

Middlebrook 7H11 Agar w/ PANTA Supplement

MP5426

Intended Use:

Recommended for the selective isolation of *Mycobacterium tuberculosis*.

Composition**

Ingredients	g / L
Tryptone	1.000
Ammonium sulphate	0.500
Potassium dihydrogen phosphate	1.500
Disodium hydrogen 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

PANTA Supplement (FD814)

Polymyxin B	10000 IU
Amphotericin B	1 mg
Nalidixic Acid	4 mg
Trimethoprim	1 mg
Azlocillin	1 mg

Middlebrook OADC Growth Supplement (FD018)

Bovine albumin fraction V	2.500g
Dextrose	1g
Catalase	0.002g
Oleic acid	0.025g
Sodium chloride	0.425g
Distilled water	50ml
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

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 Tryptone 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 (6,1). PANTA Supplement (FD814) suppress growth of contaminating microorganisms which may survive the decontamination process.

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.

Type of specimen

Clinical samples : Sputum

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

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. Further biochemical and serological tests must be carried out for further identification.

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

Sterile Middlebrook 7H11 Agar w/ PANTA Supplement in 90mm disposable plate with smooth surface and absence of black particles/cracks/bubbles.

Colour of medium

Light amber to greenish tinge coloured medium

Quantity of medium

25ml medium in 90mm disposable plate.

pH

6.40-6.80

Sterility check

Passes release criteria

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 2-4 weeks.

Organism

Growth

Mycobacterium fortuitum good-luxuriant
ATCC 6841

Mycobacterium smegmatis good-luxuriant
ATCC 14468

Mycobacterium tuberculosis good-luxuriant
H37RV (25618)

Storage and Shelf Life

On receipt store between 2-8°C Use before expiry date on the label. 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 (7,8).

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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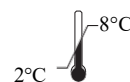
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In vitro diagnostic
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