



Middlebrook 7H11 Agar Base

M511

Middlebrook 7H11 Agar Base with the addition of supplement is recommended for isolation, cultivation and sensitivity testing of *Mycobacteria*.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	1.000
Ammonium sulphate	0.500
Monopotassium phosphate	1.500
Disodium phosphate	1.500
Sodium citrate	0.400
Magnesium sulphate	0.050
L-Glutamic acid	0.500
Ferric ammonium citrate	0.040
Pyridoxine	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

Directions

Suspend 10.25 grams in 450 ml distilled water containing 2.5 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add contents of 1 vial of Middlebrook OADC Growth Supplement (FD018). Mix thoroughly before dispensing.

Principle And Interpretation

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 7H11 Agar is a modification of Middlebrook 7H10 Agar (3) used for the isolation, cultivation and sensitivity testing of *M. tuberculosis*. It was shown by Cohn et al (4) that the addition of casein enzymic hydrolysate enhanced the growth of more fastidious *M. tuberculosis* strains, which in turn was helpful in drug susceptibility testing (5). The media is enriched by the 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, 6).

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

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 2.05% w/v aqueous solution containing 0.5% glycerol at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

M511: Cultural characteristics observed on addition of Middlebrook OADC Growth Supplement(FD018) and glycerol after an incubation at 35-37°C for 2-4 weeks.

Organism**Growth**

Mycobacterium fortuitum good-luxuriant

ATCC 6841

Mycobacterium smegmatis good-luxuriant

ATCC14468

Mycobacterium tuberculosis good-luxuriant

H37RV (25618)

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. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. 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.
3. Middlebrook G. and Cohn M. L., 1958, Am. J. Public Health, 48:844.
4. Cohn M. L., Waggoner R. F., McClatchy J. K., 1968, Am. Rev. Resp. Dis., 98:295.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
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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