

Revisions

Rev from	Rev to	ECO #
0803	2010/07	5431-10


Notes:

- BD Cat. Number 295964, 297250, 298292
- Blank (Sheet) Size: Length: 11" Width: 8.5"
 Number of Pages: 2 Number of Sheets: 1
 Page Size: Length 11" Width 8.5" Final Folded Size: N/A
- Style (see illustrations below): # 1



- See Specification Control Number N/A for Material Information
- Ink Colors: Printed two sides Yes No
 No. of Colors: 1 PMS# Black
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Proofer	Date			
Checked By	Date			
Part Number: 8801671JAA		Category and Description Package Insert, Middlebrook I-Plates	Sheet: 1 of 3 Scale: N/A	A

BD BBL™ Prepared Plated Media for the Isolation of Mycobacteria

Middlebrook 7H10//7H10 Agar, Middlebrook 7H11//7H11 Selective Agar, Middlebrook 7H10//7H11 Selective Agar

8801671JAA
2010/07

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INTENDED USE

These media are used in qualitative procedures for isolation and cultivation of mycobacteria, especially *Mycobacterium tuberculosis*, from clinical and nonclinical specimens.

Middlebrook 7H10//7H10 Agar two-sector I Plate™ dishes contain one medium divided into two sectors to permit the inoculation of two specimens.

Middlebrook 7H11//7H11 Selective Agar bi-plates provide two media, one moderately selective and one highly selective, for the recovery of mycobacteria from specimens containing mixed flora. The Middlebrook 7H11 Agar is contained in the sector differentiated by the Roman numeral "I" molded into the bottom of the sector. The 7H11 Selective Agar is contained in the sector marked "II."

Middlebrook 7H10//7H11 Selective Agar bi-plates provide two media, one moderately selective and one highly selective. The Middlebrook 7H10 Agar is contained in the sector marked "I" molded into the bottom of the sector. The 7H11 Selective Agar is contained in the sector marked "II."

SUMMARY AND EXPLANATION

Middlebrook 7H10 Agar is based on an improved formulation for the oleic acid-albumin agar that Middlebrook and Cohn developed to promote early growth of tubercle bacilli *in vitro* and to facilitate wider application of drug-susceptibility testing.¹⁻³ Because of its relatively simple chemical formulation, it is less likely to support contaminants than the egg-based media commonly used for the cultivation of mycobacterial species.⁴

Middlebrook 7H11 Agar was developed by Cohn et al. by the addition of casein hydrolysate to 7H10 Agar.⁵ 7H11 Agar provides enhanced growth of fastidious, drug-resistant strains of *M. tuberculosis* that grow poorly (or not at all) on 7H10 Agar or other widely-used media.^{5,6}

The 7H11 Selective Agar is 7H11 Agar modified by the addition of four antimicrobial agents: polymyxin B, carbenicillin, amphotericin B and trimethoprim lactate. Mitchison et al. initially developed the medium to reduce the need for decontamination procedures.⁷ They found that the alkaline agents used to reduce the growth of contaminating organisms inhibited some species of mycobacteria. McClatchy recommended reducing the concentration of carbenicillin used by Mitchinson et al. to make the medium less inhibitory to mycobacteria.⁸

PRINCIPLES OF THE PROCEDURE

Middlebrook 7H10 Agar is a defined medium consisting of oleic acid-albumin enrichment, glycerol, dextrose and inorganic compounds to supply the nutrients necessary to support the growth of mycobacterial species. Catalase destroys toxic peroxides that may be present in the medium. Malachite green acts as an inhibitory agent to provide partial inhibition of contaminating bacteria.

Middlebrook 7H11 Agar consists of 7H10 Agar supplemented with pancreatic digest of casein to enhance the growth of fastidious strains of *M. tuberculosis*.

The addition of antimicrobial agents to 7H11 Agar improves the recovery of mycobacteria from specimens containing mixed flora.⁶ Polymyxin B is a poly peptide antibiotic that selectively inhibits most species of gram-negative bacilli, including *Pseudomonas*, but not *Proteus* species.⁹ Carbenicillin is a semi-synthetic penicillin effective against gram-positive and gram-negative bacteria, including strains of *Escherichia coli* resistant to other antimicrobial agents.⁹ Amphotericin B is an antifungal antibiotic, and trimethoprim lactate is a synthetic antimicrobial agent that inhibits both gram-positive and gram-negative bacteria, including *Proteus* species.

REAGENTS

Middlebrook 7H10 Agar

Approximate Formula* Per Liter Purified Water

Magnesium Sulfate	0.05 g	Pyridoxine.....	1.0 mg
Ferric Ammonium Citrate	0.04 g	Zinc Sulfate.....	1.0 mg
Sodium Citrate.....	0.4 g	Copper Sulfate	1.0 mg
Ammonium Sulfate.....	0.5 g	Biotin	0.5 mg
Monosodium Glutamate.....	0.5 g	Calcium Chloride.....	0.5 mg
Disodium Phosphate.....	1.5 g	Malachite Green	0.25 mg
Monopotassium Phosphate.....	1.5 g	OADC Enrichment.....	100.0 mL
Agar.....	13.5 g	Glycerol.....	5.0 mL

*Adjusted and/or supplemented as required to meet performance criteria.

OADC Enrichment

Approximate Formula* Per Liter Purified Water

Sodium Chloride.....	8.5 g
Bovine Albumin (Fraction V)	50.0 g
Dextrose.....	20.0 g
Catalase.....	0.03 g
Oleic Acid.....	0.6 mL

*Adjusted and/or supplemented as required to meet performance criteria.

Middlebrook 7H11 Agar is Middlebrook 7H10 Agar modified by the addition of 1.0 g/L pancreatic digest of casein.

7H11 Selective Agar is Middlebrook 7H11 Agar with the following antimicrobial agents added per liter:

Polymyxin B	200,000 units
Carbenicillin.....	50.0 mg
Amphotericin B	10.0 mg
Trimethoprim Lactate.....	20.0 mg

Warnings and Precautions

For *in vitro* Diagnostic Use

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"¹⁰⁻¹³ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared plates, specimen containers and other contaminated materials by autoclaving.

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.¹²

Storage Instructions: On receipt, store plates in the dark at 2 to 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 to 8°C until just prior to use may be inoculated up to the expiration date and incubated for up to 8 weeks. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration.

Exposure of these media to direct or indirect sunlight has been reported to promote the formation of formaldehyde, which inhibits growth of mycobacteria.¹⁴

SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.^{4,6,15-17}

PROCEDURE

Material Provided: As ordered. Middlebrook 7H10 // 7H10 Agar, Middlebrook 7H11 // 7H11 Selective Agar or Middlebrook 7H10 // 7H11 Selective Agar.

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

Specimens collected aseptically (surgically excised tissue, aspirations from closed lesions and sterile body fluids) from patients suspected of having a mycobacterial disease may be inoculated directly onto primary isolation media. Contaminated specimens must be subjected to a digestion-decontamination procedure.⁴

N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent for sputum specimens. For detailed decontamination and digestion procedures, consult appropriate references.^{4,6,15-17}

Following inoculation, keep the plates shielded from light and incubate in an inverted position (agar side up) at 35 – 37°C in a CO₂-enriched atmosphere for up to 8 weeks. Use a dissecting microscope to examine agar surface for colonies after 5 to 7 days of incubation and once a week thereafter for 8 weeks.

User Quality Control:

1. Examine plates for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. The following test strains are recommended:

TEST STRAIN	MEDIA	EXPECTED RESULT
<i>Mycobacterium tuberculosis</i> H37Ra ATCC™ 25177	7H10 7H11 7H11 Selective	Growth
<i>Mycobacterium kansasii</i> , Group I ATCC 12478	7H10 7H11 7H11 Selective	Growth
<i>Mycobacterium scrofulaceum</i> , Group II ATCC 19981	7H10 7H11 7H11 Selective	Growth
<i>Mycobacterium intracellulare</i> , Group III ATCC 13950	7H10 7H11	Growth
<i>Mycobacterium fortuitum</i> , Group IV ATCC 6841	7H10 7H11 7H11 Selective	Growth
<i>Escherichia coli</i> ATCC 25922	7H11 Selective	Inhibition (partial to complete).
<i>Candida albicans</i> ATCC 10231	7H11 Selective	Inhibition (partial to complete).

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

RESULTS

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Colonies of mycobacteria are smooth to rough with filamentous extensions and may appear, upon microscopic examination, to be arranged in serpentine cords or other patterns. Some species produce yellow to red pigmentation. *M. tuberculosis* typically produces rough colonies with a "buff" tint arranged in serpentine cords. The way the colonies are arranged determines whether they appear opaque or translucent. Gram staining, biochemical testing and other procedures should be performed to confirm findings.

LIMITATIONS OF THE PROCEDURE

These prepared plated media are intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for further information.^{4,6,15-17}


A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

PERFORMANCE CHARACTERISTICS

Middlebrook 7H10 // 7H11 Selective Agar

Prior to release, all lots of Middlebrook 7H10/7H11 Selective Agar are tested to verify specific product characteristics. Samples are tested by spread plate with 0.01 mL of 7H9 broth suspensions of *Mycobacterium fortuitum* ATCC™ 6841, *M. intracellulare* ATCC 13950, *M. kansasii* ATCC 12478, *M. scrofulaceum* ATCC 19981 and *M. tuberculosis* ATCC 25177 (final inocula yield 10³ – 10⁴ CFUs per plate). Samples of 7H11 Selective Agar are also inoculated with 0.001 mL of saline dilutions of *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231 (final inocula yield 10³ – 10⁴ CFUs per plate). Plates are incubated at 35 – 37°C for up to 8 weeks in a CO₂-enriched atmosphere. Fair to heavy growth is observed with all mycobacteria. Failure to complete inhibition is observed with *E. coli* and *C. albicans* on 7H11 Selective Agar.

AVAILABILITY

Cat. No.	Description
295964	BBL™ Middlebrook 7H10/7H10 Agar, Pkg. of 20 plates
297250	BBL™ Middlebrook 7H11/7H11 Selective Agar, Pkg. of 20 plates
298292	BBL™ Middlebrook 7H10/7H11 Selective Agar, Ctn. of 100 plates 

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