Technical Information

Middlebrook 7H10 Agar Base

Product Code: DM 1199

Application: Middlebrook 7H10 Agar Base 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	0.025
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Pyridoxine hydrochloride	0.001
Biotin	0.0005
Malachite green	0.00025
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Dubos and Middlebrook [1] devised various formulations containing oleic acid and albumin, which protected *Mycobacterium* from toxic agents, helping for the growth of tubercle bacilli. Middlebrook 7H10 Agar Base was formulated as per Middlebrook, Cohn et al [2] revised the original oleic acid-albumin agar media and observed rapid and luxuriant growth of *Mycobacterium* species, which they called as 7H10. Kubica and Dye [3] found less contamination on 7H10 Agar than egg-based media commonly used for the cultivation of Mycobacteria. Middlebrook 7H10 Agar Base is also used for isolation, cultivation and sensitivity testing of *M. tuberculosis when enriched* with OADC Growth Supplement (MS2018) 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 (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 [4, 5].

Methodology

Suspend 9.73 grams of powder media in 450 ml distilled water containing 2-5 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
DM1199: Cultural characteristics observed with added Middlebrook OADC GrowthSupplement (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

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. Dubos R. J. and Middlebrook G., 1947, Am. Rev. Tuberc., 56:334.
2. Middlebrook G., Cohn M. L., Dye W. E., Russel W. F. and Levy D., 1960, Acta. Tuberc. Scand., 38:66.
3. 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.
4. 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.
5. 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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