

Middlebrook 7H9 Agar Base

Intended Use

Middlebrook 7H9 Agar Base is used for isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

Summary

Solid media for Mycobacterial cultivation may be agar based (Middlebrook Media) or egg-based (Lowenstein Jensen Media). Dubos and Middlebrook 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 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 and glycerol. 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.

Principle

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 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.

Formula*

Ingredients	g/L
Ammonium Sulphate	0.5
Sodium Glutamate	0.5
Sodium Citrate	0.1
Pyridoxine	0.001
Biotin	0.0005
Disodium Phosphate	2.5
Monopotassium Phosphate	1.0
Ferric Ammonium Citrate	0.04
Magnesium Sulphate	0.05
Calcium Chloride	0.0005
Zinc Sulphate	0.001
Copper Sulphate	0.001
Malachite Green	0.001
Agar	15.0
Final pH (at 25°C)	6.6 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 9.85 g in 450 mL purified / distilled water.
2. Add 1 mL glycerol if desired.
2. Heat to boiling to dissolve the medium completely
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Cool to 45°C-50°C and aseptically add 1 vial of Middlebrook OADC Growth Supplement.
5. Mix well before dispensing.

Quality Control

Dehydrated Appearance: Light yellow to light green homogeneous free flowing powder.

Prepared Appearance: Light amber coloured clear to slightly opalescent gel with greenish tinge forms in petridishes.

Cultural Response: Cultural characteristics observed with added Middlebrook OADC Growth Supplement after an incubation at 35°C-37°C for 2-4 weeks.

Organism (ATCC)

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

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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. Finegold S. M., and Baron E. J., 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.
5. Data on file: Microexpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201130510500	Dehydrated Culture Media	500 g

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
