Optochin Discs

DD009

Optochin Discs are used for identification and differentiation of *Streptococcus pneumoniae* and Viridans Streptococci.

Directions

Prepare Soyabean Casein Digest Agar (M290) w/blood or Blood Agar Base (M073) plates and streak pure culture of organism to be tested across one half of the plate. Streak a known Pneumococcus culture across the other half of the plate as positive control. Immediately place Optochin discs in the centre of the two halves of the plate and incubate at 35-37°C for 18-24 hours. Observe for zone of inhibition around the discs.

Principle And Interpretation

Alpha haemolytic (viridans) streptococci and Pneumococcus (*Streptococcus pneumoniae*) cannot be easily distinguished on Blood Agar plates as pneumococci strain shows partial clearing of blood and greenish discolouration (a-hemolysis). Optochin is inhibitory for pneumococcal growth whereas other streptococci strains show good growth or a very small zone of inhibition. Bowers and Jeffries have shown a correlation between bile solubility and full Optochin susceptibility for the differentiation of Streptococcus pneumoniae from other streptococci (1).

Hence optochin test is a useful diagnostic aid for identification / differentiation of pneumococci and viridans Streptococci.

Optochin discs are filter paper discs impregnated with optochin. The test is based on the property of viridans streptococci to grow in the presence of Optochin (ethyl hydrocuprein hydrochloride) which inhibits pneumococci. This test is performed for the diagnosis of penumococcal infections. Specimens of sputum, lung aspirate, pleural fluid, CSF, urine or blood are first examined by Gram's stain, cultured and the isolates are then subjected to Optochin Sensitivity Test.

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "Op" in continuous printing style.

Cultural response

Cultural response observed after an incubation at 35-37°C for 18-24 hours at on seeded Soyabean Casein Digest Agar (M290) with added sterile defibrinated blood, using Optochin discs.

Organism	Zone of inhibition
Streptococcus pneumoniae *ATCC 6303*	More than or equal to 15mm

Storage and Shelf Life

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

Reference

1.Bowers E.F. and Jeffries L.R., 1995, J. Clin. Path., 8:58.

Revision : 1 / 2011

CE

Bacitracin Susceptibility Test Discs

DD015

Bacitracin Susceptibility Test Discs are used for the identification and differentiation of Group A streptococci (especially *S.pyogenes*) from other beta-haemolytic streptococci.

Directions

Pure Cultures : Evenly inoculate the surface of Tryptose Blood Agar Base (M097) with pure culture of beta-haemolytic streptococci to be tested. Aseptically place a Bacitracin disc on the inoculated surface and incubate the inverted plate at 35-37°C for 18-24 hours in 10% CO2. Observe for the presence of zone of inhibition around the Bacitracin disc. A zone indicates that the Streptococcus is presumptively of Group A. If desired further confirmation can be obtained by serological grouping.

Clinical Materials : Incubate Tryptose Blood Agar Base(M097) plate with throat swab or other material. Spread the inoculum to obtain discrete colonies on some portion of the plate, so as to determine the species in mixed growth. Aseptically place a Bacitracin disc on the secondary area of inoculation and incubate the inverted plates for 18-24 hours at 35-37°C in 10% CO2. Examine for zones of inhibition. Bacitracin is inhibitory to many organisms except b-haemolytic streptococci, however the presence of a zone of inhibition does not essentially indicate Lancefield Group A streptococci. If the colonial morphology is carefully observed, it is possible to select presumptive Group A streptococci. By serological grouping, further confirmation can be obtained.

Precautions

Use known Group A and non-Group A streptococci to determine the accuracy of the discs and inoculum.

Principle And Interpretation

The growth of Group A beta-haemolytic streptococci on blood agar is inhibited by 0.04 units Bacitracin disc. Micrococci and streptococci are also inhibited by 0.04 units disc, while all coagulase-negative staphylococci are resistant (4).

Bacitracin susceptibility test discs are filter paper discs impregnated with 0.04 units of Bacitracin. Bacitracin discs can save considerable time, labour and materials if used as a screening test before serological grouping. Maxted showed that Group A streptococci were more sensitive to Bacitracin than beta-haemolytic strains of other groups (1). Hence he suggested that Bacitracin might be used as a rapid diagnostic agent for Group A streptococci.

Levinson and Frank(2) who employed Bacitracin impregnated filter paper discs for this purpose, observed that many sensitive beta-haemolytic streptococci were of Group A. Steamer et al compared Bacitracin disc, fluorescent antibody technique and Lancefield precipitin technique and found that the Bacitracin disc technique was most convenient for routine clinical laboratory (3). Bacitracin sensitivity test along with Furacin and Optochin tests are useful for distinguishing *Aerococcus viridans* and *S. milleri* from enterococci and Streptococcus mitis (2).

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "B" in continuous printing style.

Cultural response

Average diameter of zone of inhibition for S.pyogenes observed on Tryptose Blood Agar (M097) after an incubation at 35-37°C for 18-24 hours.

Organism	**Zone of inhibition (mm)**
Streptococcus pyogenes ATCC 19615	15 -20 mm

Storage and Shelf Life

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

Reference

1.Maxted W. R., 1953, J. Clin. Path., 6:234.
2.Levinson M. L. and Frank P.F., 1955, J. Bact., 69:234.
3.Streamer C.W et al, 1962, Am. J. Dis. Children, 104:157.
4.Guthof O.,1960, Ztschr. F hyg. U. Infektionskr.,146:425

Revision : 1 / 2011

Technical Data

Grams Stain-Kit K001

Intended Use

Grams Stain Kit is used for differentiation of bacteria on the basis of their gram nature.

Composition**

Ingredients

Gram's Crystal Violet (S012)(Solution A)	-
Crystal Violet	2.000 gm
Ethyl alcohol,95%	20.000 ml
Gram's Crystal Violet (S012)(Solution B)	-
Ammonium oxalate	0.800 gm
Distilled Water	80.000 ml

Solution A and B are mixed and stored for 24 hours before use.The resulting stain is stable.

Gram's Decolourizer(S032)	-
Ethyl alcohol, 95%	50.0 ml
Acetone	50.0 ml
Gram's Iodine(S013)	-
Iodine	1.000 gm
Potassium iodide	2.000 gm
Distilled water	300.000 ml
Safranin,0.5% w/v(S027)	-
Safranin O	0.500 gm
Ethyl alcohol, 95%	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms,use less crystal violet).
4. Drain the stain.
5. Flood the smear with Gram’s Iodine (S013). Allow it to remain for 1 minute.
6. Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear.
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (S027). Allow it to remain for 1 minute.
9. Wash with water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram-negative cell walls. Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined. In practice,a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain. A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin.In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram negative cells appear pink to red.

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. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. Generally the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions :

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

Limitations

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.) to confirm findings suggested by gram-stained smears.
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram-staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in a abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness.
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

Performance and Evaluation

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

Quality Control

Microscopic examination

Gram staining is carried out using gram's Stain Kit and observed under oil immersion lens.

Results

Gram-positive organisms : Violet coloured

Gram-negative organisms : Pinkish red coloured

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.

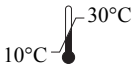
Reference

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. J.clin. Microbiol.16:1052-1056
7. Thorpe,J.E., R.P.Banghman,P.T. Frame,T.A. Wessler,and J.L. Staneck.1987.Bronchoalveolar lavage for diagnosing acute bacterial pneumoniae .J.Infect.Dis.155:855-861
8. Brown,M.S.,and T.C. Wu. 1986. The Gram stain morphology of fungi, mycobacteria, and Pneumocytis carinii. J.Med .Technol3:495-499
9. George Clark et al, 1981, 4th ed., Staining procedures: 17(375-379)
10. Godkar B. P., 1996, Textbook of medical laboratory technology: 23(309-313)

Revision : 01 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Nutrient Agar

M001

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

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

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (5,6). Nutrient Agar is ideal for demonstration and teaching purposes where a more prolonged survival of cultures at ambient temperature is often required without risk of overgrowth that can occur with more nutritious substrate. This relatively simple formula has been retained and is still widely used in the microbiological examination of variety of materials and is also recommended by standard methods. It is one of the several non-selective media useful in routine cultivation of microorganisms (1,7). 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 - Blood; Food and dairy samples; Water 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 (1,7,8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

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

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

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petri plates

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

pH
7.20-7.60

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. 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. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
6. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins,Baltimore.
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.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

Revision : 05 / 2019

IVD — In vitro diagnostic medical device

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30°C / 10°C — Storage temperature

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www.cepartner 4u.eu

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	Gms / Litre
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 (5,6). 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 (1,7). 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 - Blood; Food and dairy samples; Water 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 (1,7,8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. 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 temmperature.

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

Disposal

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

Reference

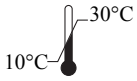
1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Isenberg, H.D. Clinical 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. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
6. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 09 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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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	Gms / Litre
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 colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink 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 USP (12), BP (2), EP (3) and AOAC (13) 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 (10).

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(11). 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. (4,7). Any increase in the oxygen content is indicated by a colour change of redox indicator, resazurin to red (8,9,10). The small amount of agar helps in maintaining low redox potential for stabilizing the medium (7).

Type of specimen

Pharmaceutical samples for sterility testing, clinical samples- blood 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 guidelines (2,3,12)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations :

1.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 on standing.

Reaction

Reaction of 2.97% 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 30-35°C for not more than 3 days.

Organism	Inoculum (CFU)	Growth
Clostridium sporogenes ATCC 19404 (00008*)	50 -100	luxuriant
Clostridium sporogenes ATCC 11437	50 -100	luxuriant
Clostridium perfringens ATCC 13124 (00007*)	50 -100	luxuriant
Bacteroides fragilis ATCC 23745	50 -100	luxuriant
Bacteroides vulgatus ATCC 8482	50 -100	luxuriant
Staphylococcus aureus subsp. *aureus ATCC 25923 (00034*)*	50 -100	luxuriant
Staphylococcus aureus subsp. *aureus ATCC 6538 (00032*)*	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant
Micrococcus luteus ATCC 9341	50 -100	luxuriant
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant

Escherichia coli NCTC 9002	50 -100	luxuriant
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant
Salmonella Abony *NCTC 6017* (00029*)	50 -100	luxuriant
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50 -100	luxuriant

Key : * Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 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 (5,6).

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598.
2.British Pharmacopoeia, 2017, The Stationery office British Pharmacopoeia
3.European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
4. Federal Register, 1992, Fed. Regist., 21:640.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. Marshall, Gunnison and Luxen, 1940, Proc. Soc. Exp. Biol. Med., 43:672.
9. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52:287.
10. Portwood, 1944, J. Bact., 48:255.
11. Quastel and Stephenson, 1926, J.Biochem., 20
12. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention, Rockville, MD.
13. Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC,Washington, D.C

Revision : 04 / 2019

IVD	In vitro diagnostic medical device
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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

1.Endo, 1904, Zentralbl. Bakteriol., Abt. 1., Orig., 35:109.
2.Levin and Schoenlein, 1930, A Compilation of Culture Media for the Cultivation of Microorganisms, Williams and Wilkins, Baltimore.
3.Greenberg, Trussell and Clesceri (ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C.
4.Richardson (ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
5.Speck M., 1984, Compendium of Methods for the Microbiological Examination of Foods, 3rd ed., APHA, Washington, D.C.

Revision : 01 / 2014

Sabouraud Dextrose Broth (Sabouraud Liquid Medium) M033

Intended Use:

Sabouraud Dextrose Broth (Sabouraud Liquid Medium) is used for cultivation of yeasts, moulds and aciduric microorganisms.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	20.000
Peptone, special	10.000
Final pH (at 25°C)	5.6±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.

Principle And Interpretation

Sabouraud Dextrose Agar is Carliers modifications (2) of the formulation described by Sabouraud (7) for the cultivation of fungi, particularly those associated with skin infections. The medium is also recommended by APHA (8). Sabouraud Dextrose Broth is also a modification by Sabouraud (6) and serves the same purpose as Sabouraud Dextrose Agar Medium 3.

Sabouraud dextrose media are peptone media supplemented with dextrose to support the growth of fungi. Peptone special provides carbon and nitrogen source, vitamins, minerals, amino acids and growth factors. Dextrose provides an energy source for the growth of microorganisms. The low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (5). The acid reaction of the final medium is inhibitory to a large number of bacteria making it particularly useful for cultivating fungi and aciduric microorganisms. For isolation of fungi from contaminated specimens, a selective medium should be inoculated simultaneously. Incubate cultures for 4 to 6 weeks before reporting as negative.

Type of specimen

Clinical : skin scrapings; Pharmaceutical 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 (1,8).

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

Warning and Precautions

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

Limitations

1. Since it is a general purpose medium, bacterial cultures will also grow .
2. Further isolation and biochemical tests should 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
Light amber coloured clear solution in tubes

Reaction
pH of 3.0% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH
5.40-5.80

Cultural Response
Cultural characteristics was observed after an incubation at 20-25°C for 3-5 days.

Organism	Inoculum (CFU)	Growth
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant
Aspergillus brasiliensis ATCC 16404 (00053*)	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 9763 (00058*)	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 2601	50 -100	good-luxuriant
Escherichia coli ATCC 8739 (00012*)	50 -100	Luxuriant (inhibited on media with low pH)
Escherichia coli ATCC 25922 (00013*)	50 -100	good-luxuriant
Escherichia coli NCTC 9002	50 -100	Luxuriant (inhibited on media with low pH)
Lactobacillus casei ATCC 334	50 -100	luxuriant

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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

2. Carlier G. I. M., 1984, Brit. J. Derm. Syph., 60:61

3.Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
6. Sabouraud R., 1892, Ann. Dermatol. Syphil. 3 : 1061.
7. Sabouraud R., Les Teignes, Paris: Masson et Cie, 1910, p 553
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.

Revision : 04 / 2019

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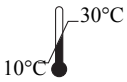

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Sabouraud Dextrose Agar

M063

Intended Use:

Recommended for the cultivation of yeasts, moulds and aciduric bacteria from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	40.000
Mycological, peptone	10.000
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.0 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.

Principle And Interpretation

Sabouraud Dextrose Agar is Carlier's modification (3) of the formulation described by is a modification of Sabouraud Dextrose Agar which is described by Sabouraud (7) for the cultivation of fungi (yeasts, moulds), particularly useful for the fungi associated with skin infections. This medium is also employed to determine microbial contamination in food, cosmetics, and clinical specimens (2).

Mycological Peptone provides nitrogenous compounds. Dextrose provides an energy source. High dextrose concentration and low pH favors fungal growth and inhibits contaminating bacteria from test samples (6).

Type of specimen

Clinical samples: skin scrapings, Food samples ; Cosmetics.

Specimen Collection and Handling

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines(1,4,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

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

Limitations

1. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.

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

3. Further biochemical tests should 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 6.5% w/v aqueous solution at 25°C (after sterilization). pH : 5.6±0.2

pH
5.40-5.80

Cultural Response
Growth Promotion was carried out in accordance with the (USP/EP/BP/JP), after an incubation at 20-25 °C for 24-48 hours.Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar and fungus growth on Sabouraud Dextrose Agar

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of ICH (USP/EP/BP/JP), after an incubation at 30-35 °C for 24-48 hours.Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar and fungus growth on Sabouraud Dextrose Agar

Growth Promoting Properties
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 24 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating >=100 cfu (at 30-35°C for 24-48 hours).

Organism	Inoculum (CFU)	Growth	Recovery
Candida albicans ATCC 10231 (00054*)	50 -100	Luxuriant (white colonies)	>=70 %
#Aspergillus brasiliensis ATCC 16404 (00053*)	50 -100	luxuriant	>=70 %
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant	>=70 %
Saccharomyces cerevisiae ATCC 9763 (00058*)	50 -100	luxuriant	>=70 %
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=70 %
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=70 %
Escherichia coli NCTC 9002	50 -100	luxuriant	>=70 %
Lactobacillus casei ATCC 334	50 -100	luxuriant	>=70 %
Trichophyton rubrum ATCC 28191		luxuriant	

Key : (*) - Corresponding WDCM numbers. (#) - Formerly known as *Aspergillus niger*

Storage and Shelf Life

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

Disposal

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

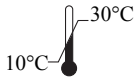
Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
3. Carlier G. I. M., 1948, Brit. J. Derm. Syph., 60:61.
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. Murray PR, Baren EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors) 2003, Manual of clinical Microbiology, 8th ed.,ASM, Washington, D.C.
7. Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061.
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.
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2018

IVD — In vitro diagnostic medical device

CE — CE Marking



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Kligler Iron Agar

M078

Intended Use:

Recommended for the differential identification of gram-negative enteric bacilli from clinical and non clinical samples on the basis of the fermentation of dextrose, lactose and H_2S production.

Composition**

Ingredients	Gms / Litre
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 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 1inch butts.

Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (9) and Russels Double Sugar Agar (7) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (3). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (3). 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 (4). 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 clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,10,11).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. 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. Else it might result in erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous 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 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	Inoculum (CFU)	Growth	Gas	H2S	Slant	Butt
Escherichia coli ATCC 25922 (00013*)	50-100	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*)	50-100	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	50-100	luxuriant	positive reaction	positive reaction, blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Proteus vulgaris ATCC 6380	50-100	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*)	50-100	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*	50-100	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*	50-100	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*	50-100	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*)	50-100	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*)	50-100	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*)	50-100	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	50-100	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*)	50-100	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

Storage and Shelf Life

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

Disposal

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

Reference

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

2.Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

3.Bailey S. F. and Lacey G. R., 1927, J. Bacteriol., 13:183.

4.Ewing, 1986, Edwards and Ewings Identification of the Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., N.Y.

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

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

7.Kligler I. J., 1917, Am. J. Publ. Health, 7:1041.

8.Kligler I. J., 1918, J. Exp. Med., 28:319.

9.Russell F. F., 1911, J. Med. Res., 25:217.

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

11. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

Revision : 02 / 2018

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
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Simmons Citrate Agar

M099

Intended Use:

Recommended for differentiation the members of *Enterobacteriaceae* on the basis of citrate utilization from clinical and non clinical samples. .

Composition**

Ingredients	Gms / Litre
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.28 grams in 1000 ml purified/ distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Precaution: Before using water, ensure pH of water is 6.5 to 7.0.Initial colour of the medium may deviate from expected colour, if the above precaution is ignored.

Principle And Interpretation

These media are used for the differentiation between *Enterobacteriaceae* and the members of aerogenes group on the basis of citrate utilization as sole carbon source. Initially the citrate medium was developed by Koser (6) containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating *Escherichia coli* and *Enterobacter aerogenes* by IMViC tests. Later on Simmons (9) modified Kosers formulation by adding agar and bromothymol blue (7). It is recommended by APHA (3).

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator. The medium should be freshly prepared because in dry conditions, changes in colour may appear even before inoculation, especially at the bottom of the slant.

Type of specimen

Isolated microorganism from clinical and non clinical samples.

Specimen Collection and Handling

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,10).

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

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

Warning and Precautions

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

Limitations

1.Before using water, ensure pH of water is 6.5 to 7.0.Initial colour of the medium may deviate from expected colour, if the above precaution is ignored.
2. The pH affects the performance of the medium and must be correctly monitored.

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
Forest green coloured slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.43% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

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

Organism	Inoculum (CFU)	Growth	Citrate utilisation
# *Klebsiella aerogenes ATCC 13048 (00175*)*	50-100	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	
Salmonella Typhi *ATCC 6539*	50-100	fair-good	negative reaction, green colour
Salmonella Typhimurium *ATCC 14028 (00031*)*	50-100	good-luxuriant	positive reaction, blue colour
Shigella dysenteriae ATCC 13313	>=10^4	inhibited	
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	positive reaction, blue colour
Salmonella Enteritidis *ATCC 13076 (00030*)*	50-100	good-luxuriant	positive reaction, blue colour

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

Storage and Shelf Life

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

Disposal

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

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, 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. Koser, 1923, J. Bact., 8:493.
7. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
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.
9. Simmons, 1926, J. Infect. Dis., 39:209.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2019

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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	Gms / Litre
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 (4) and suspected foodstuffs (1,6, 8, 9) and for microbial limit test (7). 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 H2S gas and this reductive enzyme process is attributed by thiosulphate reductase. Production of H2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H2S 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 H2S.

Type of specimen

Clinical: faeces, blood, rectal swabs; Suspected food stuffs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3,4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1.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 (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

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. 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 (2,3).

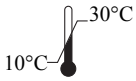
Reference

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. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed., ASM, Washington, D.C.
5. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
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. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopoeial Convention. Rockville, MD.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
9. Williams S., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed.,AOAC,Washington, D.C.

Revision : 03/ 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Mannitol Salt Agar

M118

Intended Use:

Recommended for the isolation of pathogenic Staphylococci from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
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 111.02 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, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

Note : This product contains 7.5% Sodium chloride as one of its ingredients. On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light.

Principle And Interpretation

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e *Staphylococcus aureus* is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (10). Staphylococci have the unique ability of growing on a high salt containing media (8). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5%NaCl was studied by Chapman (2). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (10, 5,3,12,11). The additional property of lipase activity of *Staphylococcus aureus* can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (4). HM peptone B and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator.

S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of *S.aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of *S. aureus* should be confirmed by performing the coagulase test [tube or slide] (10). Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion.

A possible *S.aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (9). Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (9).

Type of specimen

Clinical samples: pus, urine; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,12,13).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. A possible *S.aureus* must be confirmed by the coagulase test.
2. The organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (9).
3. Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (9).

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 11.1% 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 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Incubation temperature
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0 %		>=72 hrs
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs

Staphylococcus epidermidis ATCC 14990 (00132*)	50 -100	fair-good	30 -40 %	red	18 -72 hrs
Proteus mirabilis ATCC 12453	50 -100	none-poor	0 -10 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922 (00013*)	>=10^4	Inhibited	0%		>=72 hrs
Escherichia coli NCTC 9002	>=10^4	Inhibited	0%		>=72 hrs
# Klebsiella aerogenes ATCC 13048 (00175*)	>=10^4	Inhibited	0%		>=72 hrs

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 inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.Product performance is best if used within stated expiry period.

Disposal

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

Reference

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

2. Chapman G. H., 1945, J. Bacteriol., 50:201.

3. Davis J. G., 1959, Milk testing, 2nd Ed., Dairy Industries Ltd, London.

4. Gunn B. A., Dunkelberg W. E. and Creitz J. R., 1972, Am. J. Clin. Pathol., 57:236.

5. Hitchins A. D., Tran T. and McCarron J. E., 1995, FDA Bacteriological Analytical Manual, 8th Ed., AOAC International,Gaithersburg, Md.

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. Koch P. K., 1942, Zentralbl. Bakteriol. Parasitenkd. Abt. I Orig.149:122.

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

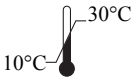
10. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed.,American Society for Microbiology, Washington, D.C.

11. Silverton R. E. and Anderson M. J., 1961, Handbook of Medical Laboratory Formulae, Butterworths, London.

12. 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.

13. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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Mueller Hinton Agar

M173

Intended Use:

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

Composition**

Ingredients	Gms / Litre
HM infusion B from #	300.000
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 infusion from

- 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 (6). 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 (9). 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 (7).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (4). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (11). 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 (2,6,9). 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 (7). 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 (7,8).

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 (5).

Type of specimen

Clinical samples : Isolated microorganisms from urine , stool, blood etc.

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

1. 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 slight opalscent 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
Cultural characteristics observed after incubation at 30-35°C for 18 -24 hours for bacterial cultures. For testing S.pneumoniae : The medium was supplemented with 5% Sheep blood and incubated at 35°C for 16-18 hours at 5% CO2 For testing *H.influenaze* : The medium was supplemented with 5g/l of Yeast extract & 2 vials /l of Haemophilus Growth Supplement (FD117 containing 15 mg/l of Haematin + 15 mg/l of NAD) and incubated at 35°C for 20-24 hours at 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	Zone of inhibition Observed
***Escherichia coli* ATCC 25922 (00013*)**	luxuriant		
Cephalothin CEP 30mcg		29-37 mm	29 -37 mm
Chloramphenicol C 30 mcg		21-27 mm	21 -27 mm
Co-Trimoxazole COT 25 mcg #		23-29 mm	23 -29 mm
Cefotaxime CTX 30 mcg		29-35 mm	29 -35 mm
Gentamicin GEN 10 mcg		19-26 mm	19 -26 mm
Sulphafurazole SF 300 mcg		15-23 mm	15 -23 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)**	luxuriant		
Co-Trimoxazole COT 25 mcg #		# 20 mm (Clear zone)	>=20 mm
Cefoxitin CX 30 mcg		23-29 mm	23 -29 mm
Erythromycin E 15 mcg		22-30 mm	22 -30 mm
Linezolid LZ 30 mcg		25-32 mm	25 -32 mm
Oxacillin OX 1mcg		18-24 mm	18 -24 mm
Pristinomycin RP 15 mcg		21-28 mm	21 -28 mm
Tetracycline TE 30 mcg $		18-25 mm	18 -25 mm
Ciprofloxacin CIP 5mcg		22-30 mm	22 -30 mm
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		
Ceftazidime CAZ 30 mcg		22-29 mm	22 -29 mm
Ciprofloxacin CIP 5mcg		30-40 mm	30 -40 mm
Tobramycin TOB 10 mcg $		19-25 mm	19 -25 mm
Amikacin AK 30 mcg $		18-26 mm	18 -26 mm
Aztreonam AT 3mcg		23-29 mm	23 -29 mm
Cephotaxime CTX 30 mcg		18-22 mm	18 -22 mm
Gentamicin GEN 10 mcg $		16-21 mm	16 -21 mm
Imipenem IPM 10 mcg		20-28 mm	20 -28 mm
Piperacillin PI 100 mcg		12-18 mm	25 -33 mm
***Escherichia coli* ATCC 35218**	luxuriant		
Amoxyclav AMC 30 mcg		18-24 mm	18 -24 mm
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm	24 -30 mm
Ticarcillin TI 75 mcg		6 mm	6 -6 mm
Ticarcillin/Clavulanic acid TCC 75/10mcg		20-28 mm	20 -28 mm
Ampicillin AMP 10 mcg		16-22 mm	16 -22 mm
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm	29 -37 mm
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		
Trimethoprim TR 5 mcg #		# 20 mm	>=20 mm
Vancomycin VA 30 mcg		17-21 mm	17 -21 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 43300 (MRSA) (00211*)**	luxuriant		
Oxacillin OX 1 mcg		Very Hazy to No Zone	No zone

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.

2. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.

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. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.

6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

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

8. National Committee for Clinical Laboratory Standards, 1986, Proposed Standards, M6-P, NCCLS, Villanova, Pa.

9. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.

10. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards.

11. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.

Revision : 04 / 2018

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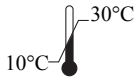

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Lactobacillus MRS Agar

M641

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 67.15 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

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

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

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

Type of specimen

Clinical samples - Faeces; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,8,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Due to nutritional variation , some strains may show poor growth.

Performance and Evaluation

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

Quality Control

Appearance

Cream to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

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

pH

6.30-6.70

Cultural Response

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

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

Key: * Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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

2. deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.

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

6. Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.

7. Menconi A, Kallapura G. Biosci Microbioto Food (2014) Identification and Characterization of Lactic Acid Bacteria in a Commercial Probiotic Culture.

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

9. 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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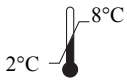

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HiCrome™ Candida Differential Agar

M1297A

Intended Use

HiCrome™ Candida Differential Agar is recommended for rapid isolation and identification of *Candida* species from mixed cultures in clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	15.000
Yeast extract	4.000
Dipotassium hydrogen phosphate	1.000
Chromogenic mixture	7.220
Chloramphenicol	0.500
Agar	15.000
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.72 grams 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

Perry and Miller (3) reported that *Candida albicans* produces an enzyme b -N-acetyl- galactosaminidase and according to Rousselle et al (4) 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 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 special and yeast extract provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Phosphate buffers the medium well. Chloramphenicol 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* colonies appear as cream to white smooth colonies, while *C.krusei* appear as purple fuzzy colonies.

Type of specimen

Clinical samples - skin scrapings, urine.

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. 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 1.5% Agar gel

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

Reaction
Reaction of 4.27% w/v aqueous solution at 25°C. pH : 6.3±0.2

pH
6.10-6.50

Cultural Response
Cultural characteristics observed 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 (1,2).

References

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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. Perry J. L. and Miller G. R., 1987, J. Clin. Microbiol., 25: 2424 -2425.
4. Rousselle P., Freydiere A., Couillerot P., de Montclos H. and GilleY., 1994, J. Clin. Microbiol. 32:3034-3036.

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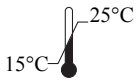

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Rappaport Vassiliadis Soya Broth (RVS Broth)

M1491

Rappaport Vassiliadis Soya Broth (RVS Broth) is recommended as a selective enrichment medium for the Salmonellae species from the food and animal feeding stuffs.

Composition**

Ingredients	Gms / Litre
Papaic digest of soyabean meal	4.500
Sodium chloride	8.000
Potassium dihydrogen phosphate	0.600
Dipotassium phosphate	0.400
Magnesium chloride. hexahydrate	29.000
Malachite green	0.036
Final pH (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.11 grams of dehydrated medium in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115°C for 15 mins.

Principle And Interpretation

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

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

Quality Control

Appearance
Light yellow to light blue homogeneous free flowing powder

Colour and Clarity of prepared medium
Greenish blue clear to slightly opalescent with a slight precipitate.

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

pH
5.00-5.40

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of colony
Cultural Response					
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	>=35	>=70 %	red with black centers
Salmonella Abony NCTC 6017	50 -100	luxuriant	>=35	>=70 %	red with black centers
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0%	
Escherichia coli ATCC 25922	50 -100	none-poor	0 -10	0 -10 %	yellow
Escherichia coli ATCC 8739	50 -100	none-poor	0 -10	0 -10 %	yellow
Salmonella Enteritidis ATCC 13076	50 -100	luxuriant	>=35	>=70 %	red with black centre
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	>=35	>=70 %	red with black centre
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	0%	
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0	0%	
E.coli +S.Typhimurium (mixed culture)					
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	>=35	>=70 %	red with black centre

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.Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:11.
2.Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:209.
3.Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523 4.McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol. 1:171

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