QUALITY CONTROL PROCEDURES

I INTRODUCTION

Middlebrook and Cohn 7H10 Agar is a culture medium for the 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 ± 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 10^5 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 vial in a 30–35 °C water bath. Dilute to 10^5 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. Assure that the agar surfaces are free of moisture before inoculation.
   b. Using sterile disposable 0.01 mL calibrated loops, inoculate test containers with mycobacterial cultures prepared as described above.
   c. Incubate all containers with loosened caps at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
   d. Include tubes of previously tested Middlebrook 7H10 Agar as controls.
2. Examine containers after 7, 14 and, if necessary, 21 days for growth, selectivity and pigmentation.

3. Expected Results

Organisms	ATCC	Recovery
**Mycobacterium tuberculosis* H37Ra	25177	Growth
**Mycobacterium kansasii*, Group I	12478	Growth
**Mycobacterium scrofulaceum*, Group II	19981	Growth
**Mycobacterium intracellulare*, Group III	13950	Growth
**Mycobacterium fortuitum*, 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 or bottles as described under "Product Deterioration."
2. Visually examine representative tubes or bottles to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes or bottles at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Middlebrook and Cohn 7H10 Agar is used in qualitative procedures for the isolation and cultivation of mycobacteria.

V SUMMARY AND EXPLANATION

Over the years, many culture media have been devised for the cultivation of mycobacteria. The early ones were egg-based formulations and included Lowenstein-Jensen Medium and Petragnani Medium. Dubos and Middlebrook were instrumental in the development of a number of formulations which 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.[1] 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.[2,3] It has been reported that the 7H10 medium tends to grow fewer contaminants than the egg-based media commonly used for the cultivation of mycobacteria.[4]

VI PRINCIPLES OF THE PROCEDURE

Middlebrook and Cohn 7H10 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. 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. Catalase destroys toxic peroxides that may be present in the medium. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

VII REAGENTS

Middlebrook and Cohn 7H10 Agar

Approximate Formula* Per Liter Purified Water

Magnesium Sulfate	0.05 g	Bovine Albumin (Fraction V)	5.0 g
Ferric Ammonium Citrate	0.04 g	Catalase	3.0 mg
Sodium Citrate	0.4 g	Pyridoxine	1.0 mg
Ammonium Sulfate	0.5 g	Zinc Sulfate	1.0 mg
Monosodium Glutamate	0.5 g	Copper Sulfate	1.0 mg
Disodium Phosphate	1.5 g	Biotin	0.5 mg
Monopotassium Phosphate	1.5 g	Calcium Chloride	0.5 mg
Agar	13.5 g	Malachite Green	0.25 mg
Sodium Chloride	0.85 g	Oleic Acid	0.06 mL
Dextrose	2.0 g	Glycerol	5.0 mL

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

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

Tubes and bottles 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"[5-8] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared tubes, 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.[7]

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

Product Deterioration: Do not use tubes or bottles 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.[9-11] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Middlebrook and Cohn 7H10 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.[9] 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.[9-12]

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

Slanted and bottled media should be inoculated in a horizontal plane until the inoculum is absorbed. Tubes and bottles 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 be *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.[9] 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 inoculation and once a week thereafter for up to 8 weeks.

Record Observations:[9]

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. Number of colonies (bottles):
   No colonies = Negative
   Less than 50 colonies = Actual Count
   50–100 colonies = 1+
   100–200 colonies = 2+
   Almost confluent (200–500) = 3+
   Confluent (more than 500) = 4+
3. 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.

Bottles may be examined by inverting the bottles on the stage of a dissecting microscope. Read at 10–60x with transmitted light. Scan rapidly at 10–20x for the presence of colonies. Higher magnification (30–60x) is helpful in observing colony morphology, i.e., serpentine cord-like colonies.

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.[9-12]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Middlebrook and Cohn 7H10 Agar containers are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are streak-inoculated with cultures diluted to contain 10^5 colony- forming units (CFU) per mL of *Mycobacterium kansasii* Group I (ATCC 21478), *M. scrofulaceum* Group II (ATCC 19981), *M. intracellulare* Group III (ATCC 13950), *M. fortuitum* Group IV (ATCC 6841) and *M. tuberculosis* (ATCC 25177). After inoculation, the containers are incubated with loosened caps at 35 ± 2 °C in an atmosphere supplemented with 5–10% carbon dioxide. Containers are read for growth and pigmentation after 7, 14 and 21 days incubation. All organisms exhibit moderate to heavy growth within 21 days. Colonial pigmentation is as follows: *M. kansasii* is white to cream yellow; *M. scrofulaceum* is medium yellow to orange; *M. tuberculosis*, *M. intracellulare* and *M. fortuitum* are cream colored.

XIII AVAILABILITY

Cat. No.	Description
220958	**BD BBL™** Middlebrook and Cohn 7H10 Agar Slants, Pkg. of 10 size A tubes
220959	**BD BBL™** Middlebrook and Cohn 7H10 Agar Slants, Ctn. of 100 size A tubes
297448	**BD BBL™** Middlebrook and Cohn 7H10 Agar Slants, Pkg. of 10 size C tubes
297396	**BD BBL™** Middlebrook and Cohn 7H10 Agar Slants, Ctn. of 100 size C tubes
297274	**BD BBL™** Middlebrook and Cohn 7H10 Agar, 1 oz. Bottles, Ctn. of 100

XIV REFERENCES

1. Dubos, R.J., and G. Middlebrook. 1947. Media for tubercle bacilli. Am. Rev. Tuberc. *56*:334-345.
2. Middlebrook, G., and M.L. Cohn. 1958. Bacteriology of tuberculosis: laboratory methods. Am. J. Pub. Health. *48*:844-853.
3. Middlebrook, G., M.L. Cohn, and W.B. Dye, W.B. Russell, Jr., and D. Levy. 1960. Microbiologic procedures of value in tuberculosis. Acta Tuberc. Scand. *38*:66-81.
4. Kubica, G.P., and W.E. Dye. 1967. Laboratory methods for clinical and public health mycobacteriology. PHS Publication No. 1547. U.S. Government Printing Office, Washington, D.C.
5. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, Pa.
6. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. *17*:53-80.
7. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
8. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
9. Kent, P.T., and G.P. Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta.
10. Cernoch, P.L., R.L. Enns, M.A. Saubolle, and R.J. Wallace, Jr. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
11. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
12. Metchock, B.G., F.S. Nolte, and R.J. Wallace, Jr. 1999. *Mycobacterium*, p. 399-437. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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