

TECHNICAL DATA SHEET

Middlebrook 7H9 Agar Base

Principle

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 7H9 Agar Base developed by Middlebrook and Cohn (3) is used for cultivation of Mycobacteria. This medium can also be used for sensitivity testing of Mycobacteria and for subculturing of stock cultures on addition of Middlebrook OADC Growth Supplement (FD018) 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 (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 (1,4). 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.

Use: For cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

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Contents*

Ingredients	Gms / Litre
Ammonium sulphate	0.500
Sodium glutamate	0.500
Sodium citrate	0.100
Pyridoxine	0.001
Biotin	0.0005
Disodium hydrogen phosphate	2.500
Potassium dihydrogen phosphate	1.000
Ferric ammonium citrate	0.040
Magnesium sulphate	0.050
Calcium chloride anhydrous	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Malachite green	0.001
Agar	15.000
Final pH (at 25°C)	6.6±0.2

* Formula adjusted for optimum performance and parameters

Directions: Suspend 9.85 grams in 450 ml purified/distilled water. 1ml glycerol may be added if desired. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 1 vial of Middlebrook OADC Growth Supplement (FD018). Mix well and distribute as desired.

Specimens' types analyzed
Clinical samples: Sputum

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Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Light yellow to light green homogeneous free flowing powder
Reaction of 1.97% solution	6.6 ± 0.2 at 25°C
pH	6.40-6.80
Color and clarity of ready medium	Light amber coloured clear to slightly opalescent gel with greenish tinge forms in Petri plates
Gelling	Firm, comparable with 1.5% Agar gel

Different Microbial Response

Cultural characteristics observed with added Middlebrook OADC Growth Supplement (FD018) after an incubation at $35-37^{\circ}\text{C}$ for 2-4 weeks

Organism	ATCC	Growth
<i>Mycobacterium tuberculosis</i> (H37RV)	25618	Luxuriant
<i>Mycobacterium fortuitum</i>	6841	Luxuriant
<i>Mycobacterium smegmatis</i>	14468	Luxuriant

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

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Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (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. Finegold S. M., and Baron E. J., 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.
5. Isenberg, (Ed.), Clinical Microbiology Procedures Handbook 2nd Edition
6. 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.

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