MIDDLEBROOK 7H10 AGAR

INTENDED USE
Remel Middlebrook 7H10 Agar is a solid medium recommended for use in qualitative procedures for the isolation and cultivation of mycobacteria.

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.[1] 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* species.[2]

PRINCIPLE
This medium contains inorganic salts which are essential to the growth of mycobacteria. 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 partially inhibits bacteria other than mycobacteria. OADC Enrichment is a supplement which contains the following additives: sodium chloride to maintain osmotic equilibrium; dextrose, an energy source; 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	Pyridoxine Hydrochloride	1.0 mg
Monopotassium Phosphate	1.5 g	Zinc Sulfate	1.0 mg
Ammonium Sulfate	0.5 g	Biotin	0.5 mg
Monosodium Glutamate	0.5 g	Calcium Chloride	0.5 mg
Sodium Citrate	0.4 g	Malachite Green	0.25 mg
Ferric Ammonium Citrate	0.04 g	•OADC Enrichment	100.0 ml
Magnesium Sulfate	25.0 mg	Glycerol	5.0 ml
Copper Sulfate	1.0 mg	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 at 35-37°C and the other set at room temperature.[3-6]

1. Using a Pasteur pipette, inoculate Middlebrook 7H10 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_2, 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_2 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 up to 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.[3-6]

Pour Tube: Melt the pour tube in a boiling water bath and cool to 45-50°C. Add OADC Enrichment (REF R450600) and mix thoroughly. Dispense into a sterile petri dish and proceed with the procedure above.

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QUALITY CONTROL

All lot numbers of Middlebrook 7H10 Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[7] 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	INCUBATION	RESULTS
**Mycobacterium fortuitum* ATCC® 6841	CO_2, up to 21 days @ 33-37°C	Growth
**Mycobacterium intracellulare* ATCC® 13950	CO_2, up to 21 days @ 33-37°C	Growth
**Mycobacterium kansasii* ATCC® 12478	CO_2, up to 21 days @ 33-37°C	Growth
**Mycobacterium scrofulaceum* ATCC® 19981	CO_2, up to 21 days @ 33-37°C	Growth
**Mycobacterium tuberculosis* ATCC® 25177	CO_2, up to 21 days @ 33-37°C	Growth

*CLSI recommended organism

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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.

IFU 1600, Revised March 17, 2011

Printed in U.S.A.

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