Middlebrook 7H10 Agar Base, Special

M196

Intended Use:

Recommended for isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis.*

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	0.500
L-Glutamic acid	0.500
Potassium dihydrogen phosphate	1.500
Disodium hydrogen phosphate	1.500
Sodium citrate	0.400
Ferric ammonium citrate	0.040
Magnesium sulphate	0.050
Pyridoxine hydrochloride	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 9.75 grams in 450 ml purified/distilled water containing 2.5 ml glycerol. Heat to boiling to dissolve the medium completely. Distribute in 180 ml amounts in flasks and sterilize at 15 lbs pressure (121°C) for 10 minutes. Cool to 45-50°C and aseptically add 50 ml Middlebrook OADC Growth Supplement (FD018). Mix well and pour into sterile screw capped tubes or containers.

Note : Keep prepared medium in the dark before and after inoculation.

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 7H10 Agar Base was formulated as per Middlebrook, Cohn et al (3) reformed the original oleic acid-albumin agar and observed rapid and luxuriant growth of *Mycobacterium* species, which they called as 7H10. Kubica and Dye (4) reported less contamination on 7H10 Agar than egg-based media commonly used for the cultivation of Mycobacteria. Middlebrook 7H10 Agar Base, Special was formulated by Middlebrook and Cohn (5). On enrichment with OADC Growth Supplement (FD018) and glycerol, it is recommended for cultivation and sensitivity testing of *M. tuberculosis*. 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 CO_2 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. For professional 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. Due to nutritional variations, some strains may show poor growth.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

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 1.95% w/v aqueous solution containing 0.5% glycerol at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

Cultural characteristics observed with added Middlebrook OADC GrowthSupplement (FD018) and glycerol after an incubation at 35 - 37°C for 2 - 4 weeks.

Organism	Growth
Mycobacterium fortuitum ATCC 6841	good-luxuriant
Mycobacterium smegmatis ATCC 14468	good-luxuriant
Mycobacterium tuberculosis H37RV (25618)	good-luxuriant

Storage and Shelf Life

Store below 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. 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

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3. Middlebrook G., Cohn M. L., Dye W. E., Russel W. F. and Levy D., 1960, Acta. Tuberc. Scand., 38:66.
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IVD ***In vitro*** **diagnostic medical device**

30°C
10°C **Storage temperature**

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