TM 213 - MIDDLEBROOK 7H9 BROTH BASE

INTENDED USE

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

PRODUCT SUMMARY AND EXPLANATION

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 Broth Base was formulated by Middlebrook and Middlebrook et al and Schaeffer. This medium with Middlebrook ADC Growth Supplement and glycerol or polysorbate 80 is also recommended for cultivation of Mycobacteria and for assaying the INH content of the patient's sera. The medium can also be used for preparing inocula for antimicrobial assays, as a basal medium for biochemical tests and for the subculture of stock strains.

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.

COMPOSITION

Ingredients	Gms / Ltr
Ammonium sulphate	0.500
Disodium hydrogen phosphate	2.500
Potassium dihydrogen phosphate	1.000
Sodium citrate	0.100
Magnesium sulphate	0.050
Calcium chloride anhydrous	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.040
L-Glutamic acid	0.500
Pyridoxine hydrochloride	0.001
Biotin	0.0005

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. Long chain fatty acids are essential for metabolism of Mycobacteria. Middlebrook ADC Growth Supplement contains bovine albumin, dextrose and catalase. 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. Mycobacteria grow more rapidly in broth media; therefore, primary isolation of all specimens can be done in Middlebrook 7H9 Broth Base. After processing the sample as required, inoculate the media with the test specimen.

INSTRUCTION FOR USE

- Dissolve 2.35 grams in 450 ml purified/distilled water. Add either 1 ml glycerol or 0.25 g polysorbate 80.
- Heat if necessary to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 10 minutes.

- Cool to 45-50°C or below and aseptically add contents of 1 vial of Middlebrook ADC Growth Supplement.
- Mix well before dispensing.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to beige homogeneous free flowing powder.
Appearance of prepared medium : Light amber coloured clear solution in tubes.
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	Incubation Temperature	Incubation Period
Mycobacterium tuberculosis	25618	50-100	Good-luxuriant	35-37°C	2-4 weeks
Mycobacterium fortuitum	6841	50-100	Good-luxuriant	35-37°C	2-4 weeks
Mycobacterium smegmatis	14468	50-100	Good-luxuriant	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

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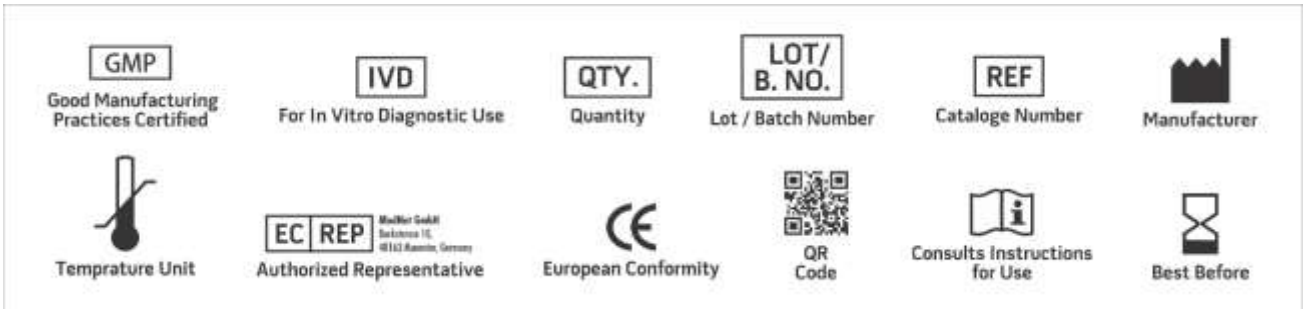

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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