Middlebrook 7H11 HiVeg™ Agar Base

MV511

Intended Use:

Recommended for isolation, cultivation and sensitivity testing of Mycobacteria.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	1.000
Ammonium sulphate	0.500
Potassium dihydrogen phosphate	1.500
Disodium hydrogen phosphate	1.500
Sodium citrate	0.400
Magnesium sulphate	0.050
L-Glutamic acid	0.500
Ferric ammonium citrate	0.040
Pyridoxine	0.001
Biotin	0.0005
Malachite green	0.001
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10.25 grams in 450 ml purified/distilled water containing 2.5 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add one vial of Middlebrook OADC Growth Supplement (FD018). Mix thoroughly and pour into sterile Petri plates or dispense as desired.

Principle And Interpretation

Solid media for Mycobacterial cultivation may be egg-based (Lowenstein Jensen Media) or agar-based (Middlebrook Media) (1). Dubos and Middlebrook (2) developed various formulations containing oleic acid and albumin, which protect *Mycobacterium* from toxic agents, helping for the growth of tubercle bacilli. Middlebrook 7H11 Agar is a modification of Middlebrook 7H10 Agar (3) used for the isolation, cultivation and sensitivity testing of *M. tuberculosis*. It was shown by Cohn et al (4) that the addition of HiVeg™ hydrolysate enhanced the growth of more fastidious *M. tuberculosis* strains, which in turn was helpful in drug susceptibility testing (5). The media is enriched by the addition of Middlebrook OADC Growth Supplement (FD018) and glycerol.

Middlebrook media consists of many inorganic salts, which help, in growth of Mycobacteria. Citric acid formed from sodium citrate helps in retaining inorganic cations in solution. Glycerol supplies carbon and energy. Middlebrook OADC Growth Supplement (FD018) contains oleic acid, bovine albumin, sodium chloride, dextrose and catalase. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Some free fatty acids are toxic to Mycobacteria but albumin binds to those fatty acids and prevents toxic action on Mycobacteria. Dextrose serves as an energy source. Catalase neutralizes toxic peroxides. Malachite green partially inhibits other bacteria (6,1).

Middlebrook 7H11 HiVeg™ Agar Base is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. Mycobacteria are strict aerobes and therefore increased CO_2 tension and aerobic conditions must be provided during incubation. Care should be taken while decontamination of the specimen. Also proper specimen collection (sputum and not saliva) should be ensured. Samples should be carefully handled to avoid contamination.

Type of specimen

Clinical samples : Sputum

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations:

1. It require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after sometime to obtain final pH result.
2. Further biochemical and serological tests need to be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to light green 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 with greenish tinge forms in Petri plates

Reaction

Reaction of 2.05% w/v aqueous solution containing 0.5% glycerol at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

Cultural characteristics observed on addition of Middlebrook OADC Growth Supplement(FD018) and glycerol after an incubation at 35-37°C for 2-4 weeks.

Organism	Growth
Mycobacterium fortuitum ATCC 6841	good-luxuriant
Mycobacterium smegmatis ATCC 14468	good-luxuriant
Mycobacterium tuberculosis H37RV (25618)	good-luxuriant

Storage and Shelf Life

Store below 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 (7,8).

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. Dubos R. J. and Middlebrook G., 1947, Am. Rev. Tuberc., 56:334.
3. Middlebrook G. and Cohn M. L., 1958, Am. J. Public Health, 48:844.
4. 4Cohn M. L., Waggoner R. F.,, McClatchy J. K., 1968, Am. Rev. Resp. Dis., 98:295.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
6. Finegold S. M., and Baron E. J., 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.

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

Revision : 03/2024