QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Middlebrook 7H9 Broth with Glycerol is a nonselective 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 (5–10%) 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 (10^8 CFU/mL) by slowly adding sterile Middlebrook 7H9 Broth with Glycerol. Shake well.
      (7) Dilute to 10^5 CFU/mL before use. Mix well and 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 1 L flask and adjust the pH, using 1N sodium hydroxide, to 6.7–7.0

           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 centrifuge 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 (10^8 CFU/mL) 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 10^5 CFU/mL before use. Mix well and 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. Using sterile disposable 0.01 mL calibrated loops, inoculate the tubes using cultures prepared as described above.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
2. Examine tubes after 7, 14 and, if necessary, 21 days for growth and pigmentation.
3. Expected Results

CLSI 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.

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 aerobically at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Middlebrook 7H9 Broth with Glycerol is a supplemented medium which supports the growth of mycobacteria, including *M. tuberculosis*. It is used primarily for growth of pure cultures of mycobacteria for use in laboratory studies.

V SUMMARY AND EXPLANATION

Middlebrook and coworkers developed the 7H9 broth base formulation during the same time period in which they devised the 7H10 agar base.[1-3] Both media types support the growth of mycobacterial species, when supplemented with nutrients such as glycerol, oleic acid, albumin, and dextrose, except for *M. bovis* which is inhibited by glycerol.

This medium is used in the preparation of inocula for antimicrobial assays, as a basal medium for biochemical tests and for the subculture of stock strains.

VI PRINCIPLES OF THE PROCEDURE

The large number of inorganic salts in this medium provide substances essential for the growth of mycobacteria. Sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. The sodium chloride, bovine albumin, dextrose and catalase are components of Middlebrook ADC Enrichment. The albumin acts as a protective agent by binding free fatty acids, which may be toxic to *Mycobacterium* species. Catalase destroys toxic peroxides that may be present in the medium. Dextrose is an energy source. Sodium chloride provides essential electrolytes. Supplementation with glycerol provides improved growth of mycobacteria.

VII REAGENTS

Middlebrook 7H9 Broth

Approximate Formula* Per Liter Purified Water

Monopotassium Phosphate	2.0 g	Ferric Ammonium Citrate	0.04 g
Disodium Phosphate	1.5 g	Magnesium Sulfate	0.05 g
Monosodium Glutamate	0.5 g	Zinc Sulfate	0.001 g
Sodium Citrate	0.1 g	Copper Sulfate	0.001 g
Ammonium Sulfate	0.5 g	Biotin	0.5 mg
Pyridoxine	0.001 g	Calcium Chloride	0.5 mg

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

The complete medium in prepared tubes, in addition to the ingredients listed above, contains, per liter, 2 mL of glycerol and the components of ADC enrichment, namely:

Sodium Chloride	0.85 g
Bovine Albumin (Fraction V)	5.0 g
Dextrose	2.0 g
Catalase	4.0 mg
Sodium Pyruvate	1.0 g

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

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

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.[4]

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

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.[5,6] 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 7H9 Broth with Glycerol

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

Test Procedure: Observe aseptic techniques.

Following inoculation, place tubes in a suitable system providing an aerobic atmosphere enriched with 5–10% carbon dioxide. Incubate at 35 ± 2 °C for up to 8 weeks. Keep the caps of the tubes loosened 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.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

Cultures should be read within 5–7 days after inoculation and once a week thereafter for up to 8 weeks. Mycobacterial growth from the broth tubes can be utilized for additional laboratory test procedures as required.

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.[5-7]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Middlebrook 7H9 Broth with Glycerol are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are inoculated with cultures diluted to contain 10^3 colony-forming units (CFU) per 0.01 mL of *Mycobacterium kansasii* Group I (ATCC 12478), *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 tubes are incubated with loosened caps at 35 ± 2 °C in an atmosphere supplemented with 5–10% carbon dioxide. Tubes are read for growth after 7, 14 and 21 days incubation. All organisms exhibit moderate to heavy growth within 21 days.

XIII AVAILABILITY

Cat. No.	Description
221832	**BD BBL™** Middlebrook 7H9 Broth with Glycerol, 5 mL

XIV REFERENCES

1. Middlebrook, G. 1955. Fitzsimmons Army Hospital Report No. 1, Denver.
2. Middlebrook, G., and M.L. Cohn. 1958. Bacteriology of tuberculosis: laboratory methods. Am. J. Public Health. *48*:844-853.
3. Middlebrook, G., M.L. Cohn, and W.B. Schaefer. 1954. Studies on isoniazid and tubercle bacilli. III. The isolation, drug-susceptibility and catalase-testing of tubercle bacilli from isoniazid-treated patients. Am. Rev. Tuberc. *70*:852-872.
4. U.S. Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott’s diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
7. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey’s Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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