QUALITY CONTROL PROCEDURES

I INTRODUCTION

Mycobactosel L-J Medium is a selective medium for the isolation of mycobacteria from specimens containing mixed flora.

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 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 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 Mycobaterium 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 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 slant surfaces using sterile disposable 0.01 mL calibrated loops with mycobacterial cultures prepared as described above and 18- to 24-h **Trypticase™** Soy Broth cultures of the other organism strains diluted to 10^6 CFU/mL before use.
   b. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere supplemented with 5–10% carbon dioxide.
   c. Include tubes of previously tested **Mycobactosel** L-J Medium as controls for the mycobacterial species, a nonselective mycology agar slant for the *Saccharomyces* culture and tubed slants of **Trypticase** Soy Agar as controls for all other non-mycobacterial organisms.
2. Examine tubes after 7, 14, and if necessary, 21 days for amount of growth and inhibition.
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
**Escherichia coli*	25922	Inhibition (partial to complete)
Additional Organisms		
Bacillus subtilis	6633	No growth to trace growth
Saccharomyces cerevisiae	9763	No growth to trace growth
Staphylococcus epidermidis	12228	No growth to trace growth
Staphylococcus aureus	25923	No growth to trace 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. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.0 ± 0.2.
4. Incubate uninoculated representative tubes aerobically at 20–25°C and 30–35°C and examine after 7 and 14 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Mycobactosel L-J Medium is used in qualitative procedures for the isolation of mycobacteria from clinical specimens.

V SUMMARY AND EXPLANATION

Mycobactosel L-J Medium is Lowenstein-Jensen Medium[1-3] plus cycloheximide, lincomycin and nalidixic acid for use with specimens likely to contain many contaminating organisms. The authors, Petran and Vera, reported that the medium permits isolation of mycobacteria from a few more specimens and reduced, by 68%, the number of cultures which otherwise would have been reported as unsatisfactory as the result of overgrowth by contaminants.[4]

VI PRINCIPLES OF THE PROCEDURE

This medium contains a variety of inorganic salts or other nitrogen sources, which provide substances essential for the growth of mycobacteria. The glycerol is an abundant source of carbon and energy. Asparagine is added to promote the initiation of growth and increase the growth rate. Egg yolk is a source of lipids for the metabolism of mycobacteria. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

Cycloheximide suppresses the growth of saprophytic fungi. Lincomycin inhibits gram-positive bacteria. Nalidixic acid inhibits some of the gram-negative bacteria encountered in clinical specimens.

VII REAGENTS

Mycobactosel L-J Medium

Approximate Formula* Per 600 mL Purified Water

Ingredient	Amount	Unit
Monopotassium Phosphate	2.5	g
Magnesium Sulfate	0.24	g
Sodium Citrate	0.6	g
L-Asparagine	3.6	g
Potato Flour	30.0	g
Malachite Green	0.4	g
Cycloheximide	0.64	g
Lincomycin	0.0032	g
Nalidixic Acid	0.056	g
Glycerol	12.0	mL
Whole Egg	1.0	L

*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"[5-8] 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.[7]

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.[9,10] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Mycobactosel L-J Medium Slants or **Mycoflask™** Bottles

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.[11] N-Acetyl-L-cysteine-sodium hydroxide (NALC- NaOH) solution is recommended as a gentle, but effective, digesting and decontaminating agent. For detailed decontamination and culturing instructions, consult an appropriate reference.[9-12]

Following inoculation, keep test containers shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide (5–10%). 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 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.[5] 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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed, bottled and **Mycoflask** brand media. The tip of the electrode should be placed in the central portion of the agar mass in solid media.

X RESULTS

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

Record Observations[11]

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.[9,10,13-16]

XII AVAILABILITY

Cat. No.	Description
221413	**BBL™ Mycobactosel™** L-J Medium Slants, Pkg. of 10 size A tubes
221414	**BBL™ Mycobactosel™** L-J Medium Slants, Ctn. of 100 size A tubes
297181	**BBL™ Mycobactosel™** L-J Medium, Ctn. of 100 **Mycoflask™** bottles

XIII REFERENCES

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Becton, Dickinson and Company
7 Loveton Circle
Sparks, Maryland 21152 USA
800-638-8663

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