the laboratory from a distance, the clot should be transferred directly to a tube of sterile ox bile and disintegrated with aseptic precautions. After overnight incubation the bile culture is examined for enteric organism in the usual manner. A method of clot culture with Streptokinase has been recommended (2). Blood is allowed to clot in 5 ml quantities in sterile screw-capped universal containers. The separated serum is removed and 15 ml of 0.5% Bile Broth Base with Streptokinase 100 units/ml is added to each bottle. The streptokinase causes rapid clot lysis with release of bacteria trapped in the clot (2).

Peptone serves as a source of nitrogen, carbon, long chain amio acids and other essential amino acids. Sodium taurocholate inhibits majority of Gram-positive species. Sodium chloride maintains the isotonicity of the medium whereas addition of streptokinase solution causes rapid clot lysis with release of bacteria trapped in the clot (2).

Type of specimen

Clinical samples - Blood clot

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

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

Warning and Precautions :

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

Limitations :

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

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 haziness

Reaction

Reaction of 3.0% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant
#*Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant
Salmonella Typhi ATCC 6539	50-100	luxuriant
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=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 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

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

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Revision : 06/ 2024

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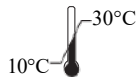
IVD ***In vitro* diagnostic medical device**



Storage temperature

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Kligler Iron Agar

M078

Intended Use:

Recommended for differential identification of gram-negative enteric bacilli from clinical and non-clinical samples on the basis of the fermentation of glucose (dextrose), lactose and hydrogen sulphide production.

Composition**

Ingredients	g / L
Peptone	15.000
HM Peptone B #	3.000
Yeast extract	3.000
Proteose peptone	5.000
Lactose	10.000
Dextrose	1.000
Ferrous sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 57.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1 inch butts. Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Note: *Avoid overheating otherwise it may produce precipitate in the medium.*

Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (1,2) and Russels Double Sugar Agar (3) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (4). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (4). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other Salmonellae and also *Salmonella* Paratyphi A from *Salmonella* Scottmuelleri and *Salmonella* Enteritidis (5). Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugar i.e. dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface of the medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced, avoids reoxidation and therefore the entire medium turns yellow. Kligler Iron Agar, in addition to Peptone, HM peptone B and yeast extract, contains lactose and glucose (dextrose), which enables the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of ferrous sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids produced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellow butt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

Pure cultures of suspected organisms from plating media such as MacConkey Agar (M081), Bismuth Sulphite Agar (M027) or Deoxycholate Citrate Agar (M065), SS Agar (M108) etc. are inoculated on Kligler Iron Agar for identification.

Type of specimen

Isolated microorganism from clinical, food, dairy and water samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Results should be noted after 18-24 hours to avoid erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous results.
4. Other biochemical and serological tests must be performed for complete 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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 5.75% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

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

Organism	Growth	Gas	H2S	Slant	Butt
Escherichia coli ATCC 25922 (00013*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
#*Klebsiella aerogenes* ATCC 13048 (00175*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Citrobacter freundii ATCC 8090	luxuriant	positive reaction	positive reaction, blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
## *Proteus hauseri* ATCC 13315	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Klebsiella pneumoniae ATCC 13883 (00087*)	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Salmonella Paratyphi A ATCC 9150	luxuriant	positive reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium

Salmonella Schottmuelleri ATCC 10719	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Typhi ATCC 6539	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Enteritidis ATCC 13076 (00030*)	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Shigella flexneri ATCC 12022 (00126*)	luxuriant	negative reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Pseudomonas aeruginosa ATCC 27853 (00025*)	luxuriant	negative reaction	negative reaction, blackening of medium	alkaline reaction, red colour of the medium	alkaline reaction,red colour of the medium
Yersinia enterocolitica ATCC 27729	luxuriant	variable reaction	negative reaction,no blackening of medium	alkaline reaction,red colour of the medium	acidic reaction, yellowing of the medium
Enterobacter cloacae ATCC 13047 (00083*)	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

Key :* Corresponding WDCM numbers

(#) Formerly known as *Enterobacter aerogenes* ## Formerly known as *Proteus vulgaris*

Storage and Shelf Life

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

Disposal

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

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

30°C
10°C **Storage temperature**

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Lauryl Sulphate Broth (Lauryl Tryptose Broth)

M080

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

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

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling:

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

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

Warning and Precautions

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

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

pH

6.60-7.00

Cultural Response

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

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

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

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

Storage and Shelf Life

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

Disposal

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

Reference

1. 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.
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. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
4. 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.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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8. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05/2024

MacConkey Agar w/ 0.15% Bile salts, CV and NaCl

M081

For the selective isolation and differentiation of coliform organisms and other enteric pathogens from clinical and non clinical samples.

Composition**

Ingredients	g / L
Gelatin peptone	17.000
Tryptone	1.500
Peptone	1.500
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.53 grams in 1000 ml purified/distilled water. Heat to boiling with gentle swirling to dissolve the agar completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45-50°C. Mix well and pour into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (1), dairy (2), food (3,4), water (5), pharmaceutical (6,7) and industrial sources (8). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for use in the performance of Microbial Limit Tests (7).

These agar media are selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. The medium M081, which corresponds with, that recommended by APHA can be used for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms (4) and for the isolation of *Salmonella* and *Shigella* species in cheese (2). Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (9), the count of coli-aerogenes and non-lactose fermenters in poultry carcasses (9), bacterial counts on irradiated canned minced chicken (10) and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (11,12).

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (13,14). The original medium contains protein, bile salts, sodium chloride and two dyes. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose-fermenting strains grow as red or pink colonies and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless, transparent and typically do not alter appearance of the medium.

Peptone, Tryptone and gelatin peptone are sources of nitrogen, carbon, long chain amino acids and other nutrients. Lactose is a fermentable carbohydrate,Sodium chloride maintains the osmotic equilibrium. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Type of specimen

Clinical - faeces, urine etc., foodstuffs and dairy samples, water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,15).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,4).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5).

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

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling clinical 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. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
2. The surface of the medium should be dry when inoculated.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
4. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural response was observed after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Corynebacterium diphtheriae type gravis	>=10^4	inhibited	0%	
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair to good	30 -40 %	colourless
Salmonella Paratyphi A ATCC 9150	50 -100	luxuriant	>=50 %	colourless
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	>=50 %	colourless
## *Proteus hauseri* ATCC 13315	50 -100	luxuriant	>=50 %	colourless
Salmonella Typhi ATCC 6539	50 -100	luxuriant	>=50 %	colourless
Staphylococcus epidermidis ATCC 12228 (00036*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=50 %	pink-red with bile precipitate

Staphylococcus aureus subsp.aureus ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	>=50 %	colourless
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=50 %	pink to red with bile precipitate
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	>=50 %	pink to red
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	>=50 %	colourless
Enterococcus faecalis ATCC 29212 (00087*)	50 -100	none - poor	<=10 %	colourless to pale pink
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	colourless
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key :- * Corresponding WDCM numbers, # Formerly known as *Enterobacter aerogenes,*
Formerly known as *Proteus vulgaris.*

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,15).

Reference

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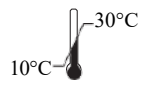
9. Medrek T. F and Barnes Ella M., 1962, J. Appl. Bacteriol., 25(2),159-168.
10. Thornley Margaret J., 1957, J. Appl. Bacteriol., 20(2), 273-285.
11. Barnes Ella M. and Shrimpton D. H., 1957, J. Appl. Bacteriol., 20(2),273-285.
12. Eddy B. P., 1960, J. Appl. Bacteriol., 23(2).216-249.
13. MacConkey A., 1905, J. Hyg., 5:333.
14. MacConkey A., 1900, The Lancet, ii:20.
15. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

Revision : 07/ 2024

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

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



Storage temperature

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

CE Marking

Do not use if package is damaged

Disclaimer :

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

MacConkey Agar w/ 0.15% Bile salts, CV and NaCl

M081

For the selective isolation and differentiation of coliform organisms and other enteric pathogens from clinical and non clinical samples.

Composition**

Ingredients	g / L
Gelatin peptone	17.000
Tryptone	1.500
Peptone	1.500
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.53 grams in 1000 ml purified/distilled water. Heat to boiling with gentle swirling to dissolve the agar completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45-50°C. Mix well and pour into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (1), dairy (2), food (3,4), water (5), pharmaceutical (6,7) and industrial sources (8). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for use in the performance of Microbial Limit Tests (7).

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Peptone, Tryptone and gelatin peptone are sources of nitrogen, carbon, long chain amino acids and other nutrients. Lactose is a fermentable carbohydrate,Sodium chloride maintains the osmotic equilibrium. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Type of specimen

Clinical - faeces, urine etc., foodstuffs and dairy samples, water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,15).
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Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

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Cultural response was observed after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
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Key :- * Corresponding WDCM numbers, # Formerly known as *Enterobacter aerogenes,*
Formerly known as *Proteus vulgaris.*

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Revision : 07/ 2024

HiMedia Laboratories Pvt. Limited,
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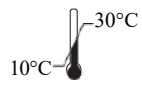
IVD ***In vitro* diagnostic medical device**



Storage temperature

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

CE Marking

Do not use if package is damaged

Disclaimer :

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HIMEDIA

Technical Data

Pseudomonas Agar Base (CN Agar) M085

Intended use :

For selective isolation of *Pseudomonas* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 16266-1:2006

Composition**

ISO specification - *Pseudomonas* agar base/CN-agar	
Ingredients	**g/ L**
Gelatin peptone	16.000
Casein hydrolysate	10.000
Potassium sulphate,anhydrous (K_2SO_4)	10.000
Magnesium chloride, anhydrous ($MgCl_2$)	1.400
Agar	11.00 - 18.00
Final pH (of solidified, complete medium)	7.1±0.2
Supplement to be added after autoclaving	**g / L**
Hexadecyitrimethyl ammonium bromide(Cetrimide)	0.200
Nalidixic acid	0.150

Pseudomonas Agar Base	**M085**
Ingredients	**g/ L**
Gelatin peptone	16.000
Tryptone	10.000
Potassium sulphate	10.000
Magnesium chloride	1.400
Agar	11.000
Final pH (at 25°C)	7.1±0.2
Cetrinix Supplement - FD029	**mg / vial**
Cetrimide	100mg
Nalidixic acid	7.500mg

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.2 grams in 500 ml purified/distilled water containing 5 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 1 vial of sterile rehydrated contents of Cetrinix Supplement (FD029). Mix well and pour into sterile Petri plates.

Note : Do not keep the molten agar for longer than 4 hours.

Principle And Interpretation

Pseudomonas Agar Baseis as per the specification laid down in ISO 16266 for testing water quality by MPN method (1). It is also a modification of Kings A medium (2) which contains magnesium chloride and potassium sulphate to enhance pigment production. CetriNix supplement is specified for the selective isolation of *Pseudomonas aeruginosa* from water samples, it suppresses the growth of *Klebsiella*, *Proteus* and *Providencia* species. Lowbury and Collins (3) studied cetrimide as a selective agent.

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

Examine inoculated plates after incubation at UV light 360 ± 20 nm. The presence of blue-green colonies with fluorescence may be considered as presumptive evidence of *Pseudomonas aeruginosa.*

Type of specimen

Clinical samples - pus, urine, Water samples.

Specimen Collection and Handling:

ISO 16266-1:2006

Preparation of test sample: Prepare tenfold dilutions of water samples(1,4).After use, contaminated materials must be sterilized by autoclaving before discarding.

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.1% Agar gel.
Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction
Reaction of 4.84% w/v aqueous solution containing 1% v/v glycerol at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Productivity : Cultural response was observed after an incubation at 36 ± 2°C for 44 ± 4 hours, with added sterile Cetrinix Supplement (FD029). Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).
Selectivity : Cultural response was observed after an incubation at 36 ± 2°C for 44 ± 4 hours, with added sterile Cetrinix Supplement (FD029).

Organisms	Inoculum (CFU)	Growth	Recovery#	Fluorescence under UV 360± 20 nm
Productivity				
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=50%	blue-green with Fluorescence
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50-100	good-luxuriant	>=50%	blue-green with Fluorescence
Pseudomonas aeruginosa ATCC 10145 (00024*)	50-100	good-luxuriant	>=50%	blue-green with Fluorescence
Selectivity				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*) *Escherichia coli* ATCC 8739 (00012*)	>=10^4	inhibited	0%	
	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers, ^ Formerly known as *Pseudomonas aeruginosa*
- Recovery obtained for productivity is >=70% when compared to a previously validated batch of Pseudomonas Agar Base (CN Agar) is used.

Storage and Shelf Life

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

Disposal

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

References

1.Water quality — Detection and enumeration of *Pseudomonas aeruginosa*-- Method by membrane filtration ; ISO 16266-1:2006

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

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

4.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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

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

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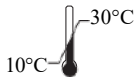

Storage temperature

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CE Marking

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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 and wastewater.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow granular media.

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

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

pH

6.80-7.20

Cultural Response

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

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

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

Storage and Shelf Life

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

Disposal

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

Reference

1. Buchbinder L., Baris Y., Aldd E., Reynolds E., Dilon E., Pessin V., Pincas L. and Strauss A., 1951, Publ. Hlth. Rep., 66:327.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
5. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
6. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05/2024

SS Agar (Salmonella Shigella Agar)

M108

Intended Use:

Recommended for the isolation of *Salmonella* and some *Shigella* species from pathological specimens, suspected foodstuffs etc.

Composition**

Ingredients	g / L
Peptone	5.000
HM peptone B #	5.000
Lactose	10.000
Bile salts mixture	8.500
Sodium citrate	10.000
Sodium thiosulphate	8.500
Ferric citrate	1.000
Brilliant green	0.00033
Neutral red	0.025
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 63.02 grams in 1000 ml purified /distilled water. Boil with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Overheating may destroy selectivity of the medium. Cool to about 50°C. Mix and pour into sterile Petri plates.

Principle And Interpretation

SS Agar medium is recommended as differential and selective medium for the isolation of *Salmonella* and *Shigella* species from pathological specimens (1) and suspected foodstuffs (2-5) and for microbial limit test (6). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

Peptone, HM peptone B provides nitrogen and carbon source, long chain amino acids, vitamins and essential growth nutrients. Lactose is the fermentable carbohydrate. Brilliant green, bile salts and thiosulphate selectively inhibit gram-positive and coliform organisms. Sodium thiosulphate is reduced by certain species of enteric organisms to sulphite and H_2S gas and this reductive enzyme process is attributed by thiosulphate reductase. Production of H_2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H_2S with ferric ions or ferric citrate, indicated in the center of the colonies.

The high selectivity of Salmonella Shigella Agar allows the use of large inocula directly from faeces, rectal swabs or other materials suspected of containing pathogenic enteric bacilli. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator-neutral red. Thus these organisms grow as red pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centers. Growth of *Salmonella* species appears as colourless colonies with black centers resulting from H_2S production. *Shigella* species also grow as colourless colonies which do not produce H_2S.

Type of specimen

Clinical: faeces, rectal swabs; Suspected food stuffs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2-5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1.The medium is highly selective and may be toxic to certain *Salmonella* or *Shigella* species. Hence it is recommended to use to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) or Deoxycholate Citrate Agar (M065) for easier isolation of *Shigella* species (3).

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 6.3% 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 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	fair	20-30%	cream pink
Escherichia coli ATCC 25922 (00013*)	50-100	fair	20-30%	pink with bile precipitate
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	>=50%	colourless with black centre
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%	colourless with black centre
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	colourless
Proteus mirabilis ATCC 25933	50-100	fair-good	30-40%	colourless, may have black centre
Shigella flexneri ATCC 12022 (00126*)	50-100	good	40-50%	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	>=50%	colourless with black centre
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50%	colourless with black centre

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed., ASM, Washington, D.C.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. 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.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
5. Williams S., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed.,AOAC,Washington, D.C.
6. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05/2024

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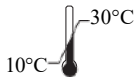

Storage temperature

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Urea Agar Base (Christensen)(Autoclavable)

M112

Intended Use:

Urea Agar Base with the addition of Urea is recommended for the detection of urease production, particularly by members of the genus *Proteus*.

Composition**

Ingredients	g / L
Peptone	1.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Disodium hydrogen phosphate	1.200
Potassium dihydrogen phosphate	0.800
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 U40 Supplement (5 ml per vial) (FD048) and mix well. Dispense into sterile tubes and allow to set in the slanting position. Do not overheat or reheat the medium as urea decomposes very easily.

Principle And Interpretation

Urea Agar is used to detect urease production. Urea Agar described by Christensen (1,2) detected urease activity by all rapidly urease-positive *Proteus* organisms and also by other members of *Enterobacteriaceae* (1) that exhibited a delayed urease reaction (3). This was 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).

Peptone is the source of 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.

Prolonged incubation may cause alkaline reaction in the medium. A medium without urea serves as negative control to rule out false positive results. Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (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.

Type of specimen

Isolated microorganism from clinical, food and water samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1.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 (6).
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 sterile U40 Supplement (5 ml per vial) (FD048) after an incubation at 35-37°C for 18-24 hours.

Organism	Urease
Escherichia coli ATCC 25922 (00013*)	negative reaction, no change
# *Klebsiella aerogenes* ATCC 13048 (00175*)	negative reaction, no change
Proteus mirabilis ATCC 25933	positive reaction, cerise colour
$ *Proteus hauseri* ATCC 13315	positive reaction, cerise colour
Salmonella Typhimurium *ATCC 14028 (00031*)*	negative reaction, no change

Key : *Corresponding WDCM numbers.

Formerly known as *Enterobacter aerogenes* $ Formerly known as *Proteus vulgaris*

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 (9,10).

Reference

1. Christensen W. B., 1946, J. Bacteriol., 52:461.
2. MacFaddin J. F, 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore, Md.
3. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.Md.

4.Farmer J. J. III, McWhorter A. C., Huntley G. A., Catignani J., J. Clin. Microbiol. 1975: 1 (1): 106-107.
5.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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.
8.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
9.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
10.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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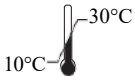

Storage temperature

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Brilliant Green Bile Broth

M121I

Intended Use:

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

Composition**

ISO Specifications : BGBLB

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

Brilliant Green Bile Broth **M121I**

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

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

Directions

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

Principle And Interpretation

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

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

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

Type of specimen

Food samples

Specimen Collection and Handling:

ISO 4831:2006 (1,2)

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate.

Reaction

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

pH

7.00-7.40

Cultural Response

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

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

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

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

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

Reference

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

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

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

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

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

Revision:04/2024

EC Broth

M127I

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

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

Directions

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

Principle And Interpretation

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

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

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

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

Type of specimen

Food samples - Food and animal feeding stuffs

Specimen Collection and Handling

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured, clear solution without any precipitate

Reaction

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

pH

6.60-7.00

Cultural Response

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

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

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

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

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

Reference

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

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

Revision : 04/2024

Columbia Blood Agar Base

M144

Intended Use:

For preparation of blood agar, chocolate agar and for preparation of various selective and identification media and isolation of organisms from clinical and non clinical samples.

Composition**

Ingredients	g / L
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.0 grams of 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 before adding heat sensitive compounds.
For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.
For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.
The medium can be made selective by adding different antimicrobials to sterile base.
For *Brucella* species: Add rehydrated contents of 1 vial of NPBCVN Selective Supplement (FD005) to 500 ml sterile molten base.
For *Campylobacter* species: Add rehydrated contents of 1 vial of Blaser-Wang Selective Supplement (FD006) or Butzler Selective Supplement (FD007) or Skirrow Selective Supplement (FD008) or VTCA Selective Supplement (FD090) or Butzler VI Selective Supplement (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Minerals Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.
For *Gardnerella* species: Add rehydrated contents of 1 vial of GNA Selective Supplement (FD056) to 500 ml sterile molten base.
For Cocci: Add rehydrated contents of 1 vial of NC Selective Supplement (FD030) or NNP Selective Supplement (FD031) or CO Selective Supplement (FD119) to 500 ml sterile molten base.

Principle And Interpretation

Columbia Blood Agar Base was devised by Ellner et al (1). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (2,3). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (4). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.
Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.
Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae,* lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.
Precaution: Brucella cultures are highly infective and must be handled carefully; incubate in 5-10% CO_2. Campylobacter species are best grown at 42°C in a micro aerophillic atmosphere. Plates with Gardenerella supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2 (2).

Type of specimen

Clinical samples : throat swabs, pus.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
2. As this medium have a relatively high carbohydrate content, beta-hemolytic *Streptococci* may exhibit a greenish hemolytic reaction which may be mistaken for the alpha haemolysis.
3. Biochemical characterization is required on colonies of pure culture for complete 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.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added 5% w/v sterile defibrinatedblood, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	gamma
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	luxuriant	>=70%	beta / gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta
Clostridium sporogenes ATCC 19404 (00008*)	50-100	luxuriant	>=50 %	
Clostridium sporogenes ATCC 11437	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 13124 (00007*)	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 12934	50-100	luxuriant	>=50 %	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
2. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
3. Fildes P., 1921, Br. J. Exp. Pathol., 2:16.
4. Chapin K. C. and Doern G. V., 1983, J. Clin. Microbiol., 17:1163.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05/2024

HiMedia Laboratories Pvt. Limited,
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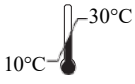
Thane (W) -400604, MS, India

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



Storage temperature

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

CE **CE Marking**

Do not use if package is damaged

Disclaimer :

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

Corn Meal Agar

M146

Intended use

Recommended for chlamydospore production by *Candida albicans* and the maintenance of fungal stock cultures.

Composition**

Ingredients	Gms / Litre
Corn meal, infusion from	50.000
Agar	15.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. If desired add 1% polysorbate 80. 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

Chlamydospore production is an accepted criterion for the identification of *Candida* species. Corn Meal Agar is a well-established mycological medium used for the cultivation of fungi and to study chlamydospores production of *Candida* species (6). Corn Meal Agar is a general purpose medium used for the cultivation of fungi and for the study of *Candida* species for chlamydospore production. Pollack and Benham (6) have described the usefulness of this medium for studying the morphology of *Candida*. Walker and Huppert (8) modified this medium by adding polysorbate 80, which then stimulated faster and plenty of chlamydospore formation of *Candida* species.

This is a very simple formulation containing only cornmeal infusion and agar. However this infusion has enough nutrients to enhance the growth of fungi. Polysorbate 80 is a mixture of oleic esters, which activates the production of chlamydospore by *Candida albicans*, *Candida stellatoides* and *Candida tropicalis* (3). Some *Candida* species lose their ability of chlamydospore formation by repeated sub culturing.

Pick a suspected colony from Sabouraud Dextrose Agar (M063) using a straight wire, and make a deep cut in the Corn Meal Agar plate. Repeat for each colony. Place a flamed sterile coverslip over the line of inoculum. After incubation for 24-48 hours at 25-30°C, the streaks are examined microscopically, through the coverslip, using low and high power objectives.

C.albicans produces mycelium bearing ball-like clusters of budding cells and characteristics thick walled round chlamydospores (2).

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8).

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 tests must be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow coarse free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured, opalescent gel forms in Petri plates

Reaction
Reaction of 1.7% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH
5.80-6.20

Cultural Response
Cultural characteristics observed after an incubation at 23-27°C for upto 4 days.

Organism	Inoculum (CFU)	Growth	Chlamydospores	Recovery
Aspergillus brasiliensis ATCC 16404 (00057)*	50-100	luxuriant	negative	
Candida albicans ATCC 10231 (00054*)	50-100	luxuriant	positive	>=70%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	luxuriant	negative	>=70%
Saccharomyces uvarum ATCC 28098	50-100	luxuriant	negative	>=70%

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,4).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Conant N. F., Smith D. T., Baker R. D., Callaway J. L. and Martin D. S., 1971, Manual of Clinical Mycology, 3rd Ed., USA
3. Cooper and Silvo-Hunter, 1985, Manual of Clinical Microbiology, Lennette, Balows, Hausler and Shadomy (Eds.), 4th ed., ASM, Washington, D.C.
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. Pollack and Benham, 1960, J. Lab. Clin. Med., 50:313.
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Walker and Huppert, 1960, Tech. Bull. Reg. Med. Technol., 30:10.

Revision : 03/2018

Acetamide Broth (Twin Pack)

M148

Intended Use:

Recommended for confirmation of *Pseudomonas aeruginosa* in water samples.

Composition**

Ingredients	Gms / Litre
Part A	-
Acetamide	10.000
Part B	-
Sodium chloride	5.000
Dipotassium hydrogen phosphate	1.390
Potassium dihydrogen phosphate	0.730
Magnesium sulphate	0.500
Phenol red	0.012
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 7.63 grams of part B in 1000 ml purified / distilled water. Add 10.0 grams of Part A. Heat if necessary to dissolve the medium completely. Dispense in 10ml amounts in tubes or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

Principle And Interpretation

Acetamide Broth is formulated as per the recommendation of Standard Methods for the Examination of Water and Wastewater (1). Acetamide is utilized by a wide variety of non-fermenting organisms (2,3). The media contains inorganic salts and acetamide a sole carbon and nitrogen source. However very few organisms growing in the medium metabolize acetamide by the process of deamination (acrylamidase activity) (4,5). This unique ability is useful in identification of various non-fermenting gram-negative organisms (6,7,8). This ability is shown by *Pseudomonas aeruginosa, Pseudomonas aciovorans* Group III *(Achromobacter xylosoxidans)* and *Alcaligenes odorans* (9). Acetamide deamination leads to the liberation of ammonia, which thereby increases the pH of the medium, leading to a subsequent colour change of the phenol red indicator from yellow orange to purplish red. Some strains require upto seven days to exhibit a positive reaction as they deaminate acrylamide slowly. However, only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. It is therefore, not advisable to rely on this test as the only criterion for identification. Phosphates in the media serve as buffering agents, Magnesium sulphate is a source of ions that stimulate metabolism whereas Acetamide serves as the sole nitrogen and carbon source. Sodium chloride maintains osmotic equilibrium. Phenol red is the pH indicator.

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 (10). 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.Only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. It is therefore, not advisable to rely on this test as the only criterion for identification.
2.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

Part A : Colourless deliquescent crystals Part B : Light yellow to light pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Orange coloured clear solution in tubes

Reaction

Reaction of the medium (Mixture of 1% w/v Part A and 0.76% w/v of Part B) 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 4-7 days.

Organism	Inoculum (CFU)	Growth	Deamination
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good - luxuriant	positive reaction, purplish red colour (within 7days)
Stenotrophomonas maltophilia ATCC 13637	50-100	good - luxuriant	negative reaction,no purplish red colour (after 7 days)

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

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Revision : 03 / 2024

Mueller Hinton Agar

M173

Intended Use:

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

Composition**

Ingredients	g / L
HM infusion solids B # (from 300g)	2.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters
- Equivalent to Beef heart infusion
- Equivalent to Casein acid hydrolysate

Directions

Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (1). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (2). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (3). Mueller Hinton Agar has been selected by the CLSI for several reasons:

i. It demonstrates good batch-to-batch reproducibility for susceptible testing.
ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
iii. It supports the growth of most non-fastidious bacterial pathogens and
iv. Many data and much experience regarding its performance have been recorded (4).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (5). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (6). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae.*

HM infusion B from and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT).

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (7,1,2). A standardized suspension of the organism is swabbed over the entire surface of the medium.

Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (4). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (4,8).

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and

capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (9). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical and Laboratory Standards Institute) Approved Standard (10).

Type of specimen

Clinical samples : Pure cultures isolated from urine , stool, blood etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,10-13).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

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 1.7% agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH

7.20-7.40

Cultural Response

Antibiotic susceptibility tests are performed in accordance with, and meet the acceptance limits of the current ISO/TS 16782 (15). Performance of the medium is checked in accordance with the CLSI/ EUCAST guidelines.

For testing *S. pneumoniae* : The medium was supplemented with 5% Horse blood and 20 mg/l NAD , incubated at 34-36°C for 18-20 hours in 5% CO_2 .

For testing *H. influenzae* : The medium was supplemented with 5% Horse blood and 20 mg/l -NAD, incubated at 34-36°C for 18-20 hours in 5% CO2 .

Antibiotic Sensitivity test

Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100).

Thymine/Thymidine Content

The zones for these discs are indicative of the Thymine/Thymidine content of the medium.

Divalent Cation Content

$ The zones for these discs are indicative of the Divalent Cation content of the medium

Organism	Growth	Standard Zone	Incubation temperature	Incubation period
***Escherichia coli* ATCC 25922 (00013*)**	luxuriant		34-36°C	16-20 hours
Cephalothin CEP 30mcg		15-21 mm		
Ampicillin AMP 10mcg		15-22 mm		
Chloramphenicol C 30 mcg		21-27 mm		
Gentamicin GEN 10mcg		19-26 mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		23-29 mm		
Sulphafurazole SF 300 mcg		15-23 mm		
Cefotaxime CTX 5 mcg		25-31 mm		
Tigecycline TGC 15mcg		20-27 mm		
Tetracycline TE 30 mcg		18-25 mm		
Amoxicillin- clavulanate AMC 30 mcg		18-24 mm		
Ciprofloxacin CIP 5mcg		29-38 mm		
***Escherichia coli* ATCC 35218**	luxuriant		34-36°C	16-20 hours
Amoxicillin- clavulanate AMC 30 mcg		17-22 mm		
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm		
Ticarcillin TI 75 mcg		6 mm		
Ticarcillin/Clavulanic acid TCC 75/10mcg		21-25mm		
Ampicillin AMP 10 mcg		6 mm		
Ampicillin/Sulbactam A/S 10/10 mcg		13-19 mm		
***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)**	luxuriant		34-36°C	16-20 hours
Erythromycin E 15 mcg		22-30 mm		
Linezolid LZ 30 mcg		24-30 mm		
Tetracycline TE 30 mcg		24-30 mm		
Ciprofloxacin CIP 5mcg		22-30 mm		
Amoxyclav(Amoxicillin/ Clavulanic acid) AMC 30 mcg		28-36 mm		
Co-Trimoxazole COT 25 mcg		24-32 mm		
Cefoxitin CX 30 mcg		23-29 mm		
Oxacillin OX 1mcg		18-24 mm		
Pristinomycin RP 15 mcg		21-28 mm		
Gentamicin GEN 10 mcg		19-27 mm		
Penicillin-G 10 units		26-37 mm		
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm		
***Staphylococcus aureus* subsp. *aureus* ATCC 29213 (00131*)**	luxuriant		34-36°C	16-20 hours
Penicillin-G P 1 unit		12-18 mm		
Cefoxitin CX 30 mcg		24-30 mm		
Erythromycin E 15 mcg		23-29 mm		
Linezolid LZ 10 mcg		21-27 mm		
Gentamicin GEN 10 mcg		19-25 mm		
Tetracycline TE 30 mcg $		23-31 mm		
Ciprofloxacin CIP 5mcg		21-27 mm		

***Staphylococcus aureus* subsp. *aureus*ATCC 43300 (MRSA) (00211*)**	luxuriant		34-36°C	24 hours
Oxacillin OX 1 mcg		Very Hazy to No Zone		
Cefoxitin CX 30 mcg		<=21 mm		
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		34-36°C	16-20 hours
Ceftazidime CAZ 30 mcg		22-29 mm		
Ciprofloxacin CIP 5mcg		25-33 mm		
Tobramycin TOB 10 mcg $		20-26 mm		
Amikacin AK 30 mcg $		20-26 mm		
Aztreonam AT 3mcg		23-29 mm		
Cephotaxime CTX 30 mcg		18-22 mm		
Gentamicin GEN 10 mcg $		17-23 mm		
Imipenem IPM 10 mcg		20-28 mm		
Piperacillin PI 100 mcg		25-33 mm		
Piperacillin Tazobactum PIT 30/6 mcg		23-29 mm		
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		34-36°C	16-20 hours
Trimethoprim TR 5 mcg #		24-32 mm		
Ampicillin AMP 2 mcg		15-21 mm		
Imipenem IPM 10 mcg		24-30 mm		
Linezolid LZ 10 mcg		19-25 mm		
Nitrofurantoin NIT 100 mcg		18-24 mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		26-34 mm		
Vancomycin VA 5 mcg		10-16 mm		
***Enterococcus faecalis* ATCC33186 (00210*)**	luxuriant		34-36°C	16-20 hours
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		<=20 mm		
***Streptococcus pneumoniae* ATCC 49619**	luxuriant		34-36°C	18-20 hours
Vancomycin VA 5 mcg		17-23 mm		
***Haemophilus influenzae* ATCC 49247**	luxuriant		34-36°C	18-20 hours
Ampicillin AMP 2 mcg		6-12 mm		
***Haemophilus influenzae* ATCC 49766**	luxuriant		34-36°C	18-20 hours
Cefixime CFM 5 mcg		29-35 mm		

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

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Revision : 06/2024

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Plot No.C-40, Road No.21Y,
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IVD ***In vitro*** **diagnostic medical device**

10°C–30°C **Storage temperature**

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Bordet Gengou Agar Base

M175

Intended use

Recommended for detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis.*

Composition**

Ingredients	g / L
Potato infusion from 125g	4.500
Peptone	10.000
Sodium chloride	5.500
Agar	20.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 grams in 1000 ml purified/distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 15-20 % sterile, fresh defibrinated blood (sheep, rabbit, human or horse). For selectivity aseptically add rehydrated contents of 2 vials of Bos Selective Supplement (FD004). Mix thoroughly, taking care to avoid incorporation of air bubbles and pour into sterile Petri plates.

Principle And Interpretation

Bordet Gengou Agar Media were originally formulated by Bordet and Gengou (1) for cultivation of *Bordetella* species. *Bordetella pertussis* is the causative agent of whooping cough and with the help of cough-plate technique, *B. pertussis* can be isolated from pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs. Kendrick and Eldering (2) modified the original media by replacing 50% human or rabbit blood with 15% sheep blood to make the medium more enriched for detection of *B.pertussis* by the virtue of its haemolytic reaction. Enrichment of the basal media with 25% human blood aids in the detection of *Mycobacterium* species from small sputum inocula and in Streptomycin sensitivity testing (3). The medium is highly nutritious thus supports luxuriant growth of *Bordetella* species and can also be used for mass cultivation of *B.pertussis* for vaccine production (4) and for maintaining stock cultures (1)

Potato infusion and peptone serve as carbon and nitrogen source, amino acids while glycerol and blood enrichment provides additional nutrients. Sodium chloride maintains osmotic equilibrium. Incubation should be carried out in a moist chamber (60% humidity) at 37°C for upto 7 days. Medium should not be over dried before use. After 40 hours *B.pertussis* colonies appear smooth, raised, glistening with a zone of haemolysis. Some strains of *Bordetella* are not haemolytic. For confirmation, serodiagnosis and biochemical test should be performed. This medium can be made more selective for *Bordetella,* by using antibiotics like penicillin (5), methicillin (4), cephalexin (2) of which, cephalexin was found to be superior. Cephalexin suppresses unwanted nasopharyngeal growth and significantly increases the isolation rate of *Bordetella* species. Cephalexin is used at a concentration of 40 mg/liter (FD004). Amphotericin B (10 µg/ml) can be added as an antifungal agent to the medium.

For isolation of *B.pertussis* from specimens, use standard procedures. Incubate the plates in a moist chamber at 35-37°C for 7 days and examine daily with or without dissecting microscope (oblique illumination) to detect the presence of *B. pertussis* . Sometimes the accompanying mold colonies can mask the *B.pertussis* colonies. Use sterile scalpel or needle to remove the portion of the agar that contains spreading colonies of moulds. *B.pertussis* colonies may not be visible without the aid of a microscope for 2-4 days. After 7 days of incubation plates may be discarded as negative. Some *Haemophilus* species will grow on *Bordetella* isolation media and cross-react with *B.pertussis* antisera. It may be prudent to rule out X and V factor dependence.

Type of specimen

Clinical samples -Pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

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

Warning and Precautions :

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

Limitations :

1. Some *Haemophilus* species will grow on *Bordetella* isolation media and cross-react with *B. pertussis* antisera.
2. *B. pertussis* colonies may not be visible without the aid of a microscope for 2-4 days.

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 2.0% agar gel.

Colour and Clarity of prepared medium

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of glycerol and 15% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

Reaction

Reaction of 4% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

Cultural characteristics observed with added Glycerol and 15% v/v sterile defibrinated blood and Bos Selective Supplement (FD004), after an incubation at 35-37°C for 3-4 days.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Bordetella bronchiseptica ATCC 4617	50-100	good-luxuriant	>=50%	gamma
Bordetella parapertussis ATCC 15311	50-100	good-luxuriant	>=50%	gamma
Bordetella pertussis ATCC 8467	50-100	good-luxuriant	>=50%	beta
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 (7,8).

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Revision : 04/2024

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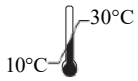
IVD ***In vitro* diagnostic medical device**



Storage temperature

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Casman Agar

M201

Intended Use:

Recommended for isolation of fastidious microorganisms from clinical specimens under reduced oxygen tension.

Composition**

Ingredients	g/ L
Proteose peptone	10.000
Tryptose	10.000
HM peptone B	3.000
Dextrose (Glucose)	0.500
Corn starch	1.000
Sodium chloride	5.000
Nicotinamide	0.050
p-Amino benzoic acid (PABA)	0.050
Agar	14.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.6 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 0.15% v/v sterile water lysed blood (water:blood:3:1) of 5% sterile blood. Alternatively add 5% partially lysed blood. Mix well and dispense as desired.

Principle And Interpretation

Fastidious microorganisms such as *Haemophilus* and *Neisseria* require the addition of X and V- growth factors for in vitro cultivation (1). Casman (1, 2, 3) described a blood-enriched medium for cultivation of *Haemophilus* and *Gonococci* (1). The medium was developed to replace the previously described formulations that required time-consuming preparations using fresh and heated blood and meat infusion to supply the essential nutrients for growth of these fastidious organisms (2, 3). Blood supplies factor-X (hemin) and factor-V (Nicotinamide Adenine Dinucleotide), which is required for growth of *Haemophilus influenzae*. Sheep blood lacks factor-V due to NADase, an enzyme that destroys factor- V (4). Horse and rabbit blood supplies both the factor X and factor V, and are relatively free of NADase activity, therefore it is preferred over sheep blood. Nicotinamide is added to medium to inhibit nucleotidase of erythrocytes that may destroy factor V.

Proteose peptone, tryptose and HM peptone B provide amino acids and other complex nitrogenous nutrients. Dextrose improves growth of pathogenic cocci. Corn starch prevents fatty acids from inhibiting the growth of *Neisseria gonorrhoeae*, without interfering with haemolytic reaction. Corn starch also neutralizes the inhibitory action of dextrose. Inoculate the medium as soon as the specimen arrives at the laboratory. After incubation *H. influenzae* produces colourless to grey colonies with a characteristic mousy odour while *N. gonorrhoeae* produces small colourless to greyish-white colonies.

Type of specimen

Clinical samples : vaginal swabs, rectal swabs, etc.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Further biochemical 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 homogeneous free flowing powder
Gelling
Firm, comparable with 1.4% Agar gel.
Colour and Clarity of prepared medium
Basal medium: Yellow coloured clear to slightly opalescent gel. After addition of 5%w/v sterile defibrinated blood : Cherry red coloured After addition of 5%w/v sterile defibrinated blood: opaque gel forms in Petri plates.
Reaction
Reaction of 4.36% w/v aqueous solution at 25°C. pH : 7.3±0.2
pH
7.10-7.50
Cultural Response
Cultural characteristics observed with added water-lysed blood, after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Haemophilus influenzae ATCC 35056	50-100	good	50-70%	none
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Streptococcus mitis ATCC 9811	50-100	luxuriant	>=70%	beta
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta

Storage and Shelf Life

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

Disposal

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

Reference

1. Casman, 1947, Am. J. Clin. Pathol., 17:281.
2. Casman, 1942, J. Bact., 43:33.
3. Casman, 1947, J. Bact., 53:561.
4. Krunveide and Kuttner, 1938, J. Exp. Med., 67:429.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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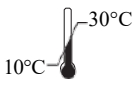

Storage temperature

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Tinsdale Agar Base

M314

Intended Use:

Recommended for selective isolation and differentiation of *Corynebacterium diphtheriae.*

Composition**

Ingredients	g / L
Peptone	20.000
Sodium chloride	5.000
L-Cystine	0.240
Sodium thiosulphate	0.430
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.67 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add Tinsdale Selective Supplement (FD073, Part A and Part B). Mix well and pour into sterile Petri plates.

Principle And Interpretation

The Corynebacteria are gram-positive, non-sporulating, non-motile rods. They are often club-shaped and frequently banded or beaded with irregularly stained granules. These bacteria are generally aerobic or facultative, but microaerophilic species do occur. *Corynebacterium diphtheriae* produces a powerful exotoxin that causes diphtheria in humans. In nature, *C.diphtheriae* occurs in nasopharyngeal area of infected persons or healthy carriers.

The three biotypes of *C.diphtheriae* are mitis, intermedius and gravis (1). The signs and symptoms of diphtheria are sore throat, malaise, headache and nausea (2). Tinsdale Agar Base Medium was developed by Tinsdale (3,4) for the selective isolation and differentiation of *C.diphtheriae* from diphtheroids. This medium was modified by Billings (2), which improved the recovery and differential qualities of *C.diphtheriae.* The present medium is according to the modified Billings Medium. Moore and Parsons (3) confirmed the halo formation as a characteristic property of *C.diphtheria* with the exception of *C.ulcerans,* which forms colony with similar features as *C.diphtheriae.*

Peptone provides nitrogenous compounds. L-cystine and sodium thiosulphate form the H_2S indicator system. Potassium tellurite from the supplement inhibits all gram-negative bacteria and most of the upper respiratory tract normal flora.

C.diphtheriae forms grayish black colonies surrounded by a dark brown halo while diphtheroids commonly found in the upper respiratory tract do not form such colonies. Dark brown halo around the colony is due to H2S production from cystine combining with the tellurite salt. Moore and Parsons (3) found Tinsdale Medium as an ideal medium for the routine cultivation and isolation of *C.diphtheriae.* They also confirmed the stability of halo formation on clear medium and its specificity for *C.diphtheriae* and *C.ulcerans. C.ulcerans* found in nasopharynx form colonies same as *C.diphtheriae* and require further biochemical confirmation (5).

Do not incubate the plates in 5-10% CO2 as it retards the development of characteristic halos (6). Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C.diphtheriae* (1). *C.ulcerans* , *C.pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar (1).Several organisms may exhibit slight browning on Tinsdale Agar in 18 hours; therefore the plates should be read after complete incubation period (48 hours) (1).

Type of specimen

Clinical samples - Throat swab

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

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

Warning and Precautions :

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

Limitations :

1. Do not incubate the plates in 5-10% CO_2 as it retards the development of characteristic halos (6).

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 4.07% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours with added Tinsdale Selective Supplement (FD073, Part A and Part B).

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
Corynebacterium diphtheriae type gravis	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type intermedius	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type mitis	50-100	good-luxuriant	>=50%	brown-black with halo
Klebsiella pneumoniae ATCC 13883 (00097*)	>=10^4	inhibited	0 %	
Streptococcus pyogenes ATCC 19615	50-100	good	40-50%	black pin point, without halo

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 (7,8).

Reference

1. Isenberg, (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.
2. Billings E., 1956, An investigation of Tinsdale Tellurite Medium: its usefulness and mechanisms of halo-formation, M.S. thesis, University of Michigan, Ann Arbor, Mich.
3. Moore M. S. and Parsons E. I., 1958, J. Infect. Dis., 102:88.
4. Tinsdale G. F. W., 1947, J. Pathol. Bacteriol., 59:461.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
6. 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
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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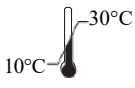

Storage temperature

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Malonate Broth

M382

Intended Use:

Recommended for differentiation of *Enterobacter* and *Escherichia* on the basis of malonate utilization from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	2.000
Dipotassium hydrogen phosphate	0.600
Potassium dihydrogen phosphate	0.400
Sodium chloride	2.000
Sodium malonate	3.000
Bromothymol blue	0.025
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Dissolve 8.02 grams in 1000 ml purified/distilled water. Dispense into sterile tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid the addition of carbon and nitrogen from other sources.

Principle And Interpretation

Leifson developed a synthetic liquid medium, which differentiated *Aerobacter* (now *Enterobacter*) from *Escherichia* species based on their ability to utilize malonate (1) where Enterobacter utilizes malonate and Escherichia does not. An organism that can simultaneously utilize sodium malonate as its carbon source and ammonium sulfate as its nitrogen source produces alkalinity due to the formation of sodium hydroxide (2). The alkali changes the color of the bromothymol blue indicator in the medium to light blue and finally to prussian blue. The color of the medium remains unchanged in the presence of an organism that cannot utilize these substances. Also some malonate-positive organisms produce only a slight alkalinity that causes the results to be difficult to interpret. Therefore these tubes should be compared with an un-inoculated malonate tube (2).

Type of specimen

Isolated Microorganism from clinical and water samples

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

1. Growth from an 18-24 hours pure culture; Kligler Iron Agar (M078) or other suitable culture (5).
2. Light inoculum must be used (2).

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 green homogeneous free flowing powder
Colour and Clarity of prepared medium
Bluish green coloured clear solution without any precipitate
Reaction
Reaction of 0.8% w/v aqueous solution at 25°C. pH : 6.7±0.2
pH
6.50-6.90
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours

Organism	Inoculum (CFU)	Growth	Malonate Utilization
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	positive reaction, dark blue colour
Escherichia coli ATCC 25922 (00013*)	50-100	poor-fair	negative reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	positive reaction, dark blue colour
Salmonella Arizonae ATCC 13314	50-100	luxuriant	positive reaction, dark blue colour
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	fair-good	negative reaction

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

Storage and Shelf Life

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

Disposal

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

Reference

1. Leifson, 1933, J. Bact., 25:329.
2. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision :03/2023

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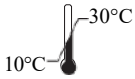

Storage temperature

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Decarboxylase Broth Base, Moeller (Moeller Decarboxylase Broth Base)

M393

Intended Use:

Recommended to differentiate bacteria from clinical and non-clinical samples on the basis of their ability to decarboxylate the amino acid.

Composition**

Ingredients	g / L
Peptone	5.000
HM peptone B	5.000
Dextrose (Glucose)	0.500
Bromocresol purple	0.010
Cresol red	0.005
Pyridoxal	0.005
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10.52 grams in 1000 ml purified / distilled water. Add 10 gm. of L-Lysine, L-Arginine, L-Ornithine or other L-amino acids. When using DL-amino acids, use 2% concentration. Heat if necessary to dissolve the medium completely. When L-Ornithine is added, readjustment of the pH is required. Dispense in 5 ml amount in screw-capped tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes.

Principle And Interpretation

Moeller Decarboxylase Broth Base is used for differentiating gram-negative enteric bacilli on the basis of their ability to decarboxylate amino acids. Moeller introduced the Decarboxylase Broth for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase (1). Prior to Moellers work, bacterial amino acid decarboxylases were studied by Gale (2) and Gale and Epps (3). Production of ornithine decarboxylase is a helpful criterion in differentiating *Klebsiella* and *Enterobacter* species. *Klebsiella* are nonmotile and do not produce ornithine decarboxylase while *Enterobacter* are motile and produce ornithine decarboxylase except *Enterobacter agglomerans* (4).

This medium contains HM peptone B and peptone which provides nitrogenous and cabonaceous compounds, long chain amino acids and other essential nutrients for the growth of bacteria. Dextrose is the fermentable carbohydrate and pyridoxal is the co-factor for the decarboxylase enzyme. Bromo cresol purple and cresol red are the pH indicators in this medium. When the medium is inoculated with the dextrose fermenting bacteria, the pH is lowered due to acid production, which changes the colour of the indicator from purple to yellow. Acid produced stimulates decarboxylase enzyme. Decarboxylation of lysine yields cadaverine while putrescine is produced due to ornithine decarboxylation. Arginine is first hydrolyzed to ornithine which is then decarboxylated to form putrescine. Formation of these amines increases the pH of the medium, changing the colour of the indicator from yellow to purple. If the organisms do not produce the appropriate enzyme, the medium remains acidic, yellow in colour. Each isolate to be tested should also be inoculated into Moeller Decarboxylase Broth Base medium tube lacking the amino acid.

Inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalinization at the surface of the medium which makes the test invalid.

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

Warning and Precautions :

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

Limitations :

1.Some fastidious organisms may show delayed reaction.
2.Overlaying with mineral oil is essential for appropraite results.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured, clear solution without any precipitate in tubes

Reaction

Reaction of 1.05% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH

5.80-6.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for upto 4 days with addition of appropriate amino acids and overlaying with sterile mineral oil.

Organism	Arginine decarboxylation	Ornithine decarboxylation	Lysine decarboxylation
Citrobacter freundii ATCC 8090	variable reaction	variable reaction	negative reaction, yellow colour
# *Klebsiella aerogenes* ATCC 13048 (00175*)	negative reaction, yellow colour	positive reaction, purple colour	positive reaction, purple colour
Escherichia coli ATCC 25922 (00013*)	variable reaction	variable reaction	positive reaction, purple colour
Klebsiella pneumoniae ATCC 13883 (00097*)	negative reaction, yellow colour	negative reaction, yellow colour	positive reaction, purple colour
Proteus mirabilis ATCC 25933	negative reaction, yellow colour	positive reaction, purple colour	negative reaction, yellow colour
## *Proteus hauseri* ATCC 13315	negative reaction, yellow colour	negative reaction, yellow colour	negative reaction, yellow colour
Salmonella Paratyphi A ATCC 9150	delayed positive reaction/positive reaction,purple colour	positive reaction, purple colour	negative reaction, yellow colour

Salmonella Typhi ATCC 6539	delayed positive reaction / negative reaction	negative reaction, yellow colour	positive reaction, purple colour
Serratia marcescens ATCC 8100	negative reaction, yellow colour	positive reaction, purple colour	positive reaction, purple colour
Shigella dysenteriae ATCC 13313	negative reaction/ delayed positive reaction	negative reaction, yellow colour	negative reaction, yellow colour
Shigella flexneri ATCC 12022 (00126*)	negative reaction/ delayed positive reaction	negative reaction, yellow colour	negative reaction, yellow colour
Shigella sonnei ATCC 25931	variable reaction	positive reaction, purple colour	negative reaction, yellow colour

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

Storage and Shelf Life

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

Disposal

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

Reference

1.Moeller V., 1955, Acta Pathol. Microbiol. Scand. 36:158.
2.Gale G. F., 1940, Biochem. J., 34:392.
3.Gale and Epps, 1943, Nature, 152:327.
4.MacFaddin J., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins,Baltimore.
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6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015)Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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8.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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10.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 05/2024

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Enterococcus Confirmatory Broth

M394

Intended Use:

Recommended for confirming the presence of Enterococci in water supplies and other sources.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	5.000
Dextrose (Glucose)	5.000
Sodium azide	0.400
Sodium chloride	65.000
Methylene blue	0.010
Final pH (at 25°C)	8.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.41 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense in 100 ml quantities in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to room temperature and add 65 units of Penicillin to each 100 ml of broth prior to use.

Principle And Interpretation

Enterococcus Confirmatory Broth is formulated by Sandholzer and Winter (4) for the detection of Enterococci in water supplies, swimming pools, sewage etc. Enterococcus Confirmatory Broth has the same formula as Enterococcus Confirmatory Agar (M392) except agar, sodium chloride and Penicillin, which is used to detect Enterococci from crabmeat and oysters etc. Enterococci are differentiated from other Streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C (1).

Tryptone, yeast extract, dextrose provide nitrogeneous and carbonaceous compounds, long chain amino acids and other essential growth nutrients for Enterococci. Sodium azide inhibits gram-negative organisms. Penicillin has inhibitory effect on Staphylococci. The positive presumptive tests are confirmed by inoculating from Enterococcus Presumptive Broth (M419) to Enterococcus Confirmatory slant-broth combination prepared with an Azide Agar medium (Enterococcus Confirmatory Agar, M392) overlaid with a Salt Azide Penicillin Broth (Enterococcus Confirmatory Broth, M394). A negative catalase test is considered confirmed positive evidence of the presence of Enterococci. Single strength medium can be used for small inoculum. Production of acid and turbidity in an azide presumptive broth when incubated at 45°C is considered positive presumptive evidence for the presence of Enterococci which is confirmed by inoculating in / on Confirmatory Broth (M394).

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 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 may have slight green tinge homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured, clear solution which aquires greenish tinge at the surface on standing

Reaction

Reaction of 8.04% w/v aqueous solution at 25°C. pH : 8.0±0.2

pH

7.80-8.20

Cultural Response

Cultural characteristics observed after an incubation at 45°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

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. 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. Sandholzer and Winter, 1946, Commercial Fisheries Leaflet T1a

Revision : 03/ 2020

Thayer Martin Medium Base

M413

Intended use

Recommended for selective isolation of Gonococci from pathological specimens.

Composition**

Ingredients	g / L
Peptone, special	23.000
Starch	1.000
Sodium chloride	5.000
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.0 grams in 450 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. Aseptically add 50 ml of sterile lysed blood and rehydrated contents of one vial of Vitamino Growth Supplement (FD025) and V.C.N Supplement (FD023) or V.C.N.T Supplement (FD024). If desired GC Supplement with Antibiotics (FD021) can be used as a single supplement. Mix well before pouring into sterile Petri plates. If Hemoglobin (FD022) is used suspend 21.0 grams of Thayer Martin Medium Base in 250 ml distilled water. Heat to boiling to dissolve the medium completely. Prepare 250 ml of 2% hemoglobin solution. Sterilize separately by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix both and add the supplements as above.

Principle And Interpretation

Carpenter and Morton reported an improved medium to isolate Gonococci in 24 hours (1). Later on the efficiency of GC medium supplemented with haemoglobin and yeast concentrate was demonstrated for isolating gonococci (2). Subsequently Thayer and Martin Medium was developed for the primary isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis* from specimens containing mixed flora collected from throat, vagina, rectum and urethra (3,4). Thayer and Martin (4) used Vancomycin, Colistin and Nystatin. Martin and Lester (5) used an additional antibiotic Trimethoprim to make the medium selective.

Special peptone provides nutrients to the organisms while starch neutralizes the toxic fatty acids if present in the agar. Haemoglobin provides the X factor whereas the V factor (N.A.D.) is provided by the added supplement. Supplement (FD025) also supplies vitamins, amino acids, coenzymes etc. which enhances the growth of pathogenic *Neisseria*. Vancomycin and colistin inhibits gram-positive and gram-negative bacteria respectively (6). Nystatin inhibits fungi. This medium may inhibit *Haemophilus* species. Some strains of *Capnocytophaga* species may grow on this medium when inoculated with oropharyngeal specimens.

Type of specimen

Clinical samples : Throat, vaginal secretions, rectum and urethra

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

1.Due to nutritional variations and fastidious nature of organisms certain strains may show poor growth.
2.Some strains of *Capnocytophaga* species may grow on this medium when inoculated with oropharyngeal specimens.
3.Further biochemical identification is necessary for confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

Basal Medium : Yellow coloured clear to slightly opalescent gel. After addition of haemoglobin or sterile lysed blood and supplements: chocolate coloured opaque gel forms in Petri plates.

Reaction

Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed with added sterile FO Growth Supplement (FD022),Vitamino Growth Supplement (FD025) and V.C.N. Supplement (FD023)/V.C.N.T. Supplement (FD024) after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Neisseria gonorrhoeae ATCC 19424	50-100	good-luxuriant	>=50%	small, grayish-white to colourless, mucoid
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	>=50%	medium to large, blue-gray, mucoid
Proteus mirabilis ATCC 25933	>=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 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 (7,8).

Reference

1. Carpenter and Morton, 1947, Proc. N.Y. State Assoc. Public Hlth. Labs., 27:58.
2. Carpenter et al, 1949, Am. J. Syphil. Gonorrh. Vener. Dis., 33:164.
3. Martin, Billings, Hackney and Thayer, 1967, Public Hlth. Rep., 82:361.
4. Thayer J. and Martin J.E. Jr., 1966, Public Health Rep., 81:559.

5. Martin J.E. Jr. and Lester A., 1971, HSMHA Hlth. Service Rep., 86(1):30.
6. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 07/2024

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3951DB Maarn, NL
www.cepartner4u.eu

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

CE Marking

10°C – 30°C **Storage temperature**

Do not use if package is damaged

Tryptone Broth (Tryptone Water)

M463

Intended use

Recommended for detection of indole producing microorganisms isolated from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Tryptone	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

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

Tryptone is a good substrate for indole production because of its high tryptophan content. Certain organisms breakdown the amino acid tryptophan with the help of enzymes that mediate the production of indole by hydrolytic activity (4). The indole produced can be detected by either Kovac's or Ehrlich's reagent (5). Indole combines with the aldehyde present in the above reagent to give red colour in the alcoholic layer. The alcohol layer extracts and concentrates the red colour complex. Tryptone Water is used in conjunction with Brilliant Green Bile Broth 2% (M121) to determine the most probable number (MPN) of *E.coli* in food sample. Growth and gas production in M121 and indole production in Tryptone Water following incubation of both media at 44 ± 1°C is used as the basis for the presumptive *E.coli* test. For determination of indole, inoculate the medium with inoculum of an 18-24 hours pure culture. Incubate the tubes at 35 ± 2° C for 18-24 hours. Add 0.5 ml of indole reagent (R008) directly to the tube and agitate. Allow the tubes to stand for 5-10 minutes. Formation of red ring at the top of the tube indicates indole production.

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

Type of specimen

Water samples, Clinical samples- faeces, urine

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations :

1.Other 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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured clear solution without any precipitate

Reaction

Reaction of 1.5% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. Add 0.2 to 0.3ml of Kovac's Reagent (R008) to each tube after incubation.

Organism	Growth	Indole reaction
Escherichia coli ATCC 25922 (00013*)	luxuriant	positive reaction, red ring at the interface of the medium
# *Klebsiella aerogenes* ATCC 13048 (00175*)	luxuriant	negative reaction, no colour development / cloudy ring
Klebsiella pneumoniae ATCC 13883	luxuriant	negative reaction, no colour development / cloudy ring

Key * -Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
2. International Organization for Standardization (ISO), 1990, Draft ISO/DIS 7251:1993.
3. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
4. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.
5. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/2024

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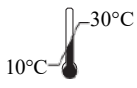
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP

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MacConkey Broth (Double strength) w/Neutral Red

M539S

MacConkey Broth (Double strength) w/Neutral Red is used for primary isolation of coliforms from large samples such as water or waste water.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	40.000
Lactose	20.000
Bile salts	10.000
Sodium chloride	10.000
Neutral red	0.140
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.14 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Distribute into test tubes with inverted Durham tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes before inoculation.

Note: Where the number of organisms is expected to be low , larger quantities of the sample may be directly added to equal amount of double strength medium in dilution bottles or flasks.

Principle And Interpretation

MacConkey Broth (Double strength) is recommended for detection of bacteria responsible for food poisoning ,for isolation, identification and enumeration of Escherichia coli (1). MacConkey Broth (Double strength) is also recommended for the primary isolation of coliforms from large samples such as water and wastewater.

MacConkey Broth has also been recommended for use in microbiological examination of foodstuffs (2) and for direct plating / inoculation of water samples for coliform counts (3). This media is also used for the Examination of Milk and Dairy Products (4) and pharmaceutical preparations (5). The selective action of this medium is attributed to bile salts, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as yellow. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium.

Quality Control

Appearance
Cream to yellow coloured homogeneous free flowing powder

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

Reaction
Reaction of 8.0% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Acid	Gas

Cultural Response

Enterobacter aerogenes ATCC 13048	50-100	luxuriant	Positive reaction	Positive reaction
Escherichia coli ATCC 25922	50-100	luxuriant	Positive reaction	Positive reaction
Salmonella choleraesuis ATCC 12011	50-100	fair to good	Negative reaction	Negative reaction
Staphylococcus aureus ATCC 25923	>=10^3	inhibited		

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.Bureau of Indian Standards IS : 5887 (Part I) - 1976, reaffirm 1986.
2. Speck M. (Ed.), 1985, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.
3. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 1992, Standard Methods for the Examination of Water and Wastewater, 18th ed., APHA, Washington, D.C.
4. Marshall R. (Ed.), 1992, Standard Methods For the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
5. The United States Pharmacopoeia XXI and the National Formulary, 16th ed., 1985, United States Pharmacopeial Convention, Inc., Washington, D.C.

Revision : 2 / 2015

Nutrient Agar pH 7.0

M561A

Intended Use

Recommended for the cultivation of *Salmonella* species.

Composition**

Ingredients	Gms / Litre
Pepone	5.000
HM extract #	3.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract

Directions

Suspend 23 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, the medium can be enriched with 5 - 10% v/v sterile defibrinated blood. Mix well and Pour into Sterile Petri plates

Principle And Interpretation

Nutrient Agar is a basic culture medium used for maintenance or to check purity of subcultures prior to biochemical or serological tests from water (1) and Dairy (7). Many bacteria have the optimum pH growth range of 6.6 to 7.0. This medium may be used as slants or plates for routine work with non-fastidious organisms. Wetmore and Gochenour (8) maintained cultures of *Malleomyces* and *Pseudomonas* on Nutrient Agar to which glycerol was added. Greenberg and Cooper (2) employed this medium in cultivation of Staphylococci for the preparation of vaccines and antigens. Nutrient Agars have relatively simple formulation which provides the necessary nutrients for the growth of many microorganisms which are not very fastidious. HM extract contains vitamins, organic nitrogen compounds, salts and little carbohydates (5). Peptone provide amino acids and long chain peptides for the organisms.

Type of specimen

Food sample

Specimen Collection and Handling

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

Warning and Precautions :

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

Limitations

1. Due to variable nutritional requirements, some strains show poor growth on this medium.
2. Further biochemical and serological testing is required for complete 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

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction
Reaction of 2.3% 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 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	>=70%
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	>=70%
Salmonella Typhi ATCC 6539	50-100	luxuriant	>=70%
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=70%
Shigella flexneri ATCC 12022	50-100	luxuriant	>=70%
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%
Yersinia enterocolitica ATCC 9610 (00038*)	50-100	luxuriant	>=70%
Yersinia enterocolitica ATCC 23715 (00160*)	50-100	luxuriant	>=70%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

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. Greenberg and Cooper, 1960, Can. Med. Assn. J., 83:143.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Pelczar, Chan and Kreig, 1986, Microbiology, 5th ed., McGraw-Hill Book Company, New York.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Standard Methods for the Examination of Dairy Products, 1978, 14th ed., APHA, Washington D.C.
8. Wetmore and Gochenour, 1956, J. Bact., 72:79.

Revision : 03/2020

HIMEDIA

Technical Data

Violet Red Bile Glucose Agar w/o Lactose

M581

Intended Use:

Recommended for enumeration of *Enterobacteriaceae* in raw food and clinical samples. The composition and performance criteria are in accordance with ISO 21528-1&2:2017 and ISO 11133:2014 /Amd.2 : 2020 (E).

Composition**

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

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

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

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

**Formula adjusted, standardized to suit performance parameters

$ -Equivalent to Enzymatic digest of animal tissues

Directions

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

Principle And Interpretation

Violet Red Bile Agar, a modification of MacConkey original formulation (1) is used for the enumeration of coli-aerogenes bacterial group. Violet Red Bile Glucose Agar w/o Lactose, a modification of VRBA (M049), was designed for the enumeration of *Enterobacteriaceae* (2). It employs the selective inhibitory components crystals violet and bile salts and the indicator system glucose and neutral red. Sought bacteria will dissimilate glucose and produce purple zones around the colonies (3). ISO committee has also recommended this medium (4,5). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/or at elevated temperature, i.e. equal to or above 42°C (6-8).

Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (9).

Type of specimen

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

Specimen Collection and Handling

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,12-14).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines & local standards (15).

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

Warning and Precautions

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

Limitations

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

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Over incubation may result in reverting of reaction.
4.Further biochemical tests must be carried out on colonies of pure culture for confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Gelling

Firm,comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.20-7.60

Cultural Response

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

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

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

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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

2. Mossel D. A. A., Eclderink I., Koopmans M. and Van Rossem F., 1978, Lab. practice, 27 No. 12: 1049.
3. Corry J. E. L., Curtis G. D. W. and Baird R. M., (Ed.), 1995, Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, Elsevier, Amsterdam.
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13. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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15. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 06/2024

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30°C
10°C **Storage temperature**

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Giolitti-Cantoni Broth Base

M584

Intended Use:

Recommended for selective enrichment of *Staphylococcus aureus* from food.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
HM peptone B #	5.000
Yeast extract	5.000
Mannitol	20.000
Sodium chloride	5.000
Lithium chloride	5.000
Glycine	1.200
Sodium pyruvate	3.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 54.2 grams in 1000 ml purified/distilled water. Warm gently to dissolve the medium completely. Dispense 19 ml amounts in 20mm x 200mm test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool rapidly to room temperature and aseptically add 0.3 ml of 3.5% Potassium Tellurite Solution (FD047) to each tube. Add 0.03 ml for testing meat and meat products. Mix well before use.

Principle And Interpretation

Giolitti-Cantoni Broth Base is a fluid medium employed for the recovery of low number of Staphylococci from foodstuffs as described by Giolitti and Cantoni (2). Giolitti- Cantoni Broth was also recommended by Mossel et.al. for detecting *Staphylococcus aureus* in dried milk, baby food and other food products (9). This medium was recommended as an enrichment medium by the International Dairy Federation (IDF) and APHA for detecting *S.aureus* in dried milk and other foods stating that the organism should be absent in 1 gram of sample (4,8). ISO committee has also recommended this medium for examination of meat and meat products (3).

Giolitti-Cantoni Broth Base contains tryptone, yeast extract and HM peptone B as sources of carbon, nitrogen, vitamins and minerals and B-complex vitamins. Mannitol and sodium pyruvate in the basal medium act as growth stimulants for *S. aureus*.

Lithium chloride inhibits gram-negative lactose fermenting bacilli. Potassium tellurite and glycine inhibit gram-positive bacilli (7). Addition of sterile paraffin wax to the inoculated medium inhibits Micrococci due to creation of anaerobic conditions. Potassium tellurite concentration must be reduced as per the weight of test sample (0.1 - 0.01 gram).

Type of specimen

Food samples

Specimen Collection and Handling

Inoculate 1 gram of sample or 1 ml of a suitable dilution of a sample into 19 ml of Giolitti-Cantoni Broth tubes in duplicate. Overlay the medium with 5 ml molten sterile paraffin wax and incubate at 37°C for 24-48 hours and examine daily. Blackening of the medium (usually at the bottom) within 48 hours indicates the presence of *S.aureus*. The blackened medium, when streaked on Baird Parker Agar (M043), shows black colonies surrounded by clear zones (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. The medium should be inoculated as soon as it has been cooled after sterilization, otherwise absorbed oxygen should be expelled by placing the tubes in free-flowing steam for 15-20 minutes.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to brownish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured, clear solution without any precipitate

Reaction

Reaction of 5.42% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural characteristics observed with added 3.5% PotassiumTellurite Solution (FD047), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Tellurite reduction
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good-luxuriant	positive, blackening at the bottom of the tubes or general blackening of the medium
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	
Micrococcus luteus ATCC 10240	>=10^4	inhibited	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	good-luxuriant	positive, blackening at the bottom of the tubes or general blackening of the medium
Bacillus cereus ATCC 11778 (00001*)	>=10^4	inhibited	
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	poor-fair	variable reaction

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

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

Reference

1. De Waart J., Mossel D. A. A., Ten Broeke R. and Van de Moosdijk A.,1968, J. Appl. Bacteriol., 31:276.
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6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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Revision : 03 /2021

Perfringens Agar Base (T. S. C. /S. F. P. Agar Base) M837

Intended Use:

Perfringens Agar Base with the addition of selective supplement and enrichment, it is used for the presumptive identification and enumeration of *Clostridium perfringens.*

Composition**

Ingredients	g / L
Tryptose	15.000
HM peptone B #	5.000
Soya peptone	5.000
Yeast extract	5.000
Sodium metabisulphite	1.000
Ferric ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 23.5 grams in 475 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°) for 15 minutes. Cool to 45-50°C. Add 25 ml of Egg Yolk Emulsion (FD045) and rehydrated contents of 1 vial of S.F.P. Selective Supplement (FD013) / T.S.C. Selective Supplement (FD014). Alternatively if fluorogenic detection is desired add rehydrated contents of CMF Selective Supplement (FD243) instead of FD013/FD014. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Tryptose Sulphite Cycloserine Agar (TSC) was originally formulated by Harmon et al (1) for the enumeration of *C. perfringens* from food. TSC Agar has been documented as one of the most useful media for the quantitative recovery of *C. perfringens* while suppressing growth of other facultative anaerobes (2). TSC Agar Base (with FD014) or SFP Agar Base (with FD013) is comparable in performance for isolation of *C.perfringens* (3,4). Perfringens Agar Base is also recommended by APHA (5). Perfringens Agar Base can be made selective either by addition of D-cycloserine (FD014) (1, 2) or Kanamycin and Polymyxin B (FD013) (6).

Tryptose, Soya peptone, yeast extract, HM peptone B provide nitrogenous compounds, carbon, sulphur, vitamin B complex and trace elements essential for clostridial growth. Sodium metabisulphite and ferric ammonium citrate act as an indicator of sulphite reduction, indicated by black coloured colonies. D-Cycloserine (FD014), Kanamycin and Polymyxin B (FD013) help in the selective isolation of *C.perfringens* by inhibiting accompanying flora. Egg yolk emulsion serves as a source of lecithin utilized by *C.perfringens* (M837).

Type of specimen

Clinical- stool, abscess; Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium :Amber coloured clear to slightly opalescent gel. After Addition of Egg Yolk Emlusion (FD045) : Yellow coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.7% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Cultural characteristics observed under anaerobic condition with added T.S.C. Selective Supplement (FD014)/S.F.P. Selective Supplement (FD013)/CMF Selective Supplement (FD243) and Egg Yolk Emulsion (FD045), after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Sulphite Reduction	Lecithinase/ Haloes	Fluorescence
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%	positive, blackening of medium	Positive reaction, opaque zone around the colony	Positive Reaction
****Paeniclostridium sordellii* ATCC 9714	>=10^4	inhibited	0%			

Key: **Formerly known as *Clostridium sordellii*

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Harmon S. M., Kauttar D.A. and Peiler J. T., 1971, Appl. Microbiol., 22:688.
2. Harmon S. M. and Kautter D.A., 1987, J. Asso. Off. Anal. Chem., 70: 994.
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7.Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
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Revision : 06/2024

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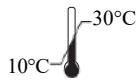

Storage temperature

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Yersinia Selective Agar Base

M843

Intended use

Recommended for the selective isolation and enumeration of *Yersinia enterocolitica* from clinical specimens and food samples.

Composition**

Ingredients	g / L
Peptone, special	20.000
Yeast extract	2.000
Mannitol	20.000
Sodium pyruvate	2.000
Sodium chloride	1.000
Magnesium sulphate	0.010
Sodium deoxycholate	0.500
Neutral red	0.030
Crystal violet	0.001
Agar	12.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.02 grams in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add reconstituted contents of 1 vial of CTN Selective Supplement (FD034). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Yersinia enterocolitica is widely distributed in lakes and reservoirs. Epizootic outbreaks of diarrhea, lymphadenopathy, pneumonia and spontaneous abortions occur in various animals. It is the most common species of *Yersinia* recovered from clinical specimens. *Y. enterocolitica* is biochemically more active at room temperature than at 37°C. Yersinia Selective Agar Base with added Yersinia Selective Supplement is used to isolate *Y. enterocolitica* from clinical and non-clinical specimens. The formulation is based on CIN Agar of Schiemann (1,2) and is recommended by ISO Committee (3). Schiemann (1) modified his previous formula of CIN medium by replacing bile salts with sodium deoxycholate.

The medium differentiates between mannitol fermenting and non-fermenting bacteria. Microorganisms that ferment the sugar mannitol acidify the medium and cause a localized drop in pH around the colonies. In presence of neutral red, the colonies take red colour. Mannitol negative organisms form colourless and translucent colonies. The medium is selective due to the of presencesodium deoxycholate and crystal violet, which inhibit gram-positive and a number of gram-negative bacteria. Addition of antibiotic supplement makes it highly selective for *Yersinia.* Typical colonies of *Y. enterocolitica* will form dark red colonies resembling bull's eye, which are normally surrounded by a transparent border. Colony size, smoothness and ratio of the border to center diameter may vary among different serotypes.

For the isolation of *Y. enterocolitica* by direct plating and pour plating, inoculate the specimen directly onto the medium. Incubate at 22-32°C for 24-48 hours or suspend the sample (food, faeces, etc.) in sterile Phosphate Buffer Saline and incubate for upto 21 days (4) at 4°C. Periodically subculture samples onto Yersinia Agar Plate and incubate as above.

Type of specimen

Clinical samples - faeces, urine, etc.; Food and dairy samples.

Specimen Collection and Handling:

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,7,8).

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

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective

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

Limitations :

1. *Serratia liquefaciens, Citrobacter freundi* and *Enterobacter agglomerans* may resemble *Y.enterocolitica* that can be further identified by biochemical tests.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.25% Agar gel.

Colour and Clarity of prepared medium

Orange red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added CTN Selective Supplement (FD034) after an incubation at 22-32°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Proteus mirabilis ATCC 25933	>=10^4	inhibited	0%	
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	0%	
Yersinia enterocolitica ATCC 27729	50-100	good-luxuriant	>=50%	transluscent with dark pink centre & bile precipitate.
Yersinia enterocolitica ATCC 23715 (00160*)	50-100	good-luxuriant	>=50%	transluscent with dark pink centre & bile precipitate.
Yersinia enterocolitica ATCC 9610 (00038*)	50-100	good-luxuriant	>=50%	transluscent with dark pink centre & bile precipitate.

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1.Schiemann D. A., 1979, Can. J. Microbiol., 25: 1298.
2.Schiemann D. A., 1980, Can. J. Microbiol., 26: 1232.
3.International Organization for Standardization (ISO), 1994 Draft ISO/DIS 10273.
4.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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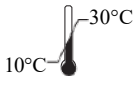

Storage temperature

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HIMEDIA®

Technical Data

HiCrome™ Candida Differential Agar Base M1297AR

Intended use

HiCrome™ Candida Differential Agar Base is selective and differential medium for rapid isolation and identification of *Candida* species from mixed cultures from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Peptone	4.000
Chromogenic mixture	13.600
Agar	13.600
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.6 gram in 500 ml purified / distilled water. Add the rehydrated contents of one vial of CH250 Selective Supplement (FD283R). Heat to boiling with frequent agitation to dissolve the medium completely. **DO NOT AUTOCLAVE**. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Perry and Miller (1) reported that *Candida albicans* produces an enzyme b -N-acetyl- galactosaminidase and according to Rousselle et al (2) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of *C. albicans* isolates directly on primary isolation. HiCrome™ Candida Differential Agar Base incorporates two chromogens X-NAG which detects the activity of hexosaminidase and BCIP which detects phosphatase activity. HiCrome™ Candida Differential Agar Base is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* species namely *C.albicans, C.krusei, C.tropicalis* and *C.glabrata* on the basis of colouration and colony morphology. On this medium results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts in Mycology and Clinical Microbiology Laboratory. Peptone provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Chloramphenicol from the supplement suppresses the accompanying bacterial flora. *C.albicans* appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to metallic blue coloured raised colonies. *C.glabrata, C.kefyr, C.parapsilosis* colonies appear as cream to white, beige/yellow due to natural pigmentation and some alkaline phosphatase activity, while *C.krusei* appear as pink-purple, fuzzy, dry colonies.

Type of specimen

Clinical samples - skin scrapings, urine, Food & dairy samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1.Variations in colour intensity may be observed for *Candida* isolates depending on the presence of enzymes.
2.Other *Candida* species may produce light mauve coloured colonies which is also produced by other yeast cells. This must be confirmed by further biochemical tests.
3.Other filamentous fungi also exhibit colour on this medium.

Performance and evaluation

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

Quality Control

Appearance

Cream to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.36% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, opaque gel forms in Petri plates

Reaction

Reaction of 3.12% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH

5.80-6.20

Cultural Response

Cultural characteristics observed with added HiCrome Candida Differential Selective Supplement (FD283R) after an incubation at 30-35°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%	light green
Candida glabrata ATCC 15126	50-100	good-luxuriant	>=50%	cream to white
#Teunomyces krusei ATCC 24408	50-100	good-luxuriant	>=50%	purple, fuzzy
Candida tropicalis ATCC 750	50-100	good-luxuriant	>=50%	blue to purple
Candida kefyr ATCC 66058	50-100	good-luxuriant	>=50%	cream to white with slight purple centre
Candida utilis ATCC 9950	50-100	good-luxuriant	>=50%	pale pink to pinkish purple
Candida parapsilosis ATCC 22019	50-100	good-luxuriant	>=50%	white to cream
Candida membranifaciens ATCC 20137	50-100	good-luxuriant	>=50%	white to cream
Candida dubliensis NCPF 3949	50-100	good-luxuriant	>=50%	pale green
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers. # - Formerly known as *Candida krusei*

Storage and Shelf Life

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

Disposal

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

Reference

1.Perry J. L. and Miller G. R., 1987, J. Clin. Microbiol., 25: 2424 -2425.
2.Rousselle P., Freydiere A., Couillerot P., de Montclos H. and GilleY., 1994, J. Clin. Microbiol. 32:3034-3036.
3.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5 American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 05/2024

HiMedia Laboratories Pvt. Limited,
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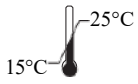
Thane (W) -400604, MS, India

IVD *In vitro* diagnostic medical device



Storage temperature

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

CE **CE Marking**

Do not use if package is damaged

Disclaimer :

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

Bifidobacterium Broth (Modified w/ 0.1% Agar)

M1395A

Bifidobacterium Broth (Modified w/ 0.1% agar) is used for the cultivation of Bifidobacterium species.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Yeast extract	10.000
Peptone	10.000
Glucose	20.000
Tomato juice (solids)	16.650
Polysorbate 80	2.000
Agar	1.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 79.65 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Distribute in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

The genus *Bifidobacterium* is the third most numerous bacterial population found in the human intestine after *Bacteroides* and *Eubacterium* . It is an anaerobic bacteria that makes up the gut microbial flora, it resides in the colon and have health benefits for their hosts. Bifidobacteria are also associated with lower incidences of allergies (1, 2). Bifidobacterium Broth is used for the cultivation and maintenance of *Bifidobacterium* species. The medium is used exclusively for the cultivation of *Bifidobacterium infantis* (3). The medium has Casein enzymic hydrolysate, Peptone and yeast extract that provides essential growth nutrients. Glucose is the energy source. Tomato juice helps in maintaining acidic pH while polysorbate 80 provides fatty acids required for metabolic activity of Bifidobacterium. Addition of 0.1% agar helps in creating favorable conditions for the growth of *Bifidobacterium* species.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Amber coloured clear to slightly opalescent solution.

Reaction
Reaction of 7.96% 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°Cfor 24-48 hours under anaerobic conditions.

Cultural Response

Organism	Inoculum (CFU)	Growth
Cultural Response		
Bifidobacterium infantis ATCC 25962	50-100	good-luxuriant

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference

1. Bjorksten B., Sepp E., Julge K., Voor T., and Mikelsaar M., 2001, J. Allergy Clin. Microbiol., Volume 108, Issue 4, 516-520.
2. Guarner F., and Malagelada J. R., 2003, The Lancet, Vol. 361, Issue 9356, 8 February 2003, 512-519.
3. Atlas R. M. 2004, 3rd Edi. Handbook of Microbiological Media, Parks, L. C. (Ed.), CRC Press, Boca Raton.

Revision : 00 / 2011

HiCrome™ UTI Agar, Modified

M1418

Intended use:

Recommended for identification, differentiation and confirmation of enteric bacteria from specimens such as urine which may contain large number of *Proteus* species as well as potentially pathogenic gram-positive organisms.

Composition**

Ingredients	**g / L**
Peptone	18.000
Tryptone	4.000
HM Peptone B#	6.000
Chromogenic mixture	12.440
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

-Equivalent to Beef extract

Directions

Suspend 55.44 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ UTI Agar, Modified is formulated on the basis of work carried out by Pezzlo (1), Wilkie et al (2), Friedman et al (3), Murray et al (4), Soriano and Ponte (5) and Merlino et al (6). These media is the modification of HiCrome™ UTI Agar (M1353), which can be used in place of MacConkey Agar for isolation and confirmation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates.

Enzymes produced by *Enterococcus* species, *Escherichia coli* and coliforms cleave the chromogenic substrates incorporated in the medium. Presence of rich source of phenylalanine and tryptophan from peptone and tryptone provides an indication of tryptophan deaminase activity, revealed with TDA Reagent (R036) indicating the presence of Proteus species, Morganella species and Providencia species, which appear brown. One chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce purple-magenta colonies due to the enzyme ß-D-galactosidase which cleaves the other chromogenic substrate. Further confirmation of *E.coli* can be done by performing indole test using DMACA Reagent (R035). Also, some strains of *Enterobacter cloacae* lacking ß-glucosidase show pink-colonies indistinguishable from *E.coli.* The DMACA reagent for indole test (should be performed on filter paper) distinguishes between *E.coli* and *Enterobacter,* and also between *Proteus mirabilis* and other species. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrates Peptone, HM Peptone B and tryptone provides nitrogenous, carbonaceous compounds and other essential growth nutrients.

Type of specimen

Clinical samples : urine, faeces, etc.; Food samples; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
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 (11).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Since it is an enzyme-substrate based reaction, the intensity of colour may vary with isolates.
2. Further biochemical and serological test must be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 5.54% 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 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	TDA (add 1-2 drops of TDA reagent)	DMACA (transfer colony on filter paper dipped in DMACA Reagent)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%	Purple to magenta	negative reaction	positive reaction, formation of blue purple colour around growth
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%	blue-green (small)	negative reaction	negative reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%	blue to purple, mucoid	negative reaction	negative reaction
Proteus mirabilis ATCC 12453	50-100	luxuriant	>=70%	light brown	positive reaction, development of brown colouration	negative reaction
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	colourless (greenish pigment may be observed)	negative reaction	negative reaction
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%	golden yellow	negative reaction	negative reaction

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Pezzlo M, (1998), Clinical Microbiology Reviews, 1:268-280
2. Wilkie M.E., Almond M.K. and Marsh F.P., (1992), British Medical Journal, 305:1137-1141.
3. Friedman M.P. et al. (1991), Journal of Clinical Microbiology, 29:2385-2389.
4. Murray P., Traynor P. and Hopson D., (1992), Journal of Clinical Microbiology, 30:1600-1601.
5. Soriano F. and Ponte C., (1992), Journal of Clinical Microbiology, 30:3033-3034.
6. Merlino et al. (1995), Abstr. Austr. Microbiol., 16(4):17-3.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. 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.
9. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
11. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 07/2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
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IVD *In vitro* diagnostic medical device

25°C 15°C Storage temperature

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

CE CE Marking

Do not use if package is damaged

Eugonic LT 100 Broth Base w/o Tween 80

M1517

Intended Use:

Recommended for the enrichment and detection of mesophilic aerobic bacteria present in cosmetic products. The composition Eugonic and performance criteria of the medium are as per the specifications laid down in ISO 21149.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Sodium chloride	4.000
L-Cystine	0.700
Sodium sulphite	0.200
Glucose	5.500
Egg lecithin	1.000
Tritox X-100	1.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.4 grams in 1000 ml purified/distilled water containing 5 grams of Polysorbate 80 (Tween 80). Heat to boiling to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Eugonic LT 100 Broth Base was developed by Pelczar and Vera (1) for cultivation of fastidious organisms like *Brucella*. Eugon media were developed to obtain eugonic (luxuriant) growth of fastidious microorganisms like *Brucella* which are otherwise difficult to cultivate (2). The unenriched medium supports rapid growth of lactobacilli associated with cured meat products, dairy products and other foods. Eugonic media is quite similar to Tryptone Soya Agar (M290) but more bacterial propagation is expected on Eugonic media. Organisms like *Bordetella* and *Neisseria* grow luxuriantly in Eugon Media because large amount of sulfur and carbon sources have been added in the formula. Eugonic LT 100 Broth Base can be used for growth of a variety of fastidious microorganisms like *Neisseria, Francisella* and *Brucella*. The composition of the medium is as per ISO (3)for the detection of mesophilic aerobic bacteria from cosmetic products.

Tryptone and soya peptone provide the nitrogen, vitamins and amino acids, which supports the growth of fastidious microbial species. The high concentration of glucose is the energy source for rapid growth of bacteria. L-Cystine and sodium sulphite are added to stimulate growth. Sodium chloride maintains the osmotic balance of the media. The high carbohydrate content along with high sulfur (cystine) content improves growth with chromogenicity (4). Lecithin and polysorbate 80 in Eugonic LT 100 Medium w/o Tween 80 neutralize antimicrobial agents hence this medium can be used as a neutralizing diluent.

Type of specimen

Cosmetic samples

Specimen Collection and Handling

For cosmetic samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (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. Certain fastidious organisms may not grow due to nutritional variation.
2. Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured, Clear to slightly opalescent solution.

Reaction

Reaction of 3.24% 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 24-48 hours (fungal cultures incubated at 25-30°C for 2-7 days).

Organism	Inoculum (CFU)	Growth
Bacillus pumilus ATCC 14884	50-100	good
Candida albicans ATCC 26790	50-100	good
Lactobacillus fermentum ATCC 9338	50-100	good
Streptococcus pneumoniae ATCC 6303	50-100	good-luxuriant (under 3-5% CO2)
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant (under 3-5% CO2)
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	50-100	good-luxuriant
Staphylococcus aureus subsp.aureus ATCC 6538 (00032*)	50-100	good
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50-100	good
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	good
Escherichia coli ATCC 8739 (00012*)	50-100	good
Candida albicans ATCC 10231 (00054*)	50-100	good
Neisseria meningitidis ATCC 13090	50-100	good

* Corresponding WDCM Numbers

Storage and Shelf Life

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

Disposal

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

Reference

1.Pelczar and Vera J., 1949, Milk Plant Monthly 38:30
2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
3.ISO 21149 (2006) Cosmetics-Microbiology- Enumeration and detection of aerobic mesophilic bacteria
4.Frank H. A., 1955, J. Bacteriol., 70:269.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 06/2023

HiCrome™ Listeria Ottaviani-Agosti Agar Base

M1540I

Intended use

Recommended for the selective and differential isolation of *Listeria monocytogenes*. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017 and ISO 11290-2:2017.

Composition**

ISO 11290 Specification / FDA BAM/ APHA - Agar Listeria according to Ottaviani and Agosti

Ingredients	g / L
Enzymatic digest of animal tissues	18.000
Enzymatic digest of Casein	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Glucose	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate (anhydrous)	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate (anhydrous)	2.500
5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside	0.050
Agar	12.00 - 18.00
Final pH (after sterilization)	7.2±0.2

M1540I - HiCrome™ Listeria Ottaviani-Agosti Agar Base

Ingredients	g / L
HM Peptone #	18.000
Tryptone ##	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Glucose(Dextrose)	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate	2.500
5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside	0.050
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Key : # - Equivalent to Enzymatic digest of animal tissues, ## - Equivalent to Enzymatic digest of casein

Supplements to be added after autoclaving	g / L
I	
Nalidixic acid sodium salt	0.020
Ceftazidime	0.020
Polymyxin B sulfate	76 700 IU
Cycloheximide OR	0.050
Amphotericin B	0.010
II	
L-α- phosphatidylinositol	2.00

FD212A - 2 vials OA Selective Supplement	mg / vial
Nalidixic acid sodium salt	10.000
Ceftazidime	10.000
Polymyxin B sulfate	38350 IU
Amphotericin B	5.000
(FD214) - 2 vials	
LP Enrichment Supplement 1	1.000g

Directions

Suspend 36.02 gram in 465 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of LP Enrichment Supplement 1 (FD214) and sterile rehydrated contents of OA Selective Supplement (FD212A). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Listeria monocytogenes is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles. The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L.monocytogenes* and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). The media is based on the formulation of Ottoviani and Agosti (1,2) for the selective and differential isolation of *L.monocytogenes* from food and animal feeds which is adopted by ISO Committee (3,4,5). It is also recommended by APHA (6) & FDA-BAM (7). HM peptone, tryptone and yeast extract supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Sodium pyruvate provide essential growth nutrients. Glucose (Dextrose) is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added

selective supplements (FD212A) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyse the chromogenic substrate (5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside) which produces blue to green coloured colonies. Differentiation of *L.monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *L.monocytogenes* colonies.

Type of specimen

Food and animal feeds, environmental samples in the area of food manufacturing and handling.

Specimen Collection and Handling

For food and animal feeds, environmental samples follow appropriate techniques for handling specimens as per established guidelines (3-7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Some strains of *L.monocytogenes* exposed to stress condition particularly acid stress may show a very weak halo (or even no halo).
2. Further biochemical tests must be carried out to differentiate between *L.monocytogenes* and *L.ivanovii*, sine both shows opaque halo of PIPLC activity.
3. Some organisms other than *Listeria* spp. may also produce blue colonies on this medium, so biochemical characterization is required for differentiation.
4. Further biochemical and serological test are need to be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Light amber coloured, opalescent gel forms in Petri plates
Reaction
Reaction of 7.2% w/v aqueous solution at 25°C.

pH
7.00-7.40
Cultural Response
Productivity : Cultural characteristics observed with added sterile OA Selective Supplement (FD212A) and LP Enrichment Supplement 1 (FD214) after an incubation at 37°±1°C for 48±4 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar). The characteristic reaction are compared with previously approved lot.
Specificity : Cultural characteristics observed with added sterile OA Selective Supplement (FD212A) and LP Enrichment Supplement 1 (FD214) after an incubation at 37°±1°C for 48±4 hours. The characteristic reaction are compared with previously approved lot.
Selectivity : Cultural characteristics observed with added sterile OA Selective Supplement (FD212A) and LP Enrichment Supplement 1 (FD214) after an incubation at 37° ± 1°C for 48 ± 4 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	PIPLC activity
Productivity					
Listeria monocytogenes ATCC 13932 (00021*)	50-100	luxuriant	>=50%	Blue-green	positive,opaque halo around the colony exhibiting phophatidylinositol specific phospholipase activity

Listeria monocytogenes ATCC 35152 (00109*)	50-100	luxuriant	>=50%	Blue-green	positive,opaque halo around the colony exhibiting phophatidylinositol specific phospholipase activity
Specificity					
Listeria innocua ATCC 33090 (00017*)	10^3-10^4	luxuriant		Blue-green	negative
Selectivity					
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited			
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited			
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited			
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited			

Storage and Shelf Life

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

Disposal

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

Reference

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Revision : 03/2024

HiCrome™ Vibrio Agar

M1682

Intended use

Recommended for isolation and selective chromogenic differentiation of *Vibrio* species from seafood and clinical samples.

Composition**

Ingredients	g / L
Peptone	10.000
Sodium chloride	25.000
Sodium thiosulphate	5.000
Sodium citrate	6.000
Sodium cholate	1.000
Chromogenic mixture	5.500
Agar	15.000
Final pH (at 25°C)	8.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 67.5 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Cool to 45-50°C. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Vibrio' s have played a significant role in human history. Outbreaks of cholera, caused by *Vibrio cholerae*, can be traced back in time to early recorded descriptions of enteric infections. The *Vibrio's* have also received the attention of marine microbiologists who observed that the readily cultured bacterial population in near-shore waters and those associated with fish and shell fish were predominantly *Vibrio* species (1). *Vibrio* species are mainly responsible for causing cholera and food poisoning in humans. *Vibrio cholerae* causes cholerae due to the intake of contaminated food such as raw oysters. *Vibrio parahaemolyticus* is a major cause of food borne infections, causing food poisoning (2). Since *Vibrio* species naturally occur in sea water, worth special mention is their need for sodium chloride, although some species can grow with minimum sodium chloride concentration (1). The widely used media for *Vibrio* isolation are TCBS Agar and Alkaline Peptone Water (3). However accompanying sucrose-fermenting bacteria pose a problem in the identification of *Vibrio* species on TCBS Agar. On HiCrome™ Vibrio Agar, the colour development by *Vibrio* species in not affected by the presence of colonies of other bacteria. This is because, the amount of colour developed depends on the reaction of the bacterial beta-galactosidase with the substrate contained in the media (4).

Peptone provides carbonaceous, nitrogenous and essential nutrients to the organisms. High concentration of sodium chloride in addition to maintaining the osmotic equilibrium also has an inhibitory action on the accompanying microflora. Sodium thiosulphate, sodium citrate and sodium cholate are used in the formulation because they can inhibit the growth of gram positive and some gram negative bacteria, but not members of *Enterobacteriaceae*. The proprietary chromogenic mixture incorporated in the medium helps in the chromogenic differentiation of *Vibrio cholerae* and *Vibrio parahaemolyticus*. The high (alkaline) pH of the medium helps in selective isolation of *Vibrio* species.

Type of specimen

Clinical samples- faeces; Food samples.

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

1. Being highly selective, some species may show poor growth due to nutritional variations.
2. Slight colour variation may be observed depending upon strains.
3. 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 expiry period when stored at the recommended temperature.

Quality Control

Appearance

Light yellow to light tan 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 6.75% w/v aqueous solution at 25°C. pH : 8.5±0.2

pH

8.30-8.70

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	>=50%	purple
Vibrio vulnificus ATCC 29306	50-100	good-luxuriant	>=50%	light purple to purple
Vibrio parahaemolyticus ATCC 17802 (00037*)	50-100	good-luxuriant	>=50%	bluish green
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=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 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

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

Reference

1. Thompson et al (ed.). 2006. The Biology of Vibrios, ASM Press, chapter 1, pg 3.
2. Alcamo. E.I, 2001. Fundamentals of Microbiology, 6th ed, Jones and Bartlett Publishers, Inc. pg 254, 244.
3. Clesceri, Greenberg and Eaton (ed.). 1998. Standard Method for the examination of Water and Waste water, 20th ed. American Public Health Association, Washington, D. C.
4. Kudo. H. Y et al, 2001. Improved Method for Detection of Vibrio parahaemolyticus in Seafood. ASM. Vol 67, No.12, pg 5819-5823.
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6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
7. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of, Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 04/2024

HiMedia Laboratories Pvt. Limited,
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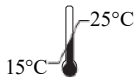
IVD *In vitro* diagnostic medical device



Storage temperature

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

CE Marking

Do not use if package is damaged

Disclaimer :

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

Cronobacter Selective Broth (CSB)

M1786I

Intended Use

Recommended for screening *Cronobacter* (formerly *Enterobacter sakazakii*) from food. The composition and performance of this media are as per specifications laid down in ISO 22964:2017(E).

Composition**

ISO 22964:2017(E) Annex B

Ingredients	g / L
Enzymatic digest of animal tissues	10.000
Meat extract	3.000
Sodium chloride (NaCl)	5.000
Bromocresol purple	0.040
Sucrose	10.000
Final pH (at 25°C)	7.4±0.2
Vancomycin hydrochloride	10mg
Water	10ml

M1786I

Ingredients	g / L
Peptone	10.000
HM Extract #	3.000
Sodium chloride	5.000
Bromocresol purple	0.040
Sucrose	10.000
Final pH (at 25°C)	7.4±0.2

FD233 Van10 Selective Supplement

Vancomycin 10mg

**Formula adjusted, standardized to suit performance parameters
Equivalent to Meat extract

Directions

Suspend 28.04 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add the contents of 1 vial of Van10 Selective Supplement (FD233). Mix well and dispense 10ml into sterile test tubes.

Principle And Interpretation

Cronobacter (formerly *Enterobacter sakazakii*) are gram-negative rod-shaped *Enterobacteriaceae* that have been implicated in outbreaks of disease causing sepsis, meningitis and necrotising enterocolitis (1). *Cronobacter* species have also been isolated from powdered infant formula as high tolerance to desiccation provides a competitive advantage in dry environments increasing the risk of contamination (2).

Cronobacter Screening Broth was specifically designed by Iversenetal (3). Cronobacter Selective Broth is recommended by ISO Committee for the isolation of *Cronobacter* species from food samples (4). Peptone and HM extract provide carbonaceous, nitrogenous and growth nutrients. Sodium chloride maintains osmotic equilibrium. Sucrose is the fermentable carbohydrate and bromocresol purple is the indicator. Sucrose is fermented by *Cronobacter*. Consequently the broth turns yellow after incubation.

Type of specimen

Food samples

Specimen Collection and Handling:

Test portion:

To prepare primary dilution, add 10g or 10ml of the test sample to 90ml of pre-enrichment medium (BPW).

Pre-enrichment:

Incubate the inoculated pre-enrichment medium between 34°C and 38°C for 18h ± 2h.

Enrichment:

After incubation of the inoculated pre-enrichment medium, mix well and transfer 0.1ml of the obtained culture into 10 ml of CSB and mix well. Incubate at 41.5°C for 24h ± 2h.

Isolation of presumptive *Cronobacter* spp.:

From enrichment culture, inoculate 10 µl onto surface of CCI Agar. Incubate at 41.5°C for 24h ± 2h.

Confirmation:

Biochemical tests are performed for confirmation.

Confirmation:
Biochemical tests are performed for confirmation.

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Colour and Clarity of prepared medium
Purple coloured clear solution forms in tubes.
Reaction
Reaction of 2.8% w/v aqueous solution at 25°C. pH : 7.4±0.2
pH
7.20-7.60
Cultural Response
Cultural characteristics observed with added Van10 Selective Supplement (FD233), after an incubation at 41.5 ± 1°C for 24 ±2 hours. The broth is recovered on HiCrome™ Cronobacter Isolation Agar (CCI Agar) (M2062I) and incubated at 41.5±1° C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Colour of medium	Colour of Colony on M2062I
Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good-luxuriant	yellow colour	blue-green
Cronobacter muytjensii ATCC 51329 (00213*)	50-100	good-luxuriant	yellow colour	blue-green
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	none-poor	purple	-
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50-100	none-poor	purple	-
Mixed cultures *Cronobacter sakazakii* ATCC 29544 (00214*) + *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*)	50-100	good-Luxuriant	yellow	blue-green
Cronobacter sakazakii ATCC 29544 (00214*) + *Staphylococcus aureus subsp. aureus* ATCC 6538 (00032*)	50-100	good-Luxuriant	yellow	blue-green

Cronobacter muytjensii ATCC 51329 (00213*) + *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*)	50-100	good-Luxuriant	yellow	blue-green
Cronobacter muytjensii ATCC 51329 (00213*) + *Staphylococcus aureus subsp. aureus* ATCC 6538 (00032*)	50-100	good-Luxuriant	yellow	blue-green

*Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Mullane et al. 2007. Minerva Pediatr. 59.137-148.
2. Lai.2001.Medicine.80.113-122.
3. Iversen et al.2008.Appl.Environ.Microbiol.74, 2550-2552.
4. International Organization for Standardization. Microbiology of the food chain- Horizontal method for the detection of *Cronobacter* spp. Draft ISO/ TS 22964, 2017 (E).
5. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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Revision : 07/ 2024

MRS Agar, Modified

M1990I

Intended Use:

Recommended isolation and enumeration of mesophilic lactic acid bacteria from food. The composition and performance criteria are in accordance with in ISO 15214:1998 and ISO 11133 : 2014 (E) /Amd. :2020.

Composition**

ISO specification - MRS Agar, Modified

Ingredients	g / L
Tryptone $	10.000
HM extract ⊖	10.000
Yeast extract	4.000
Triammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate.7H2O	0.200
Manganese sulfate. 4H2O	0.050
Dipotassium hydrogen phosphate	2.000
Dextrose (Glucose)	20.000
Polysorbate 80 (Tween 80)	1.080
Agar	15.000
Final pH (at 25°C)	5.7±0.1

M1990I - MRS Agar, Modified

Ingredients	g / L
Tryptone $	10.000
HM extract ⊖	10.000
Yeast extract	4.000
Triammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate.7H2O	0.200
Manganese sulfate. 4H2O	0.050
Dipotassium hydrogen phosphate	2.000
Dextrose (Glucose)	20.000
Polysorbate 80 (Tween 80)	1.080
Agar	15.000
Final pH (at 25°C)	5.7±0.1

**Formula adjusted, standardized to suit performance parameters.

$ -Equivalent to Enzymatic digest of casein ⊖ -Equivalent to Meat extract

Directions

Suspend 69.21 gram (equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. 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

MRS Agar, Modified is in accordance with ISO (1,2) for the enumeration of mesophilic lactic acid bacteria. Mesophilic bacteria are divided into two groups: Lactic Acid Starter bacteria (including *Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris*), which are primarily used for producing lactic acid, and Aroma Producing bacteria (including *Lactococcus lactis subsp. lactis biovar diacetylactis* and *Leuconostoc mesenteriodes subsp. cremoris*), which are primarily used for producing CO2 gas and flavor. MRS Agar, Modified (Lactobacillus Heteroferm Screen Agar) recommended by APHA (3), is used for the isolation and cultivation of *Lactobacillus* species from salad dressings (4). Tryptone, HM extract and yeast extract supply nitrogen, carbon and other elements essential for the growth of Lactobacilli. Dextrose (Glucose) is the carbohydrate source. Polysorbate 80 (Tween 80) supplies fatty acids required by Lactobacilli. Triammonium citrate, sodium acetate inhibit gram-negative organisms and certain gram-positive bacteria.

Type of specimen

Food and animal feeding stuff

Specimen Collection and Handling:

For food and animal feeding stuff samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,3).

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured slight opalescent gel forms in Petri plates

Reaction

Reaction of 6.92% w/v aqueous solution at 25°C. pH : 5.7±0.1

pH

5.60-5.80

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 30 ± 1°C for 72 ± 3 hours . Recovery rate is considered as 100% for bacteria growth on Reference Medium - previously validated MRS Agar.

Selectivity : Cultural characteristics observed after an incubation at 30 ± 1°C for 72 ± 3 hours .

Organism	Inoculum (CFU)	Growth	Recovery
Productivity			
Lactobacillus sakei ATCC 15521 (00015*)	50-100	good-luxuriant	>=70%
Lactococcus lactis ATCC 19435 00016*)	50-100	good-luxuriant	>=70%
Pediococcus pentosaceus ATCC 33316 (00158*)	50-100	good-luxuriant	>=70%
Selectivity			
Escherichia coli ATCC 8739 (00012)*	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	
Bacillus cereus ATCC 11778 (00001*)	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Product performance is best if used within stated expiry period.

Disposal

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

References

1. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of mesophilic lactic acid bacteria. ISO 15214:1998(E).
2. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd. :2020.
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5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 02/2024

HiCrome™ Chromogenic Coliform agar (CCA Agar) M1991I

Intended Use

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

Composition**

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

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

M1991I - HiCrome™ Chromogenic Coliform agar (CCA Agar)

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

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

Directions

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

Principle And Interpretation

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

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

Type of specimen

Water samples.

Specimen Collection and Handling:

Processing (1)

Filration:

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

Incubation and differentiation:

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

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates

Reaction

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

pH

6.60-7.00

Cultural Response

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

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

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

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

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

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

Formerly known as *Enterobacter aerogens*

Storage and Shelf Life

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

Disposal

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

Reference

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

Revision : 07/2024

HiCrome™ Cronobacter Isolation Agar(CCI Agar)

M2062I

Intended Use

Recommended for the isolation and identification of *Cronobacter sakazakii* from food products.The composition and performance of this media are as per specifications laid down in in ISO 22964: 2017.

Composition

ISO 22964: 2017 Specification - Chromogenic Cronobacter isolation (CCI) agar

Ingredients	g / L
Tryptic digest of casein	7.000
Yeast extract	3.000
Sodium chloride (NaCl)	5.000
Sodium desoxycholate ($C_{24}H_{39}NaO_4$	0.250
5-Bromo-4-chloro-3-indolyl α–D-glucopyranoside	0.15
Ammonium iron(III) citrate ($C_6H_8O_7FeNH_3$	1.000
Sodium thiosulfate ($N_2S_2O_3$)	1.000
Agar	9.00-18.00
Final pH after sterilization (at 25°C) 7.3±0.2	

M2062I -HiCrome™ Cronobacter Isolation Agar (CCI Agar)

Ingredients	g / L
Tryptone#	7.000
Yeast extract	3.000
Sodium chloride	5.000
Sodium deoxycholate	0.250
5-Bromo-4-chloro-3-indolyl α–D-glucopyranoside	0.15
Ammonium iron(III) citrate	1.000
Sodium thiosulfate	1.000
Agar	15.000
Final pH after sterilization (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Tryptic digest of casein

Directions

Suspend 32.4 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Enterobacter species are widely distributed in nature occurring in fresh water, soil, sewage, plants, vegetables, animal and human faeces. **Cronobacter sakazakii* has been closely associated with neonatal meningitis and sepsis (1,2). HiCrome™ Cronobacter isolation Agar is recommended by ISO Committee for the isolation and identification of **C.sakazakii* from food samples (3). The chromogenic substrate (5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside) is cleaved specifically by **C.sakazakii* resulting in the formation of blue green colonies. Other organisms, which do not cleave this substrate, produce colourless colonies. Tryptone and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Sodium deoxycholate inhibits the accompanying gram-positive flora.

Key: *: Formerly known as *Enterobacter sakazakii*

Type of specimen

ISO 22964: 2017: Food products and ingredients intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.

Specimen Collection and Handling:

Processesing : ISO 22964: 2017

Non-selective pre-enrichment in BPW (M1494I) : Samples (10 gram/ 10ml in 90 ml) are pre-enriched in Buffered Peptone Water and incubated at 34 °C and 38 °C for 18 ± 2 hours.

Selective enrichment (CSB) : 0.1 ml of enriched culture from BPW (M1494I) is then inoculated into Cronobacter Selective Broth (CSB) and incubated at 41,5 °C ± 1 °C for 24 ± 2 hours.

Identification on chromogenic agar (CCI agar) -: 10 microlitre of selectively enriched culture from CSB (M1786I) is then cultured onto CCI Agar (M2062I) and incubated at 41,5 °C ± 1 °C for 24 ± 2h.

Confirmation : Typical colonies are selected from the chromogenic agar, purified on a non-selective agar such as TSA and biochemically characterized. 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. Slight variation in colour may be observed depending on enzyme production by organism and substrate utilization from the medium.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
4. 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 recommended temperature.

Quality Control

Appearance

Cream to yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.24% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 41.5±1°C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Inoculum (CFU)	Colour of Colony
Productivity				
Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good	>=50%	blue to blue-green colonies (small to medium sized, 1 -3mm)
Cronobacter muytjensii ATCC 51329 (00213*)	50-100	good	>=50%	blue to blue-green colonies (small to medium sized, 1 -3mm)
Selectivity				
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Specificity				
Enterobacter cloacae ATCC 13047 (00083*)	10^3-10^4	growth or partial inhibition		Colonies without green or blue green colour

Key: (*) Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,4).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983
3. Microbiology of the food chain- Horizontal method for the detection of *Cronobacter* spp. International Organization for Standardization.Draft ISO/ TS 22964, 2017 (E).
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03/ 2024

MacConkey Broth

MH083

Intended use

Recommended for the selective enrichment of *E.coli* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	Gms / Litre
Gelatin peptone#	20.000
Lactose monohydrate	10.000
Dehydrated bile##	5.000
Bromo cresol purple	0.010
pH after sterilization (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Equivalent to Dehydrated Ox-bile

Directions

Suspend 34.51 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat if necessaryto dissolve the medium completely. Dispense into test tubes with inverted Durham tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

Principle And Interpretation

MacConkey Broth is a modification of MacConkey Medium (1). Childs and Allen (2) demonstrated the inhibitory effect of neutral red and therefore substituted it by the less inhibitory bromocresol purple dye. BCP is more sensitive in recording pH variation in the medium. This medium is prepared in accordance with the harmonized method of USP/BP/JP (3,4,5)

Gelatin peptone provides essential growth nutrients. Lactose is the fermentable carbohydrate. Dehydrated bile inhibits gram-positive organisms. Bromocresol purple is the pH indicator in the medium, which turns yellow under acidic condition. Lactose fermenting organisms turn the medium yellow due to the acidity produced on lactose fermentation. The colour change of the dye is observed when the pH of the medium falls below 6.8. Lactose non-fermenting organisms like *Salmonella* and *Shigella* do not alter the appearance of the medium.

Transfer homogenate in Soyabean Casein Digest Medium (MH011) containing 1 gm or 1 ml of the preparation to be examined to 100 ml MacConkey Broth Incubation is carried at 43°-45°C for 24-48 hours. For further isolation, subculture on MacConkey Agar (MH081). Growth of red generally non-mucoid colonies, sometimes surrounded by a reddish precipitation zone, indicates pressure of coliforms.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (3-6).

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

Warning and Precautions:

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

Limitations

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

3.Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
4.For further isolation, subculture on MacConkey Agar (MH081) is required.

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 with green tinge homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured clear to slightly opalescent solution in tubes

pH
7.10-7.50

Cultural Response
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. For organisms not specified in pharmacopoeia, cultural response was observed after an incubation at 30-35°C for 18-48 hours.

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 42-44°C for <= 24 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temperature for not less than longest period of time specified inoculating >=100 cfu(at 42-44°C for >= 48 hours).

Cultural Response
Cultural characteristics observed after an incubation at 30-35°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Acid	Gas	Incubation temperature	Incubation period
Growth promoting						
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	42 -44 °C	<=24 hrs
Inhibitory						
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	>=10^3	inhibited			42 -44 °C	>=48 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
# Klebsiella aerogenes ATCC 13048 (00175)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
Salmonella Choleraesuis ATCC 12011	50 -100	fair-good	negative reaction	negative reaction	30 -35 °C	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited			30 -35 °C	>=48 hrs

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 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 (7,8).

Reference

1.MacConkey A. T., 1900, The Lancet, ii: 20.
2.Childs E. and Allen, 1953, J. Hyg: Camb. 51:468-477.
3.The United States Pharmacopoeia, 2020, The United States Pharmacopeial Convention. Rockville, MD.
4.British Pharmacopoeia, 2022, The Stationery office British Pharmacopoeia
5.Japanese Pharmacopoeia, 2016.
6.European Pharmacopoeia, 2022 European Dept. for the Quality of Medicines
7.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
8.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04 / 2022

Technical Data

HiCulture™ Transport Swabs w/ Alternative Thioglycollate Medium

MS010

Recommended for transportation of aerobes, anaerobes and microaerophiles.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	15.000
Yeast extract	5.000
Dextrose (Glucose)	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500

**Formula adjusted, standardized to suit performance parameters

Directions

Remove cap from the tube. Collect sample using capped swab. Discard cap on tube, replace with capped swab.

Principle And Interpretation

Alternative Thioglycollate Medium contains sodium thioglycollate that can neutralize the bacteriostatic effect of mercurial preservatives. Absence of agar makes it suitable for testing viscous materials and devices having tubes with small lumina. Casein enzymic hydrolysate, yeast extract, dextrose, L-cystine provides nitrogenous and carbonaceous compounds, vitamin B complex, trace elements and other essential growth nutrients. The composition matches with the medium recommended in pharmacopoeias (1, 2,3). Sterile cotton swabs allow absorption of specimen material while polystyrene shaft allows semiflexibility to the swab stick, aiding in collection.

Quality Control

Appearance
Sterile Alternative Thioglycollate Medium in tubes with sterile cotton swabs.

Colour
Yellow coloured medium

Quantity of Medium
8ml of medium in tubes

Cultural response
Viability of following organisms was established for a period of 48 hours. Organisms grew luxuriantly when recovered on Tryptone Soya Agar (M290) and incubated at 35 - 37°C for 18-24 hours.

Sterility test
Passes release criteria

Cultural Response

Organism	Recovery Cultural response
*Bacteroides vulgatus ATCC 8482**	Luxuriant
*Clostridium sporogenes ATCC 11437**	Luxuriant
*Candida albicans ATCC 10231***	Luxuriant
Bacillus subtilis ATCC 6633	Luxuriant
*Bacteroides fragilis ATCC 25285**	Luxuriant

Micrococcus luteus ATCC 10240	Luxuriant
Neisseria meningitidis ATCC 13090	Luxuriant
Staphylococcus aureus ATCC 25923	Luxuriant
Streptococcus pyogenes ATCC 19615	Luxuriant

Storage and Shelf Life

Store between 5 – 25°C with caps firmly screwed. DO NOT FREEZE. Use before expiry date on label.

Reference

1.N.I.H. Memorandum, 1955: Culture Media for Sterility Tests, 4th Revision.
2.The United States Pharmacopoeia/National Formulary USP31/NF26, 2008, Asian Edition, US Pharmacopeial Convention Inc. Twinbrook Parkway, Rockville, M.D. 20852.
3.Indian Pharmacopeia, 1996, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.

Revision : 1 / 2011

Kovacs' Indole Reagent

R008

Intended use

Kovacs' Indole Reagent is used for detection of presence of indole produced by microorganisms due to tryptophan deamination.

Composition**

Ingredients

p-dimethyl amino benzaldehyde	5.0 gm
Amyl alcohol	75.0 ml
Hydrochloric Acid	25.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Take 5 ml of a 24 - 48 hours old culture of the organism under investigation.
2. Add 0.2 - 0.3 ml of Kovac's reagent.
3. Observed for a red coloured ring which indicates positive indole test.

Principle And Interpretation

Peptone Water is particularly suitable as a substrate in the study of indole production. Peptone used in Peptone Water, is rich in tryptophan content. Other peptones which contain tryptophan can be used to study indole production. Tryptone Water is also recommended by APHA for detection of indole production by coliforms, which is a key feature in differentiation of bacteria. It is used as part of the IMViC procedures. Most strains of *E. coli, P. vulgaris, P. rettgeri, M. morgani* and *Providencia* species break down the amino acid tryptophan with the release of indole. The presence of indole can be detected by the addition of Ehrlich's or Kovac's reagent (p-dimethylaminobenzaldehyde).

Kovacs reagent is a biochemical reagent consisting of isoamyl alcohol, para-dimethylaminobenzaldehyde (DMAB), and concentrated hydrochloric acid. It is used for the diagnostic test, to determine the ability of the organism to split tryptophan into indole and alpha-aminopropionic acid by hydrolytic activity of bacteria that express tryptophanase enzyme. The indole produced is indicated by formation of a red coloured ring, soluble in ether, chloroform and alcohol. This was invented by the Hungarian-Swiss Chemist, Ervin Kovac's. Indole production is used as, a test designed to distinguish among members of the family Enterobacteria.

Type of specimen

Used as biochemical reagent in diagnosis

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.

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

Warning and Precautions

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

Limitations

1. Growth media must contain an adequate amount of tryptophan. Do not use Mueller-Hinton Agar for test, because tryptophan is destroyed during the acid hydrolysis of casein.
2. Do not used media that contain dyes (e.g., EMB, MAC).
3. Do not use medium with a nitrate disc/strip to perform the indole test, as nitrate can interfere with indole test by including false positive results.

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 :** Greenish yellow coloured solution
- **Solubility :** Immiscible with water
- **Clarity :** Clear with no insoluble particles.
- **Cultural Response :** Characteristic reactions observed when Kovac's Indole Reagent is added to growth in Tryptone Broth (M463)

Cultural Response

Organism	Indole production
**Klebsiella aerogenes* ATCC 13048 (WDCM 00175)	Negative reaction , no red ring
Escherichia coli ATCC 25922 (WDCM 00013)	Positive reaction, red ring at the interface of the medium

(*) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

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

Disposal

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

Reference

1. MacFaddin J., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.

2. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
3. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.
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

Storage temperature

Do not use if package is damaged

In vitro diagnostic medical device

CE Marking

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

CEpartner4U,ESDOORNLAAN 13,3951
DB MAARN,The Netherlands,
www.cepartner4u.eu

Revision **:** 02/2022

Disclaimer :

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

Sterile Mineral Oil

R045

Intended use

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

Composition**

Ingredients

Sterile Mineral Oil

Directions

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

Principle And Interpretation

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

Type of specimen

Biological sample

Specimen Collection and Handling

Follow appropriate techniques for handling specimens as per established guidelines

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

→ **Appearance :** Colourless viscous solution.

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

Storage and Shelf Life

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

Disposal

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

Reference

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

Storage temperature

Do not use if package is damaged

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

Revision : 01/2023

Disclaimer :

Bile, Dried, Purified

RM010

Intended use

Bile, Dried, Purified is obtained from purified fresh bile desiccated under controlled conditions of temperature that helps to ensure a uniform and reproducible product. It is a greenish yellow coloured, homogeneous, fine powder having characteristic bile like odour with partly bitter, partly sweet and disagreeable taste. It is freely soluble in distilled water. The aqueous solution is clear, yellow coloured and produces foam if shaken strongly. The solution remains clear after autoclaving without developing any precipitate, or scum on surface of the liquid. It is used as a selective inhibitory agent. It is used as a selective agent used in microbiological culture media such as Brilliant Green Bile Broth 2%, Bile Esculin Agar, etc. It is equivalent to Ox Bile, Dried, Purified

Warning and Precautions

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

Limitations

1.It is biological origin product since variation in colour of powder and clarity may observed.
2.Each lot of the product has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's requirement.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium prepared by the product.

Performance and Evaluation

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

Quality Control

- **Appearance :** Greenish yellow, homogeneous, free flowing powder having bile like odour .
- **Solubility :** Freely soluble in distilled water.
- **Clarity :** 1% w/v aqueous solution is clear and free from extraneous matter.
- **pH :** pH of 1% w/v aqueous solution at 25ºC 6.5 - 7.5
- **Cultural Response :** Cultural response after an incubation at 35-37°C for 18-48 hours by preparing Brilliant Green Bile Broth 2% (M121) using Bile, Dried ,Purified as an ingredient.

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Cultural Response

Organism	Growth	Gas
Bacillus cereus ATCC 10876	Inhibited	
Escherichia coli ATCC 25922 (WDCM 00013)	Good-luxuriant	Positive reaction
**Klebsiella aerogenes* ATCC 13048 (WDCM 00175)	Good-luxuriant	Positive reaction
Enterococcus faecalis ATCC 29212 (WDCM 00087)	None-poor	Negative reaction
Staphylococcus aureus subsp. *aureus* ATCC 25923 (WDCM 00034)	Inhibited	Not Applicable

Chemical Analysis :

Cholic acid content (on dried basis) : ≥45.00 %

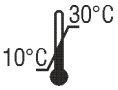
Loss on drying : ≤6.00 %

Storage and Shelf Life

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

Disposal

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



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt Limited
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Thane(W)–400604,Maharashtra,India

Revision : 04/2022

Disclaimer :

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

CELLBIO™
in service of your CELL

Technical Datasheet

Fetal Bovine Serum

Origin: South America, EU Approved
Heat inactivated
Sterile filtered

Product Code: RM9955

Product Description:

Fetal bovine serum (FBS) is a ubiquitously used essential supplement in cell culture media. FBS is a cocktail of proteins, vitamins, carbohydrates, lipids, hormones, growth factors, minerals and trace elements and is used as an universal growth supplement effective for most types of human and animal (including insect) cells. The major functions of serum in culture media are to provide:

(i) Hormonal factors stimulating cell growth and proliferation and promoting differentiated functions.
(ii) Transport proteins carrying hormones (e.g. transcortin), minerals and trace elements and lipids (e.g. lipoproteins)
(iii) Attachment and spreading factors, acting as germination points for cell attachment.
(iv) Stabilising and detoxifying factors needed to maintain pH or to inhibit proteases either directly, such as α-antitrypsin or α2-macroglobulin, or indirectly, by acting as an unspecific sink for proteases and other (toxic) molecules.

RM9955 is heat inactivated Fetal bovine serum. Heat inactivation is done to destroy heat labile components such as complement that can lead to complement mediated cell lysis. Complement proteins, antibodies and enzymes present in the serum are inactivated by heat inactivation.

Applications of Heat inactivated Serum:

- Suitable for immunoassays, enzyme assays and cytotoxicity assays
- For culture of insect cells

***Note**: Heat inactivation process can be detrimental to the growth promoting capacity of serum. When heat inactivation of serum is done, along with the complement certain amino acids, vitamins and growth factors are subjected to temperatures that could cause degradation. Hence it is recommended that researcher should experimentally determine and document the reasons for using heat inactivated serum.*

RM9955 is sourced in countries approved for import into the European Union by European Commission. Currently this includes Central and South America, USA, Canada, Australia, New Zealand and South Africa. This serum is collected and processed in facilities registered and inspected by the competent authority in the country of origin. EU approved serum can be freely moved between EU member countries and many other countries outside of Europe where the USDA or FDA regulations are not required.

Directions for Thawing of Serum:

Thawing of the sera should be done as quickly as possible in order to minimize the period of time during which elevated salt concentration prevail in the thawed liquid.

1. Remove the bottles from the freezer and allow them to acclimatize at room temperature for 10 minutes and keep in 2-8°C overnight in refrigerator.

   Note: Do not place the serum in the water bath or incubator. Avoid exposing serum to elevated temperatures as this can lead to degradation of heat labile nutrients.

2. Swirl the bottle of serum frequently during thawing to disperse the released salts and proteins uniformly in the liquid.

Note on Cryoprecipitate:

We advise our users to follow the recommended thawing procedure. Proper thawing with periodic agitation is crucial to a serum's optimum performance. If bottle of serum is not frequently swirled during thawing, the released proteins and salts tend to form crystalline or flocculent precipitates. These cryoprecipitates are not detrimental to the performance of serum but might affect serum's appearance and consistency.

Slight turbidity or small amount of flocculent material is normal in most serum products and will not affect its performance in any manner. Filtering serum to remove cryoprecipitate is not recommended and could result in loss of nutrients.

Quality Control:

Physical and Chemical analysis:

pH	: 6.8 - 8.2
Osmolality	: 280 - 340 mOsm/Kg H_2O
Endotoxin	: Value EU/ml
Hemoglobin	: < 20mg/dl
Identity	: Typical

Protein:

Total protein	: 3.0 - 4.5 g/dl
Albumin	: value g/dl
α-Globulin	: value g/dl
β-Globulin	: value g/dl
γ-Globulin	: value g/dl
IgG	: NMT 250µg/ml

Sterility Testing:

Aerobic bacteria	: Not detected
Anaerobic bacteria	: Not detected
Fungi	: Not detected
Mycoplasma	: Not detected

Virus testing:

Bovine Virus Diarrhea Virus (BVD-V)	: Not detected
Bovine Herpes Virus 1 (BHV-1)	: Not detected
Parainfluenza Type 3 (PI-3)	: Not detected

Antibody testing:

BVD-1 Antibody titer	: Value
BVD-2 Antibody titer	: Value

Growth promotion and cytotoxicity:

Each lot of serum is tested for growth promotion and cytotoxicity. Growth promotion shows the ability of the serum to support the growth of a cell line using a standardized low inoculum in media with 10% serum over a period of 10 to 14 days.

Storage and Shelf Life:

Store at -10°C to -40°C away from bright light.
Shelf life of the product is 5 years.
Thawed serum can be stored at 2- 8°C up to four weeks.
Multiple freeze thaw cycles should be avoided.
Serum should never be stored in frost free freezers. Frost free appliance undergoes intermittent warming cycles to prevent ice deposits and this might lead to multiple thawing of serum.
To avoid multiple free thaw cycles or long periods of refrigeration, we recommend freezing small aliquots which can be thawed and used as required.
Use before expiry date given on the label.

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Technical Datasheet

Fetal Bovine Serum

Origin: Brazil, EU Approved
Sterile filtered

Product Code: RM10432

Product Description:

Fetal bovine serum (FBS) is a ubiquitously used essential supplement in cell culture media. FBS is a cocktail of proteins, vitamins, carbohydrates, lipids, hormones, growth factors, minerals and trace elements and is used as an universal growth supplement effective for most types of human and animal (including insect) cells. The major functions of serum in culture media are to provide:
(i) Hormonal factors stimulating cell growth and proliferation and promoting differentiated functions.
(ii) Transport proteins carrying hormones (e.g. transcortin), minerals and trace elements and lipids (e.g. lipoproteins)
(iii) Attachment and spreading factors, acting as germination points for cell attachment.
(iv) Stabilising and detoxifying factors needed to maintain pH or to inhibit proteases either directly, such as α-antitrypsin or α2-macroglobulin, or indirectly, by acting as an unspecific sink for proteases and other (toxic) molecules.
RM10432 is sourced in Brazil which is approved for import into the European Union by European Commission. This serum is collected and processed in facilities registered and inspected by the competent authority in the country of origin. EU approved serum can be freely moved between EU member countries and many other countries outside of Europe where the USDA or FDA regulations are not required.

Directions for Thawing of Serum:

Thawing of the sera should be done as quickly as possible in order to minimize the period of time during which elevated salt concentration prevail in the thawed liquid.

1. Remove the bottles from the freezer and allow them to acclimatize at room temperature for 10 minutes and keep in 2-8°C overnight in refrigerator.

   Note: Do not place the serum in the water bath or incubator. Avoid exposing serum to elevated temperatures as this can lead to degradation of heat labile nutrients.
2. Swirl the bottle of serum frequently during thawing to disperse the released salts and proteins uniformly in the liquid.

Note on Cryoprecipitate:

We advise our users to follow the recommended thawing procedure. Proper thawing with periodic agitation is crucial to a serum's optimum performance. If bottle of serum is not frequently swirled during thawing, the released proteins and salts tend to form crystalline or flocculent precipitates. These cryoprecipitates are not detrimental to the performance of serum but might affect serum's appearance and consistency. Slight turbidity or small amount of flocculent material is normal in most serum products and will not affect its performance in any manner. Filtering serum to remove cryoprecipitate is not recommended and could result in loss of nutrients.

Quality Control:

Physical and Chemical analysis:

Appearance	: Amber liquid
pH	: 6.8 - 8.2
Osmolality	: 280 - 340mOsm/Kg H_2O
Endotoxin	: Value EU/ml
Hemoglobin	: < 20 mg/dl
Identity	: Typical

Protein:

Total protein	: 3.0 - 4.5 g/dl
Albumin	: value g/dl
α-Globulin	: value g/dl
β-Globulin	: value g/dl
γ-Globulin	: value g/dl
IgG	: < 250µg/ml

Sterility Testing:

Aerobic bacteria	: Not detected
Anaerobic bacteria	: Not detected
Fungi	: Not detected
Mycoplasma	: Not detected

Virus testing:

Bovine Virus Diarrhea Virus (BVD-V)	: Not detected
Bovine Herpes Virus 1 (BHV-1)	: Not detected
Parainfluenza Type 3 (PI-3)	: Not detected

Antibody testing:

BVD-1 Antibody titer	: Value
BVD-2 Antibody titer	: Value

Growth promotion and cytotoxicity:

Each lot of serum is tested for growth promotion and cytotoxicity. Growth promotion shows the ability of the serum to support the growth of a cell line using a standardized low inoculum in media with 10% serum over a period of 10 to 14 days.

Storage and Shelf Life:

Store at -10°C to -40°C away from bright light.
Shelf life of the product is 5 years.
Thawed serum can be stored at 2- 8°C up to four weeks.
Multiple freeze thaw cycles should be avoided.
Serum should never be stored in frost free freezers.
Frost free appliance undergoes intermittent warming cycles to prevent ice deposits and this might lead to multiple thawing of serum.
To avoid multiple freeze thaw cycles or long periods of refrigeration, we recommend freezing small aliquots which can be thawed and used as required.
Use before expiry date given on the label.

Novobiocin NV 5 mcg SD121

Novobiocin NV 5 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

*Ingredients	Concentration
Novobiocin	5 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "NV 5" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
S.aureus (25923)	22-31

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

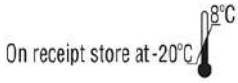
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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