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# MIDDLEBROOK 7H11 AGAR

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## INTENDED USE

Remel Middlebrook 7H11 Agar is a solid medium recommended for use in qualitative procedures for the isolation and cultivation of *Mycobacterium* species.

## SUMMARY AND EXPLANATION

Dubos and Middlebrook developed media formulations containing oleic acid and albumin which enhanced the growth of tubercle bacilli and protected the organisms against a variety of toxic agents.<sup>1</sup> In 1958, Middlebrook and Cohn improved the previous formulation of oleic acid-albumin agar to obtain 7H10 Agar which allowed faster, more luxuriant growth of *Mycobacterium* spp.<sup>2</sup> In 1968, Cohn et al. demonstrated that the addition of casein hydrolysate to 7H10 Agar stimulated the growth of mycobacteria which would otherwise not grow on 7H10 media.<sup>3</sup> This formulation is designated as Middlebrook 7H11 Agar.

## PRINCIPLE

This medium contains inorganic salts which are essential for the growth of mycobacteria. Casein hydrolysate serves as a growth stimulant for drug-resistant strains of *Mycobacterium tuberculosis*.<sup>4</sup> Glycerol is a source of carbon and energy. Sodium citrate is converted to citric acid, which holds inorganic cations in solution. Malachite-green dye is a selective agent, which inhibits bacteria other than mycobacteria. OADC Enrichment is a supplement, which contains the following additives: sodium chloride to maintain osmotic equilibrium; dextrose, a source of energy; catalase to destroy toxic peroxides that may be present in the medium; oleic acid which is required in the metabolism of mycobacteria; and albumin to protect the tubercle bacilli against toxic agents.

## REAGENTS (CLASSICAL FORMULAE)\*

Dipotassium Phosphate .....	1.5 g	Ferric Ammonium Citrate.....	0.04 g
Monopotassium Phosphate.....	1.5 g	Malachite Green .....	1.0 mg
Casein Hydrolysate .....	1.0 g	Pyridoxine Hydrochloride.....	1.0 mg
Ammonium Sulfate.....	0.5 g	Biotin .....	0.5 mg
Monosodium Glutamate .....	0.5 g	●OADC Enrichment.....	100.0 ml
Sodium Citrate .....	0.4 g	Glycerol .....	5.0 ml
Magnesium Sulfate .....	0.05 g	Agar .....	15.0 g
		Demineralized Water .....	900.0 ml

pH 6.6 ± 0.2 @ 25°C

### ●OADC Enrichment:

Albumin Fraction V .....	50.0 g	Oleic Acid .....	0.5 g
Dextrose.....	20.0 g	Catalase (Beef).....	0.04 g
Sodium Chloride.....	8.5 g	Demineralized Water .....	1000.0 ml

\*Adjusted as required to meet performance standards.

## PROCEDURE

Follow established laboratory safety procedures when working with acid-fast cultures and specimens. Consult appropriate references when necessary for detailed procedural information on specimen processing and media inoculation. Inoculate 2 sets of media for specimens obtained from skin or soft tissue from which *Mycobacterium marinum* or *Mycobacterium ulcerans* is suspected. Incubate one set of media at 35-37°C and the other set at room temperature.<sup>5-8</sup>

1. Using a Pasteur pipette, inoculate Middlebrook 7H11 Agar with 1-2 drops of decontaminated, concentrated specimen.
2. Allow inoculated media to remain at room temperature for several hours if possible, until the inoculum dries or is absorbed.
3. Incubate at 35-37°C in an atmosphere of 5-10% CO<sub>2</sub>, protected from light.
  - a. Incubate plates agar-side down until all the inoculum is absorbed. To prevent accumulation of excess moisture, do not incubate media directly on metal shelving or stack plates more than six high. If gas-permeable bags are used, incubate one plate per bag.
  - b. Incubate tubed media with caps loosened for the first week to permit the circulation of CO<sub>2</sub> and in a slanted position to allow the inoculum to be absorbed into the media. Caps should be tightened after the first week or two to prevent dehydration.
4. Examine cultures within 5-7 days after inoculation and once a week thereafter for a minimum of 8 weeks. Prolonged incubation, 10-12 weeks or more, may be necessary in selected cases or if the original smear was positive and the culture remains negative at 8 weeks.
5. Monitor cultures for growth rate, pigment production, and colony morphology.
6. If growth is detected, stain colony to confirm that isolate is acid-fast.
7. Subculture acid-fast colonies to an appropriate medium. Proceed with identification following established laboratory procedures. Consult appropriate references for further instructions if necessary.<sup>5-8</sup>

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## QUALITY CONTROL

All lot numbers of Middlebrook 7H11 Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards.<sup>9</sup> Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

### CONTROL

- \**Mycobacterium fortuitum* ATCC® 6841
- \**Mycobacterium intracellulare* ATCC® 13950
- \**Mycobacterium kansasii* ATCC® 12478
- \**Mycobacterium scrofulaceum* ATCC® 19981
- \**Mycobacterium tuberculosis* ATCC® 25177

\*CLSI recommended organism

### INCUBATION

- CO<sub>2</sub>, up to 21 days @ 33-37°C
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### RESULTS

- Growth
- Growth
- Growth
- Growth
- Growth

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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