HIMEDIA

Technical Data

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

Oxidase Discs

DD018

Oxidase Discs are used for detection of oxidase production by microorganisms like Neisseria, Alcaligenes, Aeromonas, Vibrio's, Campylobacter and Pseudomonas, which give positive reactions and for excluding Enterobacteriaceae, which give negative reactions.

Directions

Oxidase reaction is carried out by touching and spreading a well isolated colony on the oxidase disc. The reaction is observed within 5-10 seconds at 25-30°C. A change later than 10 seconds or no change at all is considered negative reaction.

Precautions

1. ,,Do not use stainless steel or nichrome inoculating wires, as false positive reaction may result from surface oxidation products formed during flame sterilization.

2. ,,Growth from media containing dyes is not suitable for testing.

3. ,,Timing is critical (5-10 sec) for interpretation of results.

4. ,,Perform oxidase test on all gram-negative bacilli.

5. ,,Cytochrome oxidase production may be inhibited byacid production. False negative reactions may be exhibited by Vibrio, Aeromonas and Plesiomonas species when grown on a medium containing fermentable carbohydrate e.g. MacConkey Agar (M081). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by auto-oxidation which may be avoided by adding 0.1% ascorbic acid (3).

Principle And Interpretation

Certain bacteria posses either cytochrome oxidase or indophenol oxidase (an iron-containing haemoprotein), which catalyzes the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). In the oxidase test, a colourless dye such as N, N-dimethy-p-phenylenediamine serves as an artificial electron acceptor for the enzyme oxidase. The dye is oxidized to form indophenol blue, a coloured compound. The test is useful in the initial characterization of aerobic gram-negative bacteria of the genera Aeromonas, Plesiomonas, Pseudomonas, Campylobacter and Pasteurella.

Oxidase discs are sterile filter paper discs impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-naphthol. These discs overcome the neccessity of daily preparation of fresh reagent. Gordon and McLeod (1) introduced oxidase test for identifying gonococci based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and a-naphthol. Gaby and Hadley (2) introduced a more sensitive method by using N, N-dimethyl-p-phenylenediamine oxalate where all staphylococci were oxidase negative. In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and a-naphthol to form the dye, indophenol blue.

Quality Control

Appearance

Filter paper discs of 10 mm diameter

Cultural response

Typical oxidase reaction given by 18-48 hour culture observed within 5-10 seconds at 25-30°C.

Organism	Reaction Observed
Pseudomonas aeruginosa ATCC 27853	positive : deep purplish blue colouration of disc

Neisseria gonorrhoeae ATCC 19424	positive : deep purplish blue colouration of disc
Escherichia coli ATCC 25922	negative : purplish blue colouration after 10 sec/ no colour change
Staphylococcus aureus ATCC 25923	negative : no colour change

Storage and Shelf Life

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

Reference

1.Gordon J. and Mcleod J.W., 1928, J. Path. Bact., 31:185
2.Gaby W.L and Hadley C., 1957. J. Bact., 74:356
3.Steel. K.J. 1962. J. Appl. Bact. 25:445

Revision : 1 / 2011

X Factor discs

DD020

Used for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of a Blood Agar (M073) plate or Brain Heart Infusion Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:

Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species.

X-factor discs are the sterile filter paper discs impregnated with growth factor X which are used for differentiating *Haemophilus* species in conjuction of V factor & X+V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium.

The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6 mm.

The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Quality Control

Appearance

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

Cultural response

Cultural characteristics observed on Brain Heart Infusion Agar (M211)or Blood Agar Base (M073) after an incubation of 24-48 hours at 35-37°C.

Cultural Response

Organism	Growth with X factor	Growth without growth factor
Cultural response		
Bordetella pertussis ATCC 8467	Positive(initial isolation on Bordet Gengou Agar (M175))	Positive(initial isolation on Bordet Gengou Agar (M175))
Haemophilus influenzae ATCC 35056	Negative	Negative
Haemophilus parainfluenzae ATCC 7901	Negative	Negative

Haemophilus haemoglobinophilus ATCC19416	Positive	Negative
Haemophilus ducreyi	Positive	Negative

Storage and Shelf Life

Store between 2-8°C. For prolonged use store at -20°C. Use before the expiry date on the label.

Reference

1.Murray PR, Baron EJ, Jorgensen J.H., Pfaller M A, Yolken R.H(Eds.),8th ed, 2003, Manual of Clinical Microbiology, ASM, Washington D.C.

Note:
Use known strains of *Haemophilus influenzae* to monitor the performance of the differentiation discs and the medium.
Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place

Revision : 1 / 2011

V Factor Discs

DD021

Used for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of a Blood Agar (M073) plate or Brain Heart Infusion Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:

Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species.

V-factor discs are the sterile filter paper discs impregnated with growth factor V which are used for differentiating *Haemophilus* species in conjuction of X factor & X+V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium.

The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6 mm.

The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Quality Control

Appearance

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

Cultural response

Cultural characteristics observed on Brain Heart Infusion Agar (M211)or Blood Agar Base (M073) after an incubation of 24-48 hours at 35-37°C.

Organism	Growth with V factor	Growth without growth factor
Bordetella pertussis ATCC 8467	Positive(initial isolation on Bordet Gengou Agar (M175))	Positive(initial isolation on Bordet Gengou Agar (M175))
Haemophilus influenzae ATCC 35056	Negative	Negative
Haemophilus parainfluenzae ATCC 7901	Positive	Negative

Haemophilus haemoglobinophilus ATCC19416	Negative	Negative
Haemophilus ducreyi	Negative	Negative

Storage and Shelf Life

Store below -10°C. Use before the expiry date on the label.

Reference

1.Murray PR, Baron EJ, Jorgensen J.H., Pfaller M A, Yolken R.H(Eds.),8th ed, 2003, Manual of Clinical Microbiology, ASM, Washington D.C.

Note:
Use known strains of *Haemophilus influenzae* to monitor the performance of the differentiation discs and the medium.
Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place

Revision : 1 / 2011

DMACA Indole Discs

DD040

The DMACA Indole Discs are used for Indole test to determine the ability of an organism to split indole from the tryptophan molecule, and thus to aid differentiation between *Escherichia coli* from *Klebsiella.*

Directions

Place the DMACA Indole Disc on suspected colony from HiCrome UTI Agar (M1353) or HiCrome UTI Agar, Modified (M1418) plate. Observe for appearance of blue-purple colour within 10 - 30 seconds.

Principle And Interpretation

In the presence of oxygen, some bacteria are able to split tryptophan into indole and alpha-aminopropionic acid. The presence of indole can be detected by the addition of DMACA (p-Dimethylaminocinnamaldehyde) reagent indicated by formation of bluish-purple colour (1).

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters 'Dm' in continuous printing style.

Cultural response

The indole production by organisms was tested after an incubation of 18-24 hours at 35-37°C, using HiCrome UTI Agar (M1353).

Cultural Response

Organism	**Indole production**
Cultural response	
Escherichia coli ATCC 25922	Positive reaction, blue-purple colour formation
Klebsiella pneumoniae ATCC 13883	Negative reaction.
Pseudomonas aeruginosa ATCC 27853	Negative reaction.

Storage and Shelf Life

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

Reference

1.MacFaddin J. F., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.

Revision : 1 / 2011

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

Co-Trimoxazole Ezy MIC™ Strip (COT) (0.002-32 mcg/ml) EM021

(Trimethoprim/ Sulphamethoxazole) (1:19)

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Co-Trimoxazole in a concentration gradient manner, capable of showing MICs in the range of 0.002 mcg/ml to 32 mcg/ml, on testing against the test organism.

Introduction

Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1, 3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1, 3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).
Also, direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland. This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, *Bacteroides* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood. For *Haemophilus* spp, Haemophilus Test Agar Base (M1259) with added supplement (FD117) is to be used.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate pre-spread with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bacteriostatic drugs such Trimethoprim/ sulphamethoxazole (Co-Trimoxazole), Tetracycline, Azithromycin, Fluconazole, Linezolid and, Chloramphenicol read MICs at 80% inhibition for homogenously sensitive strains such as QC control strains.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values "in-between" two fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Co-Trimoxazole showing reading of 0.75 mcg/ml should be rounded up to next concentration ie. 1.0 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL:

Interpretation

Table 1 : Use following interpretive criteria for susceptibility categorization.

When testing	Incubation	Interpretative Criteria		
		≤ S	I	≥ R
Enterobacterales (Excluding *Salmonella/Shigella* spp), *Acinetobacter* spp, *B. cepacia, S. maltophila,* Other Non-*Enterobacterales, Staphylococcus* spp, *Salmonella* and *Shigella* spp	35-37°C for 18 hrs.	2	-	4
Haemophilus spp, *S. pneumoniae*	35-37°C for 20-24 hrs with 5% CO_2	0.5	1-2	4
N. meningitidis	35-37°C for 20-24 hrs with 5% CO_2	0.12	0.25	0.5

Quality control

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC Cultures recommended by CLSI on suitable medium incubated appropriately.
Table 2 : Following are the reference MIC values (mcg/ml) range for Co-Trimoxazole.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
E.coli ATCC 25922	Mueller Hinton Agar	35-37°C for 18 hrs.	≤ 0.5
S.aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	≤ 0.5
P.aeruginosa ATCC 27853	Mueller Hinton Agar	35-37°C for 18 hrs.	8-32
E.faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	≤ 0.5
H. influenzae ATCC 49247	Haemophilus Test Medium	35-37°C for 20-24 hrs with 5% CO_2	0.032 - 0.064 - 0.12 - 0.25
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24 hrs with 5% CO_2	0.125 - 0.25 - 0.5 - 1.0

Storage & Shelf Life:

1. Once the consignment is received, store applicators at room temperature and Ezy MIC™ Strip container at 2-8°C, for prolonged use store below -20°C or below.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
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. Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.

Packing:

Each Pack contains following material packed in air-tight plastic container with a desiccator capsule.
1. Co-Trimoxazole Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2. Applicator sticks
3. Package insert

Revision: 05/2024

CE

On receipt store at -20°C 8°C

IVD In vitro diagnostic medical device

Plot No. C-40,
Road No. 21Y, MIDC,
Wagle Industrial Estate,
Thane (W) - 400604,
Maharashtra, India

Indicates a single sterile barrier system with protective packaging outside

Do not re-use

EC REP CEpartner4U,
ESDOORNLAAN 13,
3951DB MAARN, NL
www.cepartner 4u.eu

Do not use if package is damaged

Penicillin Ezy MIC™ Strip (PEN) (0.002-32 mcg/ml) EM084

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Penicillin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.002 mcg/ml to 32 mcg/ml, on testing against the test organism.

Introduction:
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4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1, 3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1, 3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).
Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland. This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood and for *Neisseria gonorrhoeae*, GC Agar Base (M434) with 1% defined growth supplement (FD025) is recommended. For *B. fragilis*, Brucella Agar with Hemin and Vitamin K1, supplemented with 5 % v/v defibrinated sterile sheep blood is recommended.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self-sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bactericidal drugs such as Penicillin and other members of β-lactams class of drugs, Amikacin, Vancomycin, Gentamicin, always read the MIC at the point of complete inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values “in-between” two-fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Penicillin showing reading of 0.75 mcg/ml should be rounded up to next concentration ie. 1.0 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL:

Interpretation:
Table 1: Use following interpretive criteria for susceptibility categorization.

When testing	Incubation	Interpretive Criteria		
		≤ S	I	≥ R
Staphylococcus spp	35-37°C for 18 hrs.	0.12	-	0.25
Enterococcus spp	35-37°C for 18 hrs.	8	-	16
S.pneumoniae (Non Meningitis) (Parenteral)	35-37°C for 20-24hrs with 5% CO_2	2	4	8
S.pneumoniae (Meningitis) (Parenteral)	35-37°C for 20-24hrs with 5% CO_2	0.06	-	0.12
Streptococcus spp. Beta haemolytic group	35-37°C for 20-24hrs with 5% CO_2	0.12	-	-
Streptococcus spp. Viridans group	35-37°C for 20-24hrs with 5% CO_2	0.12	0.25-2	4
N. gonorrhoeae, S. pneumoniae (Oral)	35-37°C for 20-24hrs with 5% CO_2	0.06	0.12-1	2
N. meningitidis	35-37°C for 20-24hrs with 5% CO_2	0.06	0.12-0.25	0.5
Anaerobes	35-37°C for 24-48hrs under anaerobic condition.	0.5	1	2

Quality control

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC cultures recommended by CLSI on suitable medium incubated appropriately.
Table 2: Following are the reference MIC values (mcg/ml) range for Penicillin

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S. aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25-0.5-1.0-2.0
E. faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	1.0 - 2.0 - 4.0
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24hrs at 5% CO_2	0.25 - 0.5 - 1.0
Neisseria gonorrhoeae ATCC 49226	GC Agar Base (M434) with 1% defined growth supplement (FD025)	35-37°C for 20-24hrs at 5% CO_2	0.25 - 0.5 - 1.0
B.fragilis ATCC 25285	Brucella Agar with Hemin and Vitamin K1, supplemented with 5 % v/v defibrinated sterile sheep blood	35-37°C for 24-48 hrs under strict anaerobic condition	8.0 – 16.0 – 32.0

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at -20°C or below.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
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. Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.

Packing:

Each Pack contains following material packed in sealed glass vial with a desiccator capsule.

1) Penicillin Ezy MIC™ strip (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package Insert

Revision: 05/2024

CE

On receipt store at -20°C

IVD In vitro diagnostic medical device

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Indicates a single sterile barrier system

Do not re-use

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Do not use if package is damaged

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

FD045/FD045L

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

Composition

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

Directions:

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

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

OR

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

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

OR

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

OR

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

OR

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

OR

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

OR

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

OR

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

OR

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

Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - faeces, urine etc. ; Food samples

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

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

Reference

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

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

2°C 8°C **Storage temperature**

CE **CE Marking**

Do not use if package is damaged

Technical Data

Tinsdale Selective Supplement (Part A & Part B) FD073

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

Composition

Per vial sufficient for 1000 ml medium

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

Directions:

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

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

Type of specimen

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

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

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

Reference

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

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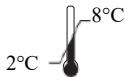
CE Marking



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Technical Data

Neo30 Selective Supplement FD149

An antibiotic supplement recommended for selective isolation of *Streptococcus* species.

Composition

Per vial sufficient for 940 ml medium

*Ingredients	Concentration
Neomycin sulphate	30mg

Directions:

Rehydrate the contents of 1 vial aseptically with 10 ml of sterile distilled water. Mix gently to dissolve. Aseptically add the contents to 940 ml of sterile, molten, cooled (45-50ºC) Anaerobic Blood Agar Base M1345 along with 50 ml of sterile defibrinated sheep blood. Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - Throat samples, skin samples, urine, vaginal or rectal swab etc.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

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

Reference

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

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

CE **CE Marking**

2°C – 8°C **Storage temperature**

Do not use if package is damaged

U5 Supplement

FD157

Filter sterilized urea solution recommended for the isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis*.

Composition

Per vial sufficient for 425 ml medium

Ingredients	Concentration
Urea	0.250g
Distilled water	5ml
Final pH (at 25°C)	8.0±0.2

Directions:

Warm up the refrigerated Urea solution to room temperature and aseptically add 1 vial to 425 ml of sterile, molten, cooled (45-50ºC) Mycoplasma Urogenital Broth Base M1374 along with 1 vial of Vitamino Growth Supplement FD025 and 1 vial of PAN Selective Supplement FD175 and 50 ml of Horse Serum RM1239. Mix well and dispense as desired.

Type of specimen

Isolated microorganism from clinical, food and water samples.

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

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

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1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

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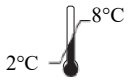

Storage temperature

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AzCe Selective Supplement

FD226

Recommended to differentiate *Enterococcus faecium* from *Enterococcus faecalis*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cephalexin	25mg
Aztreonam	37.500mg

Directions:

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500 ml of sterile, molten, cooled (45-50°C) Arabinose Agar Base M1576/ HiCrome™ Enterococcus faecium Agar Base M1580 /HiCrome™ Enterococccus Faecium HiVeg™ Agar Base MV1580/ HiCrome™ Enterococcus faecium HiCynth™ Agar Base MCD1580. Mix well and pour into sterile petri plates

Type of specimen

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

Specimen Collection and Handling

For Food & Water samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterili ed by autocla ing before discarding.

Warning & Precautions

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

Storage and Shelf Life

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

Disposal

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

Reference

1. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
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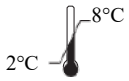
CE Marking



Storage temperature

Do not use if package is damaged

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Technical Data

Coagulase Plasma (0.1gm per vial) FD248

It is recommended for studying coagulase reaction in diagnosis of Staphylococci.

Composition

Per vial sufficient for 6 tests medium

Ingredients	Concentration
Coagulase Plasma	0.100g

Directions:

Rehydrate the contents of one vial aseptically with 3 ml sterile distilled water. Add 0.5 ml of rehydrated FD248 in a tube. To this add approximately 0.05 ml of overnight broth culture of test organisms or 2-3 pure colonies picked from a non-inhibitory agar plate. Mix gently & incubate at 37°C in incubator or water bath for up to 4 hours. Observe for clot formation in the tube at regular intervals. Any degree of clotting within 4 hours is considered as positive results.

Type of specimen

Clinical- skin, throat samples etc; Food samples

Specimen Collection and Handling

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

Warning & Precautions

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

Storage and Shelf Life

Store at 2-8°C. For unopened vial, use before the expiry date on the label. The rehydrated solution can be stored for up to 2 weeks at 2-8°C

Disposal

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

Reference

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

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2°C 8°C **Storage temperature**

CE Marking

Do not use if package is damaged

Sabouraud Dextrose Agar, Granulated

GM063

Intended Use:

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

Composition**

Ingredients	g / L
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 (1) of the formulation described by is a modification of Sabouraud Dextrose Agar which is described by Sabouraud (2) 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 (3). 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 (4).

Type of specimen

Food and dairy samples ; Clinical samples: skin scrapings

Specimen Collection and Handling

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

Warning and Precautions:

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

Limitations

1. 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 coloured granular media.
Gelling
Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium
Light yellow to 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
Cultural response was observed after an incubation at 20-25°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 (8,9).

Reference

1.Carlier G. I. M., 1948, Brit. J. Derm. Syph., 60:61.
2.Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061.
3.Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
4. Murray PR, Baren EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors) 2003, Manual of clinical Microbiology, 8th ed., ASM, Washington, D.C.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.8.
8.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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Revision : 05/2024

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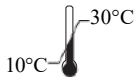

Storage temperature

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CE **CE Marking**

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Lactobacillus MRS Agar (MRS Agar), Granulated

GM641

Intended use

Recommended for isolation and cultivation of all *Lactobacillus* species.

Composition**

Ingredients	g / L
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 media are based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (2), dairy products (3), foods (2), faeces (4,5) and other sources (6).

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. Lactobacilli isolated on MRS Agar should be further confirmed biochemically.

Type of specimen

Clinical samples - urine, faeces, etc.; Food and dairy 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 (2,7,8).

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

Warning and Precautions :

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

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further Biochemical and serological testing is required for complete identification.

Performance and Evaluation

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

Quality Control

Appearance

Cream to light yellow coloured granular medium.

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Medium to dark amber coloured 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 rhamnosus ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
^*Lactobacillus delbrueckii* subsp. *lactis ATCC 7830*	50-100	luxuriant	>=50%
# *Lactiplantibacillus plantarum ATCC 8014*	50-100	luxuriant	>=50%
Lactobacillus saki ATCC 15521(00015)*	50-100	luxuriant	>=50%
Lactobacillus lactis ATCC 19435(00016)*	50-100	luxuriant	>=50%
Pediococcus pentosaceas ATCC 33316 (00158)*	50-100	luxuriant	>=50%

Key: (*) Corresponding WDCM numbers.

#Formerly known as *Lactobacillus plantarum*

^ Formerly known as *Lactobacillus leichmannii*

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 (4,5).

References

1. deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.
2. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
3. Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th 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. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, 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 : 06/2024

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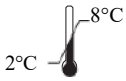

Storage temperature

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Technical Data

HiCombi™ Dual Performance Medium

LQ012

Intended use

Recommended for rapid growth of *Enterobacteria, Pseudomonas*, Staphylococci and *Candida*. Combination of solid (20ml) and liquid (40ml) media in single bottle.

Composition**

Ingredients	g / L
Solid	20.000 ml
HM infusion powder #	12.500
BHI powder	5.000
Proteose peptone	10.000
Dextrose (Glucose)	2.000
Sodium chloride	5.000
Disodium hydrogen phosphate	2.500
Agar	15.000
Liquid	40.000 ml

Same as solid media without Agar

**Formula adjusted, standardized to suit performance parameters
Equivalent to Calf brain infusion from

Directions

Label the ready to use blood culture bottle. Do not unscrew the cap. Remove the top seal of the cap. Disinfect the part of the rubber stopper which is now exposed. Draw patient's blood with the sterile or disposable needle and syringe. Transfer the blood sample immediately into the culture bottle by puncturing the rubber stopper with the needle and injecting the blood. Incubate the bottle for 4-6 hours at 30 -35°C. For adsorption on solid surface. DO NOT SHAKE OR HOLD MORE THAN 15 SECONDS. Revert into an upright position and incubate for 18-24 hours at 30-35°C or longer if necessary. Venting: Use sterile venting needle (LA038). Keep the bottle in an upright position preferably in a biological safety cabinet, place an alcohol swab over the rubber stopper and insert the venting needle with filter through it. Insertion and withdrawal of the needle should be done in a straight line. discard the needle and mix the contents by gently inverting the bottle 2-3 times. Do Not vent the bottle for anaerobic cultures. Incubate at 30-35°C for 18-24 hours and further for seven days. Recommended volume of blood to be tested in LQ012: 8-10 ml (For Adult use).

Principle And Interpretation

BHI Medium is useful for cultivating a wide variety of microorganisms since it is a highly nutritive medium. It is also used to prepare the inocula for antimicrobial susceptibility testing. BHI Broth is a modification of the original formulation of Rosenow, where he added pieces of brain tissues to dextrose broth (1). BHI Broth is also the preferred medium for anaerobic bacteria, yeasts and moulds (2,3). This medium is nutritious and well buffered to support the growth of wide variety of organisms (1,4,5). With the addition of 10% defibrinated sheep blood, it is useful for isolation and cultivation of *Histoplasma capsulatum* (6) and other fungi. For selective isolation of fungi, addition of gentamicin and/or chloramphenicol is recommended (7).

Proteose peptone, HM infusion powder and BHI powder serve as sources of carbon, nitrogen, essential growth factors, amino acids and vitamins. Dextrose serves as a source of energy. Disodium phosphate helps in maintaining the buffering action of the medium whereas sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples : Blood

Specimen Collection and Handling

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

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling

specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

In a sterile glass bottle combination of broth and one agar coated surface.

Colour of Agar medium
Yellow coloured media

Colour of liquid medium
Amber coloured solution

Quantity of medium

20ml of medium in glass bottle 40ml of medium in glass bottle

pH of Agar medium
7.20- 7.60

pH of liquid medium
7.20- 7.60

Sterility Check

Passes release criteria

Cultural response

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

Organism	Inoculum (CFU)	Growth on agar medium	Growth on liquid medium
Candida albicans ATCC 10231 (00054*)	50-100	Luxuriant	Luxuriant
Haemophilus influenzae ATCC 19418	50-100	Luxuriant	Luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	Luxuriant	Luxuriant
Streptococcus pyogenes ATCC 19615	50-100	Luxuriant	Luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	Luxuriant	Luxuriant
Neisseria meningitidis ATCC 13090	50-100	Luxuriant	Luxuriant
Streptococcus pneumoniae ATCC 6303	50-100	Luxuriant	Luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 15-30°C. 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 (8,9).

Reference

1.Rosenow, 1919, J. Dental Research, 1:205.
2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
3.Atlas R. M., 1993, Handbook of Microbiological Media, 147-153, CRC Press, Boca Raton, FL.
4.Roseburg T. et al, 1944, J. Inf. Dis., 74:
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6.Howard B., Keiser J. F., Weissfeld A. et al, 1994, Clinical and Pathogenic Microbiology, 2nd Ed., Mosby Co.
7.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology,8th Ed., American Society for Microbiology, Washington, D.C.
8.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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Revision : 03/2023

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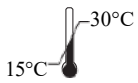

Storage temperature

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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

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

Principle And Interpretation

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

Type of specimen

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

Specimen Collection and Handling:

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

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

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

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.20-7.60

Cultural Response

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

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

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1.Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
2.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins,Baltimore.
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8.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 07/2024

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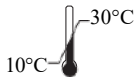

Storage temperature

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Do not use if package is damaged

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Nutrient Broth

M002

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

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

Principle And Interpretation

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

Type of specimen

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

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

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

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

pH
7.20-7.60

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
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4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C
5. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 11/2024

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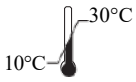

Storage temperature

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Fluid Thioglycollate medium (Thioglycollate medium Fluid) M009

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.75 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 25°C and store in a cool dark place preferably below 25°C. Note : If more than the upper one-third of the medium has acquired a pink-purple colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink-purple colour disappears.

Principle And Interpretation

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

Type of specimen

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

Specimen Collection and Handling:

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

Warning and Precautions

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

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

Reaction

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

pH

6.90-7.30

Growth Promotion Test

As per USP/EP/BP/IP

Growth promoting properties

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

Sterility Testing + Validation

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

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

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

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

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

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

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

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

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

Disposal

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

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598.
2. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
3. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
4. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022
5. Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC,Washington, D.C
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10. Marshall, Gunnison and Luxen, 1940, Proc. Soc. Exp. Biol. Med., 43:672.
11. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
12. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
13. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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IVD *In vitro* diagnostic medical device

10°C–30°C Storage temperature

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CE CE Marking

Do not use if package is damaged

Triple Sugar Iron Agar

M021

Intended Use:

Recommended for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

Composition**

Ingredients	g / L
Peptone	10.000
Tryptone	10.000
Yeast extract	3.000
HM Peptone B#	3.000
Lactose	10.000
Sucrose	10.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Ferrous sulphate	0.200
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 64.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt about 1 inch long.

Note: For better results, the medium can be sterilized by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Principle And Interpretation

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett (1) and modified by Hajna (2) for identifying *Enterobacteriaceae*. This medium complies with the recommendation of APHA, for the examination of meat and food products (3), for the examination of milk and dairy products (4) and for microbial limit test for confirming the presence of *Salmonellae* (5,6) and in the identification of gram-negative bacilli (5,7).

Tryptone, peptone, yeast extract and HM peptone B provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous ions make H_2S indicator system. Phenol red is the pH indicator. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amount of acids are liberated in butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amounts of acid present in the butt. Thus the appearance of an alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to glucose, produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO_2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H_2S combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube. Triple Sugar Iron Agar should be used in parallel with Urea Agar/Broth (M112/M111) to distinguish between *Salmonella* and *Proteus* species. The reactions can be summarized as follows:

Alkaline slant / acid butt-only glucose fermented
Acid slant / acid butt-glucose and sucrose fermented or glucose and lactose fermented or all the three sugars, glucose, lactose and sucrose fermented.
Bubbles or cracks present-gas production
Black precipitate present-H_2S gas production

Type of specimen
Pure bacterial isolate from water, food, or clinical sample.

Specimen Collection and Handling:
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

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

Limitations :
1.Some members of the *Enterobacteriaceae* and H_2S producing *Salmonella* may not be H_2S positive on TSI Agar. Some bacteria may show H_2S production on Kligler Iron Agar but not on TSI Agar. This can happen because utilization of sucrose in TSI Agar suppresses the enzymic pathway that result in H_2S production.

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 1.2% Agar gel.
Colour and Clarity of prepared medium
Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.
Reaction
Reaction of 6.45% w/v aqueous solution at 25°C. pH : 7.4±0.2
pH
7.20-7.60
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H_2S
Citrobacter freundii ATCC 8090	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

$ Proteus hauseri ATCC 13315	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
Salmonella Paratyphi A ATCC 9150	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Salmonella Typhi ATCC 6539	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative, no blackening of medium
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

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

Storage and Shelf Life

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

Disposal

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

Reference

1.Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
2.Hajna A.A., 1945, J. Bacteriol, 49:516.
3.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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8.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

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

Revision : 04/2024

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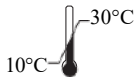

Storage temperature

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Bismuth Sulphite Agar (BS)

M027

Intended Use:

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

Composition**

ISO 6579-1 Specification -Bismuth Sulphite Agar

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

Bismuth Sulphite Agar

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

**Formula adjusted, standardized to suit performance parameters

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

Directions

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

Principle And Interpretation

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

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

Type of specimen

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

Specimen Collection and Handling

Processesing : (8)

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

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTT broth (M1496I) and incubated at 37± 1°C for 24 ± 3 hours. In-case of *Salmonella* Typhi and *Salmonella* Paratyphi A selective enrichment is carried out in Selenite Cystine broth and then incubated at 37± 1°C for 24 h ± 3 h and 48 h ± 3 h.

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

Confirmation : Biochemical and serological tests are performed for confirmation.

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,9).

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.50-7.90

Cultural Response

Cultural response was observed after an incubation at 37 ± 1°C for 24 ± 3 hours. The plates are further incubated for an additional 24 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Productivity				
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Selectivity & Specificity				
Escherichia coli ATCC 8739 (00012*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen
Escherichia coli ATCC 25922 (00013*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (7, 9).

Reference

1. Tindall B. J., Crimont P. A. D., Gorrity G. M., EUZESY B. P., 2005, Int. J. Sys. Evol. Microbiol., 55:521.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Wilson and Blair, 1926, J. Pathol. Bateriol., 29:310.
4. Wilson and Blair, 1927, J. Hyg., 26:374.
5. Wilson and Blair, 1931, J. Hyg., 31:138.
6. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
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8. Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of *Salmonella*- Part I Detection of *Salmonella* . International Organization for Standardization (ISO), ISO/ DIS 6579-1:2017.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

Revision : 02/2024

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IVD *In vitro* diagnostic medical device

10°C–30°C Storage temperature

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

CE CE Marking

Do not use if package is damaged

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

Kligler Iron Agar

M078

Intended Use:

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

Composition**

Ingredients	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 tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1 inch butts. Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

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

Principle And Interpretation

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

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

Type of specimen

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

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Red coloured, clear to slightly opalescent gel forms in tubes as slants
Reaction
Reaction of 5.75% w/v aqueous solution at 25°C. pH : 7.4±0.2
pH
7.20-7.60
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18 - 48 hours.

Organism	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. Product performance is best if used within stated expiry period.

Disposal

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

Reference

1.Kligler I. J., 1917, Am. J. Publ. Health, 7:1041.
2.Kligler I. J., 1918, J. Exp. Med., 28:319.
3.Russell F. F., 1911, J. Med. Res., 25:217.
4.Bailey S. F. and Lacey G. R., 1927, J. Bacteriol., 13:183.
5.Ewing, 1986, Edwards and Ewings Identification of the Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., N.Y.
6.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
7.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
8.Salfinger Y., and Tortorello M.L., 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.
10.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
11.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1

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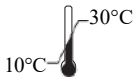
IVD ***In vitro* diagnostic medical device**



Storage temperature

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

CE **CE Marking**

Do not use if package is damaged

Disclaimer :

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Simmons Citrate Agar

M099

Intended Use:

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

Composition**

Ingredients	g / L
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 (1) 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 (2) modified Kosers formulation by adding agar and bromothymol blue . 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 water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5,6).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1.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	Growth	Citrate utilisation
# *Klebsiella aerogenes* ATCC 13048 (00175*)	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922 (00013*)	inhibited	negative reaction, green colour
Salmonella Typhi ATCC 6539	fair-good	negative reaction, green colour
Salmonella Typhimurium ATCC 14028 (00031*)	good-luxuriant	positive reaction, blue colour
Shigella dysenteriae ATCC 13313	inhibited	negative reaction, green colour
Salmonella Enteritidis ATCC 13076 (00030*)	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 (7,8).

Reference

1. Koser, 1923, J. Bact., 8:493.
2. Simmons, 1926, J. Infect. Dis., 39:209.
3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

4.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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8.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05/2024

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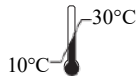
IVD ***In vitro* diagnostic medical device**



Storage temperature

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

**Formula adjusted, standardized to suit performance parameters
- Equivalent to Beef extract

Directions

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

Principle And Interpretation

SS Agar medium is recommended as differential and selective medium for the isolation of *Salmonella* and *Shigella* species from pathological specimens (1) and suspected foodstuffs (2,3,4,5) and for microbial limit test (6). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

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

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

Type of specimen

Clinical: faeces, rectal swabs; Suspected food stuffs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,3,4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

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

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 6.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

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

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

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

Storage and Shelf Life

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

Disposal

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

Reference

1. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed., ASM, Washington, D.C.
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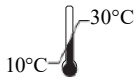

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Columbia Blood Agar Base

M144

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.0 grams of in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding heat sensitive compounds.
For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.
For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.
The medium can be made selective by adding different antimicrobials to sterile base.
For *Brucella* species: Add rehydrated contents of 1 vial of NPBCVN Selective Supplement (FD005) to 500 ml sterile molten base.
For *Campylobacter* species: Add rehydrated contents of 1 vial of Blaser-Wang Selective Supplement (FD006) or Butzler Selective Supplement (FD007) or Skirrow Selective Supplement (FD008) or VTCA Selective Supplement (FD090) or Butzler VI Selective Supplement (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Minerals Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.
For *Gardnerella* species: Add rehydrated contents of 1 vial of GNA Selective Supplement (FD056) to 500 ml sterile molten base.
For Cocci: Add rehydrated contents of 1 vial of NC Selective Supplement (FD030) or NNP Selective Supplement (FD031) or CO Selective Supplement (FD119) to 500 ml sterile molten base.

Principle And Interpretation

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

Type of specimen

Clinical samples : throat swabs, pus.

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.10-7.50

Cultural Response

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

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

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
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5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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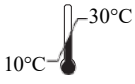

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Mueller Hinton Agar

M173

Intended Use:

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

Composition**

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

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

Directions

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

Principle And Interpretation

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

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

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

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

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

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

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

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

Type of specimen

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

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.20-7.40

Cultural Response

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

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

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

Antibiotic Sensitivity test

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

Thymine/Thymidine Content

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

Divalent Cation Content

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

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

References

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11.ISO/TS 16782:2016, Confirmed in 2021 Clinical laboratory testing - Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing
12.European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 14.0, valid from 2024-01-01.
13.European Committee on Antimicrobial Susceptibility Testing Routine and extended internal quality control as recommended by EUCAST Version 14.0, valid from 2024-01-01.

Revision : 06/2024

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

10°C–30°C **Storage temperature**

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Anaerobic Agar

M228

Intended Use:

Recommended for the cultivation of anaerobic bacteria, especially *Clostridium* species and other anaerobic organisms from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Tryptone	20.000
Dextrose (Glucose)	10.000
Sodium chloride	5.000
Sodium thioglycollate	2.000
Sodium formaldehyde Sulfoxylate	1.000
Methylene blue	0.002
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58.0 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

Anaerobic Agar was originally designed for surface cultivation of members of the genus *Clostridium* and other anaerobic organisms on plates (1). This medium is suitable for isolation of facultative and obligate anaerobes and for the study of colonial morphology as colonies can be readily seen on the light coloured agar and are easily accessible (2,3). Anaerobic bacteria vary in their sensitivity to oxygen and nutritional requirements (3). Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor (4).

This medium contains sodium thioglycollate and sodium formaldehyde sulphoxylate that provide adequate anaerobiosis which is indicated by methylene blue present in the medium which yields blue colour to medium in presence of oxygen. Tryptone and dextrose provide essential nutrients while sodium chloride maintains osmotic equilibrium.

Dispense 50-60 ml medium per 95 x 20 mm plate. For best results, use porous tops for the plates during solidification to get the dry surface. Inoculation can be done by streaking or smearing. Cover the inoculated plate with sterile Brewer Anaerobic Petri dish cover. Incubate aerobically, as desired. When standard plates are used, dispense 0.1 to 1.0 ml of inoculum into plates and mix with 20 - 25 ml of sterile medium. After solidification, incubate anaerobically as required by particular organism under study. Methylene blue is inhibitory to some anaerobic microorganisms.

Type of specimen

Clinical- stool, abscess

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

1.Ensure that the clinical samples are properly transported under anaerobic conditions.
2.Proper anaerobic conditions must be maintained for optimal recovery of organisms
3.Methylene blue is toxic to certain anaerobes.
4.Further biochemical and serological tests must be performed for confirmation.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in Petri plates that becomes greenish due to aeration on standing

Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%
Clostridium butyricum ATCC 13732	50-100	good-luxuriant	>=50%

Storage and Shelf Life

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

Disposal

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

Reference

1. Brewer J. H., 1942, Science, 95:587.
2. Vera J., 1942, J. Bacteriol., 44:497.
3. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
4. Baron E. J., Peterson and Finegold S. M., Bailey & Scotts Diagnostic Microbiology, 9th Ed., 1994, Mosby-Year Book, Inc., St. Louis, Mo.
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IVD *In vitro* diagnostic medical device

30°C / 10°C Storage temperature

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CE CE Marking

Do not use if package is damaged

Tinsdale Agar Base

M314

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

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

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

C. diphtheriae forms grayish black colonies surrounded by a dark brown halo while diphtheroids commonly found in the upper respiratory tract do not form such colonies. Dark brown halo around the colony is due to H_2S production from cystine combining with the tellurite salt. Moore and Parsons (3) found Tinsdale Medium as an ideal medium for the routine cultivation and isolation of *C. diphtheriae*. They also confirmed the stability of halo formation on clear medium and its specificity for *C. diphtheriae* and *C. ulcerans*. *C. ulcerans* found in nasopharynx form colonies same as *C. diphtheriae* and require further biochemical confirmation (5).

Do not incubate the plates in 5-10% CO_2 as it retards the development of characteristic halos (6). Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C. diphtheriae* (1). *C. ulcerans* , *C. pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar (1).Several organisms may exhibit slight browning on Tinsdale Agar in 18 hours; therefore the plates should be read after complete incubation period (48 hours) (1).

Type of specimen

Clinical samples - Throat swab

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.20-7.60

Cultural Response

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Isenberg, (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.
2. Billings E., 1956, An investigation of Tinsdale Tellurite Medium: its usefulness and mechanisms of halo-formation, M.S. thesis, University of Michigan, Ann Arbor, Mich.
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5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
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IVD ***In vitro* diagnostic medical device**

CE Marking

10°C – 30°C **Storage temperature**

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Candida BCG Agar Base

M355

Intended Use:

Recommended for primary isolation and identification of *Candida* species.

Composition**

Ingredients	g / L
Peptone	10.000
Yeast extract	1.000
Dextrose (Glucose)	40.000
Bromocresol green	0.020
Agar	15.000
Final pH (at 25°C)	6.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 66.02 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 and add sterile neomycin to a concentration of 500 µg/ml of medium. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Candida albicans is most frequently isolated from clinical specimens. Species of *Candida*, other than *C. albicans* are normal flora of cutaneous and mucocutaneous surfaces and are only rarely incriminated as agents of clinical disease (1). Of the many media used for isolating and differentiating *Candida*, Pagano Levin Base (M1390) employes TTC (Triphenyl Tetrazolium Chloride) as an indicator. Harold and Snyder (2) observed that the TTC used greatly retards the growth of some *Candida* species, while completely inhibiting the rest. Therefore to overcome this difficulty, they formulated Candida BCG Agar, which employs bromocresol green instead of TTC as the indicator.

Candida BCG Agar Base is used to obtain pure yeast colonies from mixed cultures on the basis of colony morphology (3, 4). Peptone along with yeast extract and dextrose serve as sources of essential nutrients, amino acids and vitamins. Dextrose also serves as a source of energy by being the fermentable carbohydrate. Bromocresol green is non-toxic indicator incorporated to visualize the fermentation reaction. Selectivity is obtained by the addition of neomycin. Neomycin is incorporated to inhibit gram-negative bacteria and some gram-positive bacteria. Neomycin is an aminoglycoside antibiotic that is active against aerobic and facultatively anaerobic gram-negative bacteria and certain gram-positive bacteria. Bromocresol green is the indicator. Acid production due to fermentation lowers the pH of the medium and subsequently the colour of medium changes to yellow, indicated by yellow zones around the dextrose-fermenting colonies. *C.albicans* appears as blunt conical colonies with smooth edges and yellow to blue green colour. Other *Candida* species appear as smooth to rough colonies, with either convex or cone shaped colonies (5). Standard methods should be followed for inoculating the plates of Candida BCG Agar.

Presumptive *Candida* colonies should be further identified by gram staining, biochemical and serological testing (6,7,8).

Type of specimen

Clinical samples - skin scraping from the infected body site.

Specimen Collection and Handling:

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

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

Warning and Precautions :

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

Limitations:

1.Standard methods should be followed for inoculating the plates of Candida BCG Agar.

2.Presumptive Candida colonies should be further identified by gram staining, biochemical and serological testing(6,7,8).

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 light green homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Bluish green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.6% w/v aqueous solution at 25°C. pH : 6.1±0.2

pH

5.90-6.30

Cultural Response

Cultural characteristics observed with added sterile Neomycin (500 mcg/ml of medium) after an incubation at 25-30°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of medium
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%	yellow
Candida glabrata ATCC 15126	50-100	good-luxuriant	>=50%	yellow
Candida kruisei ATCC 24408	50-100	good-luxuriant	>=50%	yellow
Candida tropicalis ATCC 1369	50-100	good-luxuriant	>50%	yellow
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Konemann E. W., Allen S. D., Janda M. W., Schreckenberger P.C, Winn C. W. Jr., 1992, Colour Atlas and Text book of Diagnostic Microbiology,4th Ed. J. B. Lippincott Company.
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3. Haley L. D., and Callaway C. S., 1978, Laboratory Methods in Medical Mycology, 4th Ed., U.S. Government Printing Office, Washington, D.C.
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9.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
10.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision :04 / 2024

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

CE Marking

10°C – 30°C **Storage temperature**

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Lactobacillus MRS Broth (MRS Broth)

M369

Intended Use

Recommended for cultivation of Lactobacilli from clinical and non-clinical samples.

Composition**

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

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef Extract

Directions

Suspend 55.15 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes, bottles or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Lactobacilli MRS media are based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all *Lactobacilli* from oral cavity (1) dairy products (2,3), faeces (4,5) and other sources (6).

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.

Type of specimen

Clinical samples - faeces, swab from oral cavity; Food and dairy 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 (2,7,8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder, having tendency to form soft lumps which can be easily broken down to powder form

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent solution in tubes

Reaction

Reaction of 5.51% 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% CO_2)

Organism	Inoculum (CFU)	Growth
Lactobacillus fermentum ATCC 9338	50-100	luxuriant
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant
Lactobacillus plantarum ATCC 8014	50-100	luxuriant
Lactobacillus casei ATCC 9595	50-100	luxuriant
Lactobacillus saki ATCC 15521 (00015*)	50-100	luxuriant
Lactobacillus lactis ATCC 19435 (00016*)	50-100	luxuriant
Pediococcus pentosaceas ATCC 33316 (00158*)	50-100	luxuriant

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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3. Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington,D.C.
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2°C – 8°C **Storage temperature**

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Bile Esculin Azide Agar

M493

Intended Use:

For selective isolation and presumptive identification of faecal Streptococci.

Composition**

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

Equivalent to Beef extract ## - Equivalent to Oxgall

**Formula adjusted, standardized to suit performance parameters

Directions

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

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

Principle And Interpretation

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

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

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

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

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

Type of specimen

Clinical- Faeces, Food samples

Specimen Collection and Handling:

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

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
2. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
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IVD *In vitro* diagnostic medical device

30°C
10°C
Storage temperature

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CE **CE Marking**

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HIMEDIA

Technical Data

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

Principle And Interpretation

Perry and Miller (1) reported that *Candida albicans* produces an enzyme b -N-acetyl- galactosaminidase and according to Rousselle et al (2) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of *C.albicans* isolates directly on primary isolation. HiCrome™ Candida Differential Agar 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; Food & dairy samples

Specimen Collection and Handling

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

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

Warning and Precautions

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

Limitations

1. 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 (3,4).

References

1. Perry J. L. and Miller G. R., 1987, J. Clin. Microbiol., 25: 2424 -2425.

2. Rousselle P., Freydiere A., Couillerot P., de Montclos H. and GilleY., 1994, J. Clin. Microbiol. 32:3034-3036.

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

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

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

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

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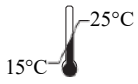

Storage temperature

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Anaerobic Blood Agar Base

M1345

Intended Use:

Recommended for isolation and cultivation of Group A and Group B Streptococci from throat cultures and other clinical samples.

Composition**

Ingredients	g / L
Tryptone	14.500
Soya peptone	5.000
Sodium chloride	5.000
Growth Factors	1.500
Agar	14.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40 grams in 990 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure(121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Neomycin Supplement (FD149), and 5% v/v sterile defibrinated sheep blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Group B streptococcus (GBS) infection is a common bacterial infection that is rarely serious in adults, but can be life-threatening to newborns. Group A Streptococci commonly causes strep throat and rarely, a potentially deadly destruction of flesh. Anaerobic Blood Agar Base with Neomycin Supplement is used for the isolation of Group A and Group B Streptococci from clinical specimens (1). This medium was originally formulated by Blanchette and Lawrence (2), by addition of the antibiotic Neomycin to sheep blood agar. This addition improved the detection of Group A & B Streptococci, while inhibiting the growth of the other accompanying haemolytic organisms.

Tryptone and soya peptone in the medium provide carbon and nitrogenous compounds, long chain amino acids, vitamins and other essential growth nutrients. Growth factors and defibrinated sheep blood together supply enrichment for growth of fastidious organisms. Sodium chloride helps in maintaining the osmotic equilibrium. Addition of Neo Selective Supplement (FD149) helps to suppress the normal flora thereby enhancing recovery of Group A and Group B Streptococci.

Type of specimen

Clinical samples - Throat samples, skin samples, urine, vaginal or rectal swab etc.

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 Precaution

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

Limitation

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 1.4% Agar gel.

Colour and Clarity of prepared medium

Basal medium : Yellow coloured clear to slightly opalescent gel.

After addition of 5%v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates

Reaction

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

pH

7.10-7.50

Cultural Response

Cultural characteristics observed in presence of 5-10% CO2 with added 5%v/v sterile defibrinated sheep blood and Neo Selective Supplement (FD149), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor	<=10%	none
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	50-100	none-poor	<=10%	none
Streptococcus agalactiae ATCC 13813	50-100	good-luxuriant	>=50%	beta
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=50%	beta

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.). 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Blanchette and Lawrence, 1967, Am. J. Clin. Pathol., 48-411
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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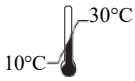

Storage temperature

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HiCrome™ UTI Agar

M1353

Intended use

Recommended for presumptive identification and confirmation of microorganisms mainly causing urinary tract infections, can also be used for testing water, food, environmental and other clinical samples.

Composition**

Ingredients	g / L
Peptone, special (containing phenylalanine and tryptophan)	15.000
Chromogenic mixture	2.450
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.45 gram in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli,* but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome™ UTI Agar is formulated on basis of work carried out by Pezzlo (2) Wilkie et al (3), Friedman et al (4), Murray et al (5), Soriano and Ponte (6) and Merlino et al (7). These media are recommended for the detection of urinary tract pathogens where HiCrome™ UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink colonies due to the enzyme ß-D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E.coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus, Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptone special provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

Type of specimen

Clinical samples : urine, faeces, Food samples, Water samples.

Specimen Collection and Handling

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10,11). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12).

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

Warning and Precautions

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

Limitations

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Since it is an enzyme-substrate based reaction, the intensity of colour may vary with isolates.
4.Further biochemical and serological test are necessary for confirmation.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in Petri plates
Reaction
Reaction of 3.24% w/v aqueous solution at 25°C. pH : 6.8±0.2
pH
6.60-7.20
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%	blue (0.5 to 1.5 mm)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%	pink-purple
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%	blue to purple, mucoid
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	colourless (greenish pigment may be observed)
Proteus mirabilis ATCC 12453	50-100	luxuriant	>=70%	light brown
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	>=70%	cream to golden yellow
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	>=70%	blue to purple
Salmonella Typhi ATCC 6539	50-100	luxuriant	>=70%	colourless to amber
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=70%	colourless to amber

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

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 (8,9).

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Revision : 07/2024

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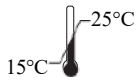
IVD *In vitro* diagnostic medical device



Storage temperature

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

CE **CE Marking**

Do not use if package is damaged

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