MIDDLEBROOK 7H11 AGAR (7244)

Intended Use

Middlebrook 7H11 Agar is used with glycerol and OADC Enrichment for the cultivation of *Mycobacterium* spp.

Product Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] Non-tuberculous mycobacteria infections have also increased since 1985.[2] There are two types of solid culture media for the primary isolation of mycobacteria, coagulated egg as a base (Lowenstein formulations) and an agar base (Middlebrook formulations). The use of agar-based media for primary isolation of mycobacteria have the following significant advantages:

1. Agar-based media do not usually liquefy in the presence of contaminating proteolytic organisms.[2]
2. Agar-based media are recommended for specimens from nonsterile sites, because colonies of mycobacteria can be viewed in a clear medium after 10 – 12 days incubation using a stereo microscope even if contaminating organisms are present.[3]
3. Agar- based media retain exact concentrations of added drugs because the medium is solidified with agar rather than by inspissation of the egg. There is less drug inactivation when egg ingredients are absent.

Middlebrook 7H11 Agar is a modification of Middlebrook 7H10 Agar Special as recommended by Cohn, Waggoner, and McClately.[4] Cohn et al. added an enzymatic digest of casein and found organism growth was stimulated for fastidious strains of *Mycobacterium tuberculosis* and provided improved susceptibility testing.[4]

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, vitamins, and amino acids in Middlebrook 7H11 Agar. Ammonium Sulfate, Sodium Citrate, Pyridoxine, Monosodium Glutamate, and Biotin supply growth factors. Magnesium Sulfate, Ferric Ammonium Citrate, Zinc Sulfate, and Copper Sulfate are sources of trace ions required for growth of Mycobacteria spp. Disodium Phosphate and Monopotassium Phosphate help maintain the pH of the medium. Malachite Green inhibits contaminating organisms. Agar is a solidifying agent. Glycerol enhances the growth of *Mycobacterium avium* and other *Mycobacterium* spp.[3] OADC Enrichment contains Dextrose and Oleic Acid as carbon sources.

Formula / Liter

Enzymatic Digest of Casein	1 g
Disodium Phosphate	1.5 g
Monopotassium Phosphate	1.5 g
Ammonium Sulfate	0.5 g
Monosodium Glutamate	0.5 g
Sodium Citrate	0.4 g
Ferric Ammonium Citrate	0.04 g
Magnesium Sulfate	0.05 g
Copper Sulfate	0.001 g
Pyridoxine	0.001 g
Zinc Sulfate	0.001 g
Biotin	0.0005 g
Malachite Green	0.00025 g
Agar	13.5 g

Final pH: 6.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glycerol, 5 mL
OADC Enrichment, 100 mL

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 19 g of the medium in 900 mL of purified water containing 5 mL of glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Cool to 45 - 50°C and aseptically add 100 mL of OADC Enrichment.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and pale to light green to gray-white.

Expected Cultural Response: Cultural response on Middlebrook 7H11 Agar at incubated under CO_2 at 35 ± 2°C and examined for growth after 3 – 28 days incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Mycobacterium fortuitum Group IV ATCC® 6841	Heavy	Growth
Mycobacterium intracellulare Group III ATCC® 13950	Heavy	Growth
Mycobacterium kansasii Group I ATCC® 12478	Heavy	Growth
Mycobacterium scrofulaceum Group II ATCC® 19981	Heavy	Growth
Mycobacterium tuberculosis H37Ra ATCC® 25177	Heavy	Growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate specimen onto the medium. Incubate tubes for up to eight weeks. Examine tubes for growth at regular intervals. Refer to specific procedures for a complete discussion on the isolation and identification of *Mycobacterium* spp.

Results

Observe colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Mycobacterium* spp.
2. Negative culture results do not rule out an active mycobacterial infection.

Packaging

Middlebrook 7H11 Agar	**Code No.**	**7244A**	**500 g**
		7244B	**2 kg**
		7244C	**10 kg**

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. Clinical Microbiology Reviews. **8:**496-514.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1 American Society for Microbiology, Washington, D.C.
4. **Cohn, M. L., R. F. Waggoner, and J. K. McClatchy.** 1968. The 7H11 Medium for the cultivation of mycobacteria. Am. Rev. Resp. Dis. **98:**295.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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