Technical Information

Middlebrook 7H10 Agar Base, Special

Product Code: DM 1196

Application: 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

Principle & Interpretation

Ther are two types' Solid media for Mycobacterial cultivation which may either be egg-based (Lowenstein Jensen Media) or agar-based (Middlebrook Media) [1]. Dubos and Middlebrook [2] tried various combinations of oleic acid and albumin, which protect *Mycobacterium* from toxic agents, helped in the growth of tubercle bacilli.

Middlebrook 7H10 Agar Base was formulated by Middlebrook [3] Cohn et al[4] further reformed the original oleic acid-albumin agar and found rapid and luxuriant growth of *Mycobacterium* species. They called this medium as 7H10. This media Kubica and Dye [5] observed less contamination on 7H10 Agar than egg-based media commonly used for the cultivation of Mycobacteria. On enrichment of middle book media with OADC Growth Supplement (MS2018) and glycerol, it was used 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 (MS2018) 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].

Methodology

Suspend 9.75 grams of powder media in 450 ml distilled water containing 5 ml glycerol. Shake well & heat 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 (MS2018). Mix well and pour into sterile screw capped tubes or containers.

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

Quality Control

Physical 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 0.5% glycerol at 25°C. pH : 6.6±0.2

pH Range:- 6.40-6.80

Cultural Response/Characteristics
DM 1196: Cultural characteristics observed with added Middlebrook OADC GrowthSupplement (MS2018) 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

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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. 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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