remel

MIDDLEBROOK 7H11 AGAR (THIN POUR)

INTENDED USE

Remel Middlebrook 7H11 Agar (Thin Pour) is a solid medium recommended for use in qualitative procedures for the isolation of *Mycobacterium* spp., particularly unusually fastidious strains of tubercle bacilli which arise following treatment of tuberculosis with secondary antituberculosis drugs. The thin pour of this medium facilitates rapid detection of mycobacteria utilizing the microcolony method.

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 formulation and developed 7H10 Agar which allowed faster, more luxuriant growth of *Mycobacterium* spp.[2] In 1968, Cohn et al. demonstrated that after supplementation with casein hydrolysate 7H10 Agar would stimulate the growth of mycobacteria which otherwise would not grow.[3] This formulation was designated 7H11 Agar. In 1992, Welch et al. developed a technique for early detection of mycobacteria on solid media utilizing a reduced pour Middlebrook 7H11 Agar plate.[4,5] Inoculated plates were sealed, incubated, and examined microscopically at regular intervals for the appearance of microcolonies. Use of this method has been shown to reduce the detection time for a positive culture to an average of 11 days. Additionally, species recognition and the presence of mixed infections based on microscopic colony morphology was shown to facilitate implementation of identification testing.

PRINCIPLE

This medium contains casein hydrolysate which serves as a growth stimulant for drug-resistant strains of *Mycobacterium tuberculosis* and inorganic salts which are essential for the growth of mycobacteria. Glycerol is an energy source and sodium citrate is converted to citric acid which holds inorganic cations in solution. Malachite green dye is a selective agent which partially inhibits contaminating bacteria. Supplemental OADC Enrichment contains the following additives required by mycobacteria: sodium chloride to maintain osmotic equilibrium; dextrose for an energy source; catalase to destroy toxic peroxides that may be present in the medium; oleic acid, a fatty acid utilized in the mechanism of mycobacteria; albumin to protect the tubercle bacilli against toxic agents.

REAGENTS (CLASSICAL FORMULAE)*

Monopotassium Phosphate	1.5 g
Dipotassium Phosphate	1.5 g
Casein Hydrolysate	1.0 g
Monosodium Glutamate	0.5 g
Ammonium Sulfate	0.5 g
Sodium Citrate	0.4 g
Magnesium Sulfate	0.05 g
Ferric Ammonium Citrate	0.04 g
Pyridoxine Hydrochloride	1.0 mg
Malachite Green	1.0 mg
Biotin	0.5 mg
Glycerol	5.0 ml
•OADC Enrichment	100.0 ml
Agar	15.0 g
Demineralized Water	1000.0 ml

pH 6.6 ± 0.2 @ 25°C

•OADC Enrichment:

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

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after their use. Directions should be read and followed carefully**.**

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use. Protect from light, as malachite green is photosensitve.[6]

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed, (4) the expiration date has passed, or (5) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[7]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Microscope, (7) Shrink Seals (REF R522600), gas permeable strips, (8) Biological safety equipment, (9) Pasteur pipettes, (10) Acid-fast stain reagents.

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.[7-9]

1. Using a Pasteur pipette, inoculate the agar with 1-2 drops of decontaminated, concentrated specimen and streak for isolation.
2. Following inoculation, seal the plate with Shrink Seal or gas permeable strip.
3. Incubate plate at 35°C in an atmosphere of 5-10% CO_2.
4. Examine sealed plates microscopically (total magnification 40 X to 100 X) on alternate days for the first two weeks and less frequently thereafter.
5. Invert plate on the stage of a conventional microscope and focus objective on the surface of the agar. Look for streak lines or inoculum through the bottom of the plate.
6. Scan the area of heavy inoculum (first and second quadrants) for evidence of growth.
7. If microcolonies are detected, pick and stain for acid-fastness. Subculture acid-fast colonies to suitable media. Proceed with identification following established laboratory procedures.
8. Continue incubation of plates with no growth for 4 weeks before discarding, unless the culture becomes overgrown with bacteria or fungus.

QUALITY CONTROL

All lot numbers of Middlebrook 7H11 Agar (Thin Pour) 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.[10] 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 11 days @ 35°C	Microcolony detection
	CO_2, up to 21 days @ 35°C	Growth
**Mycobacterium intracellulare* ATCC® 13950	CO_2, up to 11 days @ 35°C	Microcolony detection
	CO_2, up to 21 days @ 35°C	Growth
**Mycobacterium kansasii* ATCC® 12478	CO_2, up to 11 days @ 35°C	Microcolony detection
	CO_2, up to 21 days @ 35°C	Growth
**Mycobacterium scrofulaceum* ATCC® 19981	CO_2, up to 11 days @ 35°C	Microcolony detection
	CO_2, up to 21 days @ 35°C	Growth
**Mycobacterium tuberculosis* ATCC® 25177	CO_2, up to 11 days @ 35°C	Microcolony detection
	CO_2, up to 21 days @ 35°C	Growth

*CLSI recommended organism

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PACKAGING

Middlebrook 7H11 Agar (Thin Pour):
REF R01606, 10 X 100 mm Plate 15/Pk

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

IFU 1606-PI, Revised January 13, 2011 Printed in U.S.A.

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