Middlebrook 7H10 Agar Base, Special

M196

Middlebrook 7H10 Agar Base, special is recommended for isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis* .

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	0.500
L-Glutamic acid	0.500
Monopotassium phosphate	1.500
Disodium phosphate	1.500
Sodium citrate	0.400
Ferric ammonium citrate	0.040
Magnesium sulphate	0.050
Pyridoxine hydrochloride	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 9.75 grams in 450 ml distilled water containing 2.5 ml glycerol. Heat to boiling to dissolve the medium completely. Distribute in 180 ml amounts in flasks and sterilize at 15 lbs pressure (121°C) for 10 minutes. Cool to 45-50°C and aseptically add 50 ml Middlebrook OADC Growth Supplement (FD018). Mix well and pour into sterile screw capped tubes or containers.

Note : Keep prepared medium in the dark before and after inoculation.

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 7H10 Agar Base was formulated as per Middlebrook, Cohn et al (4) reformed the original oleic acid-albumin agar and observed rapid and luxuriant growth of *Mycobacterium* species, which they called as 7H10. Kubica and Dye (5) reported less contamination on 7H10 Agar than egg-based media commonly used for the cultivation of Mycobacteria. Middlebrook 7H10 Agar Base, Special was formulated by Middlebrook and Cohn (3). On enrichment with OADC Growth Supplement (FD018) and glycerol, it is recommended for cultivation and sensitivity testing of *M. tuberculosis* .

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 (1, 6).

Mycobacteria are strict aerobes and therefore increased CO2 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.

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 1.95% w/v aqueous solution containing 0.25% glycerol at 25°C. pH : 6.6±0.2

pH
6.40-6.80

Cultural Response
M196: Cultural characteristics observed with added Middlebrook OADC GrowthSupplement (FD018) and glycerol after an incubation at 35 - 37°C for 2 - 4 weeks.

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

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. 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.
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4. Middlebrook G., Cohn M. L., Dye W. E., Russel W. F. and Levy D., 1960, Acta. Tuberc. Scand., 38:66.
5. Kubica G. P. and Dye W. E., 1967, Laboratory Methods for Clinical and Public Health Mycobacteriology, PHS Publication No. 1547, U.S. Govt. Printing Office, Washington, D.C.
6. Finegold S. M., and Baron E. J., 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.

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