HiCombi™ Blood -Mannitol Salt Agar Plate HB009

Intended Use

Recommended for isolation of *Neiserria* and other fastidious microorganisms along with potentially pathogenic Gram positive organisms especially pathogenic Staphylococci.

Composition**

Blood Agar Base

Ingredients	g / L
HM peptone B#	10.000
Tryptose	10.000
Sodium chloride	5.000
Agar	15.000
Blood	50ml
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef Heart peptone

Mannitol Salt Agar

Ingredients	g / L
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Egg Yolk Emulsion (FD045).	50ml
Final pH (at 25°C)	7.4±0.2

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

Directions

Streak the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation

Blood Agar Base

Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. It can also be used as general-purpose media without the addition of blood. Blood Agar Base media can be used with added phenolphthalein phosphate (1) for the detection of phosphate producing Staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass (2) and to determine salinity range of marine *Flavobacteria* (3). It can also be used for preparation of *Salmonella* Typhi antigens (4). Blood Agar Base is recommended by APHA (5) and Standard Methods (6,7) for testing of food samples.

HM peptone B and tryptose provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A Streptococci (8). But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* (9).

Mannitol Salt Agar

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e Staphylococcus aureus is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (9). Staphylococci have the unique ability of growing on a high salt containing media (10). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5% NaCl was studied by Chapman (11).

The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive Staphylococci from cosmetics, milk, food and other specimens (5,9,12,13,15). The additional property of lipase activity of *Staphylococcus aureus* can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (16). HM peptone B and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator.

S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of *S.aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of *S. aureus* should be confirmed by performing the coagulase test [tube or slide] (9). Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion.
A possible *S.aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (16). Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (16).

Type of specimen

Clinical samples - Pus, urine;Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (19,20). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,17,18). 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 :

Blood Agar Base

1. Sheep blood is recommended to detect haemolysis. This medium does not support the growth of *H.haemolyticus.*
2. Haemolytic pattern varies with the source of blood used.

Mannitol Salt Agar

1. A possible *S.aureus* must be confirmed by the coagulase test.
2. The organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (20).
3. Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (17).

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
Sterile Blood and Mannitol Salt Agar in 90 mm disposable biplates with smooth surface and absence of black particles/ cracks/bubbles
Colour of Blood Agar Base
Red coloured medium
Colour of Mannitol salt Agar
Red coloured medium
Quantity of medium
10ml of each medium in biplate
pH of Blood Agar Base
7.10- 7.50

pH of Mannitol salt Agar
7.20- 7.60
Sterility Check
Passes release criteria
Cultural response
Cultural characteristics observed after incubation at 35-37°C for 18-48 hours.

Organism	Growth on Mannitol Salt Agar	Colour of colony on Mannitol Salt Agar	Colour of colony on Blood Agar Base	Haemolysis
# *Klebsiella aerogenes ATCC 13048* (00175*)	Inhibited	-	-	-
Escherichia coli ATCC 25922 (00013*)	Inhibited	-	-	-
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	luxuriant	yellow/white colonies surrounded by yellow zone	-	-
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	luxuriant	yellow/white colonies surrounded by yellow zone	-	-
Staphylococcus epidermidis ATCC 12228 (00036*)	Fair- Good	Red	-	-
Staphylococcus epidermidis ATCC 14990 (00132*)	Fair- Good	Red	-	-
Proteus mirabilis ATCC 12453	None-Poor	yellow	-	-
Streptococcus pyogenes ATCC 19615	-	-	luxuriant	beta
Streptococcus pneumoniae ATCC 6303	-	-	luxuriant	alpha

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

Storage and Shelf Life

On receipt store between 2-8°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 (19,20).

Reference

1.Noble W. C., 1962, J. Clin, Pathol., 15:552.

2.Hansen N. H., 1962, J. Appl. Bacteriol., 25:46.

3.Hayes P. R., 1963, J. Gen. Microbiol., 30:1.

4.Schuber J. H., Edwards P. R. and Ramsere C. H., 1959, J. Bacteriol., 77:648.

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.U.S. Food and Drug Administration, 1995, Bacteriological Analytical Manual, 8th Ed., AOAC International, Gaithersburg, Md.

7.Atlas R. M., 1993, Handbook of Microbiology of Microbiological Media, CRC Press, Boca Raton, Fla.

8.Snavely J. G. and Brahier J., 1960, Am. J. Clin. Pathol., 33:511.
9.Murray P. R., Baron J. H., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
10.Koch P. K., 1942, Zentralbl. Bakteriol. Parasitenkd. Abt. I Orig.149:122.
11.Chapman G. H., 1945, J. Bacteriol., 50:201.
12.Hitchins A. D., Tran T. and McCarron J. E., 1995, FDA Bacteriological Analytical Manual, 8th Ed., AOAC International,Gaithersburg, Md.
13.Davis J. G., 1959, Milk testing, 2nd Ed., Dairy Industries Ltd, London.
14.Silverton R. E. and Anderson M. J., 1961, Handbook of Medical Laboratory Formulae, Butterworths, London.
15.Gunn B. A., Dunkelberg W. E. and Creitz J. R., 1972, Am. J. Clin. Pathol., 57:236.
16.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
17.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
18.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
19.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
20.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03/2024

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

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

IVD *In vitro* diagnostic medical device

2°C 8°C Storage temperature

CE CE Marking

Do not use if package is damaged

Disclaimer :

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