TM 590 - MIDDLEBROOK 7H9 AGAR BASE

INTENDED USE

For isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

PRODUCT SUMMARY AND EXPLANATION

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

COMPOSITION

Ingredients	Gms / Ltr
Ammonium sulphate	0.500
Sodium glutamate	0.500
Sodium citrate	0.100
Pyridoxine	0.001
Biotin	0.0005
Disodium phosphate	2.500
Monopotassium phosphate	1.000
Ferric ammonium citrate	0.040
Magnesium sulphate	0.050
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Malachite green	0.001
Agar	15.000

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.

INSTRUCTION FOR USE

- Dissolve 9.85 grams in 450 ml distilled water. 1 ml glycerol may be added if desired.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.

- Cool to 45-50°C and aseptically add I vial of Middlebrook OADC Growth Supplement.
- Mix well and distribute as desired.

QUALITY CONTROL SPECIFICATIONS

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

Appearance of prepared medium : Light amber coloured clear to slightly opalescent gel with greenish tinge forms in Petri plates.

pH (at 25°C) : 6.6±0.2

INTERPRETATION

Cultural characteristics observed with added Middlebrook OADC Growth Supplement after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Mycobacterium tuberculosis	25618	50-100	Good-luxuriant	>=50%	35-37°C	2-4 weeks
Mycobacterium fortuitum	6841	50-100	Good-luxuriant	>=50%	35-37°C	2-4 weeks
Mycobacterium smegmatis	14468	50-100	Good-luxuriant	>=50%	35-37°C	2-4 weeks

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

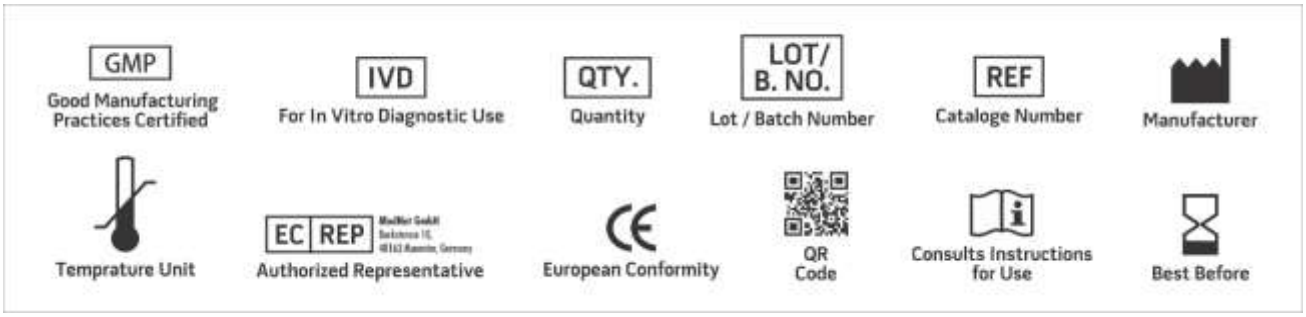
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. 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.
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



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

Revision: 08 Nov., 2019