

## CM 21186 - MIDDLEBROOK 7H10 AGAR BASE, 'TBL'

### 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 7H10 Agar Base was formulated as per Middlebrook, Cohn et al reformed the original oleic acid-albumin agar and observed rapid and luxuriant growth of *Mycobacterium* species, which they called as 7H10. Kubica and Dye 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. On enrichment with OADC Growth Supplement and glycerol, it is recommended for cultivation and sensitivity testing of *M. tuberculosis*.

*Mycobacteria* are strict aerobes and therefore increased CO<sub>2</sub> 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     |
| L-Glutamic acid          | 0.500     |
| Monopotassium phosphate  | 1.500     |
| Disodium phosphate       | 1.500     |
| Sodium citrate           | 0.400     |
| Ferric ammonium citrate  | 0.040     |
| Magnesium sulphate       | 0.025     |
| Pyridoxine hydrochloride | 0.001     |
| Biotin                   | 0.0005    |
| Malachite green          | 0.00025   |
| 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 19.46 grams in 900 ml distilled water containing 5 ml glycerol.

Heat to boiling to dissolve the medium completely.

Sterilize at 15 psi pressure (121°C) for 10 minutes.

Cool to 45-50°C and aseptically add 100 ml Middlebrook OADC Growth Supplement (TS 060).



Mix well and pour into sterile screw capped tubes or containers.

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

#### 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 and glycerol 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 10-25°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., Tenover F. C., 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. Middlebrook G., Cohn M. L., Dye W. E., Russel W. F. and Levy D., 1960, Acta. Tuberc. Scand., 38:66.
5. Kubica G. P. and Dye W. E., 1967, Laboratory Methods for Clinical and Public Health Mycobacteriology, PHS Publication No. 1547, U.S. Govt. Printing Office, Washington, D.C.

