

**QUALITY CONTROL PROCEDURES****I INTRODUCTION**

Seven H11 Agar is a culture medium for the isolation and cultivation of mycobacteria.

**II PERFORMANCE TEST PROCEDURE****A. Procedure for Preparation of Inocula**

1. Inoculate Lowenstein-Jensen Medium slants with stock cultures of the pertinent mycobacterial strains using sterile inoculating sticks.
2. Incubate tubes with loosened caps in an aerobic atmosphere supplemented with carbon dioxide at  $35 \pm 2$  °C until heavy growth is obtained (usually within 2 – 3 weeks).
3. Harvest the growth with a sterile sharpened applicator stick by gently removing the cells from the surface of the medium with care being taken not to include culture medium with the cell crop.
  - a. For *Mycobacterium tuberculosis* ATCC® 25177:
    - (1) Transfer growth to 5.0 mL Middlebrook 7H9 Broth with Glycerol in a sterile screw-capped glass tube containing sterile glass beads.
    - (2) Vortex well (several minutes) until suspension is free of large clumps.
    - (3) Compare this suspension to a McFarland #1 nephelometer standard. The suspension should be more turbid than the standard.
    - (4) Place the tube in a rack for 2 – 3 h at room temperature to allow large particles to settle to the bottom.
    - (5) Transfer the supernatant to a sterile container.
    - (6) Adjust the turbidity of the suspension to the McFarland #1 standard by slowly adding sterile Middlebrook 7H9 Broth with Glycerol. Shake well.
    - (7) Dilute to 105 CFU/mL before use. Mix well and streak-inoculate the test medium using a 0.01 mL calibrated loop.
  - b. For all other mycobacterial strains:
    - (1) Transfer the growth to a sterile 50 mL screw-capped centrifuge tube containing 8 – 12 sterile glass beads (2 mm diameter) and 5 mL of Mycobacterium Diluent prepared as follows:
      - Mix the following ingredients in a 1L flask and adjust the pH, using 1N sodium hydroxide, to 6.7 to 7.0
      - Bovine Albumin (fatty acid free).....1.0 g
      - Polysorbate 80 .....0.1 mL
      - Purified Water.....500 mL
      - Sterilize by membrane filtration (0.2 µ filter)
      - Aseptically dispense in 5.5 mL amounts into sterile screw-capped tubes.
    - (2) Emulsify the mycobacterial growth on the sidewall of a screw-capped centrifuge tube using an applicator stick. Mix the growth with the diluent.
    - (3) Cap the tube and “vortex” approximately 10 min until the growth is well suspended and free of large clumps.
    - (4) Add 15 mL of sterile Mycobacterium Diluent and mix thoroughly.
    - (5) Compare this suspension to a McFarland #1 nephelometer standard. The suspension should be more turbid than the standard.
    - (6) Place the tube in a rack for 2 – 3 h at room temperature to allow large particles to settle to the bottom.
    - (7) Aspirate the supernatant and transfer it to a sterile container. The suspension must be more turbid than a McFarland #1 standard and free of large particles. If large particles still are present, mix and allow to stand for an additional 1 h. Transfer the supernatant to a sterile container.
    - (8) Adjust the turbidity of the suspension to the McFarland #1 standard by slowly adding sterile Mycobacterium Diluent. Shake well.
    - (9) Dispense aliquots of the suspension into freezer vials labeled to contain organism identification and date of preparation.
    - (10) Freeze the suspensions by placing the vials in a low-temperature freezer at -60 °C. The vials can be stored for up to 6 months.
    - (11) For use, remove the frozen vial from the freezer and quick-thaw the contents by placing the tube in a 30 – 35 °C water bath. Dilute to 105 CFU/mL before use. Mix well and streak-inoculate the test medium using a 0.01 mL calibrated loop.

**B. Procedure for Testing Medium**

1. Inoculate representative samples with the cultures listed below.
  - a. Inoculate the test containers with a sterile disposable 0.01 mL calibrated loop using cultures prepared as described above.
  - b. Incubate containers with loosened caps at  $35 \pm 2$  °C in an aerobic atmosphere supplemented with carbon dioxide.
  - c. Include containers of previously tested Middlebrook 7H10 Agar as controls.
2. Examine tubes after 7, 14 and, if necessary, 21 days for growth and selectivity.

### 3. Expected Results

Organisms	ATCC	Recovery
* <i>Mycobacterium tuberculosis</i> H37Ra	25177	Growth
* <i>Mycobacterium kansasii</i> , Group I	12478	Growth
* <i>Mycobacterium scrofulaceum</i> , Group II	19981	Growth
* <i>Mycobacterium intracellulare</i> , Group III	13950	Growth
* <i>Mycobacterium fortuitum</i> , Group IV	6841	Growth

\*Recommended organism strain for User Quality Control.

**NOTE:** Must be monitored by users, according to CLSI M22-A3.

### III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20 – 25 °C and 30 – 35 °C and examine after 7 days for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

Seven H11 Agar is used in qualitative procedures for the isolation and cultivation of mycobacteria.

### V SUMMARY AND EXPLANATION

Many culture media have been devised for the cultivation of mycobacteria. The early ones were egg-based formulations and included Lowenstein-Jensen Medium and Petraghani Medium. Dubos and Middlebrook were instrumental in the development of a number of formulations that contained oleic acid and albumin as key ingredients to aid in the growth of the tubercle bacilli and to protect the organisms against a variety of toxic agents.<sup>1</sup> Subsequently, Middlebrook and Cohn improved the formulation of oleic acid-albumin agar and obtained faster, more luxuriant growth of *Mycobacterium* species on their medium designated as 7H10.<sup>2,3</sup>

Cohn et al. modified the 7H10 Agar formulation by the addition of one gram of pancreatic digest of casein per liter in order to enhance the growth of strains of *Mycobacterium tuberculosis* that were observed to grow poorly (or not at all) on 7H10 and other conventional isolation media.<sup>4</sup> This formulation is designated as Seven H11 Agar.

### VI PRINCIPLES OF THE PROCEDURE

Seven H11 Agar contains a variety of inorganic salts that provide substances essential for the growth of mycobacteria. The sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. Glycerol is an abundant source of carbon and energy. The pancreatic digest of casein is a rich source of nitrogen for the growth of tubercle bacilli and provides a number of additional growth factors.<sup>1</sup> Oleic acid, as well as other long-chain fatty acids, can be utilized by tubercle bacilli and plays an important role in the metabolism of mycobacteria. The primary effect of albumin is that of protection of the tubercle bacilli against toxic agents and, therefore, it enhances their recovery on primary isolation. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

### VII REAGENTS

#### Seven H11 Agar

Approximate Formula\* Per 900 mL Purified Water

Pancreatic Digest of Casein .....	1.0 g
Magnesium Sulfate.....	0.05 g
Ferric Ammonium Citrate .....	0.04 g
Sodium Citrate .....	0.4 g
Ammonium Sulfate .....	0.5 g
Monosodium Glutamate .....	0.5 g
Disodium Phosphate .....	1.5 g
Monopotassium Phosphate .....	1.5 g
Agar .....	13.5 g
Pyridoxine .....	1.0 mg
Zinc Sulfate .....	1.0 mg
Copper Sulfate .....	1.0 mg
Biotin .....	0.5 mg
Calcium Chloride .....	0.5 mg
Malachite Green .....	0.25 mg

The complete medium in prepared tubes, in addition to the ingredients listed above, contains, per liter, 5 mL of glycerol and the components of Middlebrook OADC Enrichment, namely:

Sodium Chloride .....	0.85 g
Dextrose .....	2.0 g
Bovine Albumin (Fraction V) .....	5.0 g
Catalase .....	3.0 mg
Oleic Acid .....	0.06 mL

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

**Warnings and Precautions:** For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>5-8</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

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.<sup>7</sup>

**Storage Instructions:** On receipt, store tubes in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times including up to 8 weeks for mycobacteriology media. Allow the medium to warm to room temperature before inoculation.

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

## VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.<sup>9-12</sup> Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Seven H11 Agar

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

**Test Procedure:** Observe aseptic techniques.

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.<sup>9</sup> N-Acetyl-L-cysteine-sodium hydroxide (NALC- NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.<sup>9-12</sup>

Following inoculation, keep tubes shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide and incubate at 35 ± 2 °C.

Slanted media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes should have screw caps loose for the first 3 weeks to permit circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosen briefly once a week. Stand tubes upright if space is a problem.

**NOTE:** Cultures from skin lesions suspected to contain *M. marinum* or *M. ulcerans* should be incubated at 25 – 33 °C for primary isolation; cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40 – 42 °C.<sup>9</sup> Incubate a duplicate culture at 35 – 37 °C.

**User Quality Control:** See "Quality Control Procedures."

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 CLSI guidance and CLIA regulations for appropriate Quality Control practices.

## X RESULTS

Cultures should be read within 5 – 7 days after incubation and once a week thereafter for up to 8 weeks.

Record Observations<sup>9</sup>

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days. Slow growers require more than 7 days for mature colony forms.
2. Pigment production  
White, cream or buff = Nonchromogenic (NC)  
Lemon, yellow, orange, red = Chromogenic (Ch)  
Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

## XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>10-12</sup>

## XII PERFORMANCE CHARACTERISTICS

Cohn et al. performed a study comparing Seven H11 Agar to 7H10 Agar. Of 96 clinical isolates of *M. tuberculosis* tested, 13 failed to grow on the 7H10 medium in the first three weeks. Ten of the 13 cultures grew on Seven H11 in three weeks; the other three required an additional three-week incubation for the appearance of visible growth.<sup>4</sup>

## XIII AVAILABILITY

**Cat. No. Description**

- 221391 **BD BBL™** Seven H11 Agar Slants, Pkg. of 10 size A tubes  
221392 **BD BBL™** Seven H11 Agar Slants, Ctn. of 100 size A tubes  
296105 **BD BBL™** Seven H11 Agar Slants, Pkg. of 10 size C tubes  
297704 **BD BBL™** Seven H11 Agar Slants, Ctn. of 100 size C tubes

## XIV REFERENCES

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