Middlebrook 7H9 Broth Base

M198

Intended Use:

Recommended for cultivation and sensitivity testing of *Mycobacterium tuberculosis.*

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	0.500
Disodium hydrogen phosphate	2.500
Potassium dihydrogen phosphate	1.000
Sodium citrate	0.100
Magnesium sulphate	0.050
Calcium chloride anhydrous	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.040
L-Glutamic acid	0.500
Pyridoxine hydrochloride	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 purified/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 45-50°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) (7). Dubos and Middlebrook (1) 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 (5) and Middlebrook et al and Schaeffer (4,6). 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. Long chain fatty acids are essential for metabolism of Mycobacteria. Middlebrook ADC Growth Supplement (FD019) contains bovine albumin, dextrose and catalase. 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 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

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Mycobacteria are strict aerobes and therefore increased CO_2 tension and aerobic conditions must be provided during incubation.

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
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

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 inorder 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Dubos R. J. and Middlebrook G., 1947, Am. Rev. Tuberc., 56:334.
2. Isenberg, (Ed.), Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. Middlebrook G. and Cohn M. L., 1958, Am. J. Public Health, 48:844.
5. Middlebrook G., Fitzsimmons Army Hospital, Denver, Co, Report 1, 1955
6. Middlebrook G., Cohn, M. L. and Schaeffer W. B.,1954, Am. Rev. Tuber, 70, 852
7. 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.

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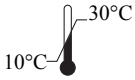
IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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