BBL™ Lowenstein-Jensen Medium
BBL™ Lowenstein-Jensen Medium with 5% Sodium Chloride

L007464 • Rev. 08 • January 2007

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Lowenstein-Jensen Medium is used for the isolation and cultivation of mycobacteria. The medium tubed as deeps is used for the semi-quantitative catalase test as an aid to the classification 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 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 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 10^5 CFU/mL before use. Mix well and streak-inoculate the test medium using a 0.01 mL calibrated loop.

B. Procedures for Testing Media

Lowenstein-Jensen Medium Deeps

1. Inoculate butt surfaces with a sterile disposable 0.01 mL inoculating loop using cultures prepared as described above.
2. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
3. After 14 days incubation, to each culture add 1.0 mL of polysorbate 80-peroxide mixture prepared as follows:
   a. 30% hydrogen peroxide. Store in refrigerator.
   b. 10% polysorbate 80 prepared as follows:
      (1) Mix 10 mL polysorbate 80 with 90 mL purified water.
      (2) Autoclave at 121°C for 10 min.

(3) Store in refrigerator.

c. Immediately before performing the test, mix equal parts of the two solutions.

4. Keep the cultures in an upright position for 5 min at room temperature.
5. Measure (mm) the height of the column of bubbles above the surface of the medium.
6. Expected Results

Column of bubbles greater than 45 mm in height.

**Mycobacterium kansasii*, Group I
ATCC 12478

Mycobacterium scrofulaceum, Group II
ATCC 19981

Mycobacterium fortuitum, Group IV
ATCC 6841

Column of bubbles less than 45 mm in height.

**Mycobacterium tuberculosis* H37Ra
ATCC 25177

Mycobacterium intracellulare, Group III
ATCC 13950

*Recommended organism strain for User Quality Control.

Lowenstein-Jensen Medium Slants and Bottles

1. Inoculate representative samples with the cultures listed below.
   a. Using sterile disposable 0.01 mL calibrated loops, inoculate the slants or bottles using mycobacterial cultures prepared as described above.
   b. Incubate containers with loosened caps at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
2. Examine tubes or bottles after 7, 14 and 21 days for growth, selectivity and pigmentation.
3. Expected Results
   a. For Lowenstein-Jensen Medium

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

   b. For Lowenstein-Jensen Medium with 5% Sodium Chloride

Organisms	ATCC	Recovery
**Mycobacterium fortuitum*	6841	Growth
**Mycobacterium kansasii*	12478	No Growth

*Recommended organism strain for User Quality Control.

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. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.0 ± 0.2.
4. Incubate uninoculated representative tubes or bottles at 20–25°C and 30–35°C and examine after 7 and 14 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Lowenstein-Jensen Medium is used for the cultivation of *Mycobacterium tuberculosis* and other mycobacterial species.

V SUMMARY AND EXPLANATION

Lowenstein originally formulated a medium for cultivation of mycobacteria in which congo red and malachite green were incorporated for the partial inhibition of other bacteria.[1,2] These dyes were similarly used by other investigators, notably Sonnenschein[3] and Hohn.[4] In the United States the gentian violet media of Corper[5] and Petroff[6] were popular, along with the medium of Petragnani, which contained malachite green. The present formula, developed by Jensen,[7] has a slightly different citrate and phosphate content, does not contain congo red, and has an increased malachite green concentration.

BBL prepared products of Lowenstein-Jensen Medium include tubed slants for general use in the cultivation of *Mycobacterium* species, bottles for use when a greater surface area is desired and tubed deeps for the performance of the semi-quantitative catalase test. This latter procedure was developed by Wayne[8] and is useful for the classification of mycobacteria.

Additionally, the medium is available with the addition of 5% sodium chloride since the ability to tolerate 5% sodium chloride is a characteristic of certain strains of mycobacteria (e.g., *M. fortuitum* and *M. chelonae* subsp. *abscessus*).[9] Most rapid growers, the slowly growing *M. triviale* and some strains of *M. flavescens* also grow on NaCl-containing media. The inability of *M. chelonae* subsp. *chelonae* to grow helps differentiate it from other members of the *M. fortuitum* complex (e.g., *M. chelonae* subsp. *abscessus*).[9,10]

VI PRINCIPLES OF THE PROCEDURE

Lowenstein-Jensen Medium Base is a relatively simple formulation that requires supplementation in order to support the growth of mycobacteria. Glycerol and egg mixture are added prior to the inspissation process. These substances provide fatty

acids and protein required for the metabolism of mycobacteria. The coagulation of the egg albumin during sterilization provides a solid medium for inoculation purposes.

VII REAGENTS

Lowenstein-Jensen Medium

Approximate Formula* Per 600 mL Purified Water

Monopotassium Phosphate	2.5 g	Potato Flour	30.0 g
Magnesium Sulfate	0.24 g	Malachite Green	0.4 g
Sodium Citrate	0.6 g	Glycerol	12.0 mL
L-Asparagine	3.6 g	Whole Egg	1000.0 mL

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

Lowenstein-Jensen Medium with 5% Sodium Chloride contains the above ingredients with, per 600 mL, 80 g of sodium chloride.

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"[11-14] 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.[13]

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.[15,16] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Lowenstein-Jensen Medium or Lowenstein-Jensen Medium with 5% Sodium Chloride

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.[10] 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.[10,16-18]

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

Slanted and bottled media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes and bottles should have screw caps loose for the first 3 weeks to permit the 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.[10] Incubate a duplicate culture at 35–37°C.

The recommended procedure for the semi-quantitative catalase test using agar deeps of Lowenstein-Jensen Medium is as follows:[10]

1. Inoculate the surface of the medium with either 0.1 mL of a 7-day broth culture or a loopful of growth from an actively growing slant of each test strain. Also inoculate tubes with a strong catalase-producing culture, e.g., *M. kansasii*, and a weak enzyme strain, e.g., *M. intracellulare*, as controls.
2. Incubate, with cap loosened, at 35 ± 2°C for 2 weeks.
3. Prepare a polysorbate 80-peroxide mixture by admixing equal parts of:
   a. An autoclaved 10% solution of polysorbate 80 in distilled water or a dilution of 1 mL of sterile polysorbate 80 in 9 mL of distilled water.
   b. Hydrogen peroxide (30%).
4. Add 1 mL of the polysorbate 80-peroxide mixture to each culture. After 5 min, record the height in mm of the columns of bubbles.

The recommended procedure for the sodium chloride tolerance test is as follows:[10,17]

1. Make a suspension of an actively growing subculture in Middlebrook 7H9 Broth equal to a McFarland no. 1 turbidity standard.

2. Inoculate 0.1 mL of the standardized culture onto a slant of Lowenstein-Jensen Medium with 5% Sodium Chloride. Similarly inoculate a slant of the medium without the NaCl as a growth control tube.
3. Incubate with caps loose in a CO_2-enriched atmosphere, initially in a flat rack for 1 week at 28–30°C for rapid growers or 35 ± 2°C for slow growers.
4. Examine weekly for growth. Continue incubation for three more weeks if necessary.

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.

X RESULTS

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

Record Observations:

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.

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.

In the semi-quantitative catalase test, most mycobacteria fall into two groups.[8,10,16]

1. Column of bubbles more than 45 mm in height.
   M. chelonae
   M. fortuitum
   M. gordonae
   M. kansasii (clinically significant)
   M. scrofulaceum
2. Column of bubbles less than 45 mm in height.
   M. avium
   M. bovis
   M. gastri
   M. haemophilum
   M. intracellulare
   M. kansasii (clinically insignificant)
   M. malmoense
   M. marinum
   M. tuberculosis
   M. xenopi

The presence or absence of growth in the tube of medium containing 5% NaCl aids in the differentiation of mycobacterial isolates. The salt tolerance test is positive when numerous colonies appear on the control medium and more than 50 colonies grow on the medium containing 5% NaCl.[10,17] Colonies on the control medium, but no visible growth on the test medium after a total of 4 weeks of incubation constitutes a negative test.[10,16,17]

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.[15,16,19]

XII PERFORMANCE CHARACTERISTICS

Lowenstein-Jensen Medium

In a study by Palaci et al., 85 respiratory specimens were inoculated onto Lowenstein-Jensen (LJ) slants and into **BBL™ MGIT™** tubes by standard procedures. Twenty-five (25) specimens were found to be positive for *M. tuberculosis*. Culture sensitivity for both LJ and **MGIT** was 96.1% (25 of 26 positive cultures). Although the time to detection was significantly shorter in the **MGIT** tubes, there was no significant difference in the sensitivity of detection of *M. tuberculosis* between the two methods.[20]

Lowenstein-Jensen Medium Deeps

Prior to release, all lots of Lowenstein-Jensen Medium Deeps are tested for specific product characteristics. Samples are tested with *M. fortuitum* ATCC 6841, *M. intracellulare* ATCC 13950, *M. kansasii* ATCC 12478, *M. scrofulaceum* ATCC 19981 and *M. tuberculosis* ATCC 25177 by inoculating with 0.2 mL of Middlebrook 7H9 Broth suspensions. Tubes are incubated with loose caps for up to 3 weeks at 35–37°C. A polysorbate 80-peroxide mixture is prepared and 1 mL is added to each culture. After 5 min, the height in mm of the columns of bubbles is recorded. A positive catalase reaction is a column of bubbles greater than 45 mm in height. A negative reaction is a column of bubbles less than 45 mm in height. A positive catalase reaction is observed with *M. fortuitum*, *M. kansasii* and *M. scrofulaceum*. A negative catalase reaction is observed with *M. intracellulare* and *M. tuberculosis*.

Lowenstein-Jensen Medium with 5% Sodium Chloride

Prior to release, all lots of Lowenstein-Jensen Medium with 5% Sodium Chloride are tested for specific product characteristics. Samples are tested with cell suspensions of *M. fortuitum* ATCC 6841 and *M. kansasii* ATCC 12478 diluted in **BBL** Middlebrook 7H9 Broth to yield 10^3–10^4 CFU. Tubes are incubated with loose caps at 35–37°C for 7–14 days in a CO_2-enriched atmosphere. Moderate to heavy growth is observed with *M. fortuitum*. *M. kansasii* is inhibited.

XIII AVAILABILITY

Cat. No.	Description
221115	**BBL™** Lowenstein-Jensen Medium **Mycoflask™**, Pkg. of 10 bottles
221116	**BBL™** Lowenstein-Jensen Medium **Mycoflask™**, Ctn. of 100 bottles
220908	**BBL™** Lowenstein-Jensen Medium Slants, Pkg. of 10 size A tubes
220909	**BBL™** Lowenstein-Jensen Medium Slants, Ctn. of 100 size A tubes
221387	**BBL™** Lowenstein-Jensen Medium Slants, Pkg. of 10 size C tubes
221388	**BBL™** Lowenstein-Jensen Medium Slants, Ctn. of 100 size C tubes
221257	**BBL™** Lowenstein-Jensen Medium Deeps, Pkg. of 10 size A tubes
295701	**BBL™** Lowenstein-Jensen Medium, 1 oz. Bottle, Ctn. of 100 bottles
221896	**BBL™** Lowenstein-Jensen Medium with 5% Sodium Chloride Slants, Pkg. of 10 size C tubes

XIV REFERENCES

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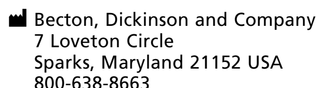

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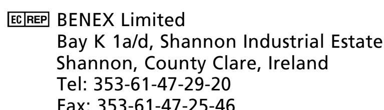

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