# Lowenstein Media
# Lowenstein Medium Base • Lowenstein-Jensen Medium • Lowenstein-Jensen Medium, Gruft Lowenstein-Jensen Medium with Iron Lowenstein-Jensen Medium with Pyruvic Acid Lowenstein-Jensen Medium with 5% Sodium Chloride

## Intended Use

Lowenstein Medium and Lowenstein-Jensen (LJ) Medium are used for the isolation and cultivation of mycobacteria and as bases for selective, differential and enriched media for mycobacteria.

LJ Medium, tubed as deeps, is used for the semi-quantitative catalase test.

LJ Medium, Gruft, is a selective medium used for the isolation and cultivation of mycobacteria.

LJ Medium with Iron is used to determine iron uptake for differentiation and identification of mycobacteria.

LJ Medium with Pyruvic Acid is an enrichment medium used for enhanced growth of mycobacteria.

LJ Medium with 5% sodium chloride is used to characterize certain strains of mycobacteria.

## Summary and Explanation

LJ Medium is an inspissated, egg-based medium developed from Jensen's modification of Lowenstein's formula.[1,2]

Gruft modified LJ Medium by adding penicillin and nalidixic acid for selective isolation of mycobacteria.[3] Gruft also found that the addition of ribonucleic acid (RNA) increased the percentage of tubercle bacilli recovered from clinical specimens compared to recovery on the standard LJ Medium.[4]

Wayne and Doubek differentiated rapidly-growing from slow-growing mycobacteria based on iron intake.[5] The rapid-growing mycobacteria take up iron in the medium, producing rusty-brown colonies and a tan discoloration in the medium.[6] *M. chelonae* and slow-growing species do not take up the iron.[7]

Hughes [8] and Dixon and Cuthbert [9] reported that the addition of pyruvic acid to egg-based media resulted in improved recovery of tubercle bacilli compared to recovery on egg-based media supplemented only with glycerol. Dixon and Cuthbert recommended using pyruvic acid-egg medium in addition to media supplemented with glycerol for optimum recovery of tubercle bacilli from clinical specimens.[9]

Additionally, the medium is available with the addition of 5% sodium chloride. Most rapid growers, the slowly growing *M. triviale* and some strains of *M. flavescens* 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*).[6,10]

In the semi-quantitative catalase test, mycobacteria can be differentiated into groups, based upon catalase activity. [6,11,12]

## User Quality Control

### *Identity Specifications*

**Difco™ Lowenstein Medium Base**

| | |
|---|---|
| Dehydrated Appearance: | Medium to dark green-blue, free flowing, homogeneous. |
| Solution: | 37.4 g/600 mL solution containing 12 mL of glycerol, soluble in purified water upon boiling. Solution is opalescent, viscous, dark blue-green. |
| Prepared Appearance: | Opalescent, viscous, dark blue green. |

### *Cultural Response*

**Difco™ Lowenstein Medium Base**

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for up to 21 days.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY |
|---|---|---|---|
| *Escherichia coli* | 25922 | $10^3$-2×$10^3$ | Partial inhibition |
| *Mycobacterium tuberculosis* H37Ra | 25177 | $10^2$-3×$10^2$ | Good |
| *Mycobacterium tuberculosis* | 27294 | $10^2$-3×$10^2$ | Good |
| *Mycobacterium kansasii* Group I | 12478 | $10^2$-3×$10^2$ | Good |
| *Mycobacterium scrofulaceum* Group II | 19981 | $10^2$-3×$10^2$ | Good |
| *Mycobacterium intracellulare* Group III | 13950 | $10^2$-3×$10^2$ | Good |
| *Mycobacterium fortuitum* Group IV | 6841 | $10^2$-3×$10^2$ | Good |

Uninoculated Tube

*Mycobacterium fortuitum* ATCC™ 6841

## Principles of the Procedure

Lowenstein 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. Malachite green selectively inhibits contaminants.

Low-level concentrations of penicillin (50.0 units/mL) and nalidixic acid (35.0 mg/mL) are included in the LJ Medium, Gruft, to inhibit gram-positive as well as some gram-negative bacterial contaminants. The addition of RNA (0.05 mg/mL) enhances the recovery of tubercle bacilli.

In the iron uptake test, most rapid growers take up the iron salt in the medium (ferric ammonium citrate, 25 mg/mL), producing rusty brown colonies and a tan discoloration in the surrounding medium. Slow-growing species and most strains of *M. chelonae* do not take up the iron in the medium.[6,7]

Pyruvic acid (2.5 mg/mL) enhances the growth of tubercle bacilli.

The ability to tolerate 5% sodium chloride is a characteristic of certain strains of mycobacteria (e.g., *M. fortuitum* and *M. chelonae* subsp. *abscessus*).[10]

Catalase is an intracellular, soluble enzyme capable of splitting hydrogen peroxide into water and oxygen. The oxygen bubbles into the reaction mixture creating a column of bubbles. With a column height breakpoint of 45 mm, the mycobacteria can be divided into groups: those producing less than 45 mm (*M. tuberculosis, M. marinum, M. avium* complex and *M. gastri*); and those producing more than 45 mm *(M. kansasii, M. simiae*, most scotochromogens, the nonphotochromogenic saprophytes and the rapid growers).[6]

## Formula

**Difco™ Lowenstein Medium Base**

| Approximate Formula* Per 600 mL | | |
|---|---|---|
| Asparagine | 3.6 | g |
| Monopotassium Phosphate | 2.4 | g |
| Magnesium Sulfate | 0.24 | g |
| Magnesium Citrate | 0.6 | g |
| Potato Flour | 30.0 | g |
| Malachite Green | 0.4 | g |

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

## Precaution[13]

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.

## Directions for Preparation from Dehydrated Product

1. Suspend 37.4 g of the powder in 600 mL of purified water containing 12 mL of glycerol. Do not add glycerol if bovine tubercle bacilli or other glycerophobic organisms are to be cultivated. Mix thoroughly.
2. Heat with frequent agitation just until the medium boils.
3. Autoclave at 121°C for 15 minutes. Cool to approximately 50°C.
4. Meanwhile, prepare 1,000 mL of whole eggs collected aseptically and mixed thoroughly, without introducing air bubbles.
5. Admix base and egg gently until mixture is uniform and without bubbles.
6. Distribute in suitable sterile containers such as screw-capped tubes.
7. Arrange tubes in slanted position, then coagulate and inspissate at 85°C for 45 minutes.
8. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[6] 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.[6,7,12,14,15]

Following inoculation, keep test containers shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate 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; loosened 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.[6] Incubate a duplicate culture at 35-37°C.

For LJ Medium with Iron, specimens must first be isolated in pure culture on an appropriate solid medium. Inoculate LJ Medium with Iron with one drop of a barely turbid suspension of the culture to be tested.

For the semi-quantitative catalase test, 1 mL of a 1:1 mixture of 10% polysorbate 80 and 30% hydrogen peroxide is added to each inoculated tube after 2 weeks of incubation. The height of the column of bubbles is recorded after 5 minutes as <45 mm or >45 mm.[6,7]

## Expected 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 to 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 10-60× with transmitted light. Scan rapidly at 10-20× for the presence of colonies. Higher magnification (30-60×) is helpful in observing colony morphology; i.e., serpentine cord-like colonies.

Examine LJ Medium with Iron for rusty-brown colonies with a tan discoloration in the surrounding medium, indicating uptake of the iron.

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.[6,15] 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.[6,12,15]

In the semi-quantitate catalase test, mycobacteria fall into two groups with *M. tuberculosis* falling into the group producing a column of bubbles less than 45 mm in height.[6]

## Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased $CO_2$ tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of $CO_2$. Screw caps on tubes or bottles should be handled as directed for exchange of $CO_2$.

## References


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15. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.


## Availability

### Difco™ Lowenstein Medium Base

| | | |
|---|---|---|
| Cat. No. | 244420 | Dehydrated – 500 g |
| | 244410 | Dehydrated – 2 kg |

### BBL™ Lowenstein-Jensen Medium

BS12 CMPH2 EP MCM9 SMWW

| | | |
|---|---|---|
| Cat. No. | 220908 | Prepared Slants (A Tubes) – Pkg. of 10* |
| | 220909 | Prepared Slants (A Tubes) – Ctn. of 100* |
| | 221387 | Prepared Slants (C Tubes) – Pkg. of 10* |
| | 221388 | Prepared Slants (C Tubes) – Ctn. of 100* |
| | 221257 | Prepared Deeps (A Tubes) – Pkg. of 10* |
| | 221115 | **Mycoflask**™ Bottle – Pkg. of 10* |
| | 221116 | **Mycoflask**™ Bottles – Ctn. of 100* |
| | 295701 | Bottles, 1 oz – Ctn. of 100* |

### BBL™ Lowenstein-Jensen Medium, Gruft

BS12 CMPH2 MCM9

| | | |
|---|---|---|
| Cat. No. | 297608 | Prepared Slants (A Tubes) – Pkg. of 10* |
| | 297653 | Prepared Slants (A Tubes) – Ctn. of 100* |
| | 297211 | Prepared Slants (C Tubes) – Pkg. of 10* |
| | 297703 | Prepared Slants (C Tubes) – Ctn. of 100* |

### BBL™ Lowenstein-Jensen Medium with Iron

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|---|---|---|
| Cat. No. | 297206 | Prepared Slants (C Tubes) – Pkg. of 10* |

### BBL™ Lowenstein-Jensen Medium with Pyruvic Acid

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|---|---|---|
| Cat. No. | 297270 | Prepared (Transgrow-style bottle) – Ctn. of 100* |

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

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|---|---|---|
| Cat. No. | 221896 | Prepared Slants (C Tubes) – Pkg. of 10* |

**Store at 2-8°C.*