Middlebrook 7H9 Broth Base

M198

Middlebrook 7H9 Broth Base with added enrichment is recommended for cultivation and sensitivity testing of *Mycobacterium tuberculosis* .

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	0.500
Disodium phosphate	2.500
Monopotassium phosphate	1.000
Sodium citrate	0.100
Magnesium sulphate	0.050
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.040
L-Glutamic acid	0.500
Pyridoxine	0.001
Biotin	0.0005
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 2.35 grams in 450 ml distilled water. Add either 1 ml glycerol or 0.25 g polysorbate 80. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Cool to 45°C or below and aseptically add contents of 1 vial of Middlebrook ADC Growth Supplement (FD019). Mix well before dispensing.

Principle And Interpretation

Media for Mycobacterial cultivation may be egg-based (Lowenstein Jensen Media) or agar-based (Middlebrook Media) (4). Dubos and Middlebrook (5) developed various formulations containing oleic acid and albumin, which protect *Mycobacterium* from toxic agents, helping for the growth of tubercle bacilli. Middlebrook 7H9 Broth Base was formulated by Middlebrook (2) and Middlebrook et al and Schaeffer (1, 3). This medium with Middlebrook ADC Growth Supplement (FD019) and glycerol or polysorbate 80 is also recommended for cultivation of Mycobacteria and for assaying the INH content of the patients sera. The medium can also be used for preparing inocula for antimicrobial assays, as a basal medium for biochemical tests and for the subculture of stock strains.

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. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Middlebrook ADC Growth Supplement (FD019) contains bovine albumin, dextrose, catalase and sodium chloride. 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.

Mycobacteria grow more rapidly in broth media; therefore primary isolation of all specimens can be done in Middlebrook 7H9 Broth Base. After processing the sample as required, inoculate the media with the test specimen.

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

Cream to beige homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured clear solution in tubes

Reaction
Reaction of 0.47% w/v aqueous solution (containing either Glycerol or Polysorbate 80) at 25°C. pH : 6.6±0.2

pH
6.40-6.80

Cultural Response
Cultural characteristics observed with added Middlebrook ADC Growth Supplement (FD019) with added glycerol or Polsorbate 80 after an incubation at 35-37°C for 2-4 weeks

Cultural Response

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. Middlebrook G. and Cohn M. L., 1958, Am. J. Public Health, 48:844.
2. Middlebrook G., Fitzsimmons Army Hospital, Denver, Co, Report 1, 1955
3. Middlebrook G., Cohn, M. L. and Schaeffer W. B.,1954, Am. Rev. Tuber, 70, 852
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. Dubos R. J. and Middlebrook G., 1947, Am. Rev. Tuberc., 56:334.

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