

Loeffler Blood Serum • Loeffler Medium

Intended Use

Loeffler media are used primarily for the cultivation of corynebacteria.

Summary and Explanation

In 1887, Loeffler devised a culture medium containing horse serum, meat infusion and dextrose for use in the cultivation of corynebacteria and for differentiating them from other organisms.¹ A number of modifications of the original formulation have been made; those of Perry and Petran² and Buck³ are incorporated in Loeffler Medium.

The primary value of these media is for the promotion of growth and morphological characterization of members of the genus, *Corynebacterium*; formation of metachromatic granules is enhanced within the cells of these organisms. These media are also useful for demonstrating colonial pigmentation, ascospore production and the proteolytic activity of microorganisms.⁴

Principles of the Procedure

Bovine serum powder and peptones provide the amino acids and other complex nitrogenous substances necessary to support growth of corynebacteria. Sodium chloride supplies essential ions. Dextrose is a source of fermentable carbohydrate.

User Quality Control

Identity Specifications

Difco™ Loeffler Blood Serum

Dehydrated Appearance:	Medium beige, free-flowing, homogeneous.
Solution:	8.0% solution, soluble in purified water upon warming to 42-45°C. Solution is opaque.
Prepared Appearance:	Opaque, white-cream colored (coagulated).
Reaction of 8.0% Solution (after coagulation) at 25°C:	pH 7.1 ± 0.2

Cultural Response

Difco™ Loeffler Blood Serum

Prepare the medium per label directions. Dispense into tubes and coagulate for 10 minutes at approximately 100°C. Autoclave tubes, cool, inoculate and incubate at 35 ± 2°C for 18-24 hours. Prepare heat-fixed slides of growth from the medium and stain with Loeffler's alkaline methylene blue. Stained cells should show bipolar granules, club cells and some cells with general granulation.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Corynebacterium diphtheriae</i> biotype mitis	8024	30-300	Good
<i>Corynebacterium diphtheriae</i> biotype intermedius	8032	30-300	Good
<i>Corynebacterium diphtheriae</i> biotype gravis	8028	30-300	Good

In addition to the beef serum, eggs are included in the prepared tube formulation. These components cause the medium to coagulate during the sterilization process and are sources of protein utilized for the metabolism of corynebacteria and other organisms.

Formula

Difco™ Loeffler Blood Serum

Approximate Formula* Per Liter

Bovine Serum Powder	67.0	g
Beef Extract	3.0	g
Pancreatic Digest of Casein	2.0	g
Dextrose	2.5	g
Proteose Peptone No. 3	4.25	g
Sodium Chloride	1.25	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 80 g of the powder in 1 L of purified water warmed to 42-45°C.
2. Check pH. Adjust to pH 7.1, if necessary.
3. Dispense into tubes having screw caps or other tightly sealing closures.
4. Slant the tubes in the autoclave, close the door loosely and coagulate the medium in constantly flowing steam for 10 minutes.
5. Close the door tightly and autoclave at 121°C for 15 minutes.
6. Allow the autoclave pressure to fall to zero before removing tubes.
7. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the tubed medium as soon as possible after specimen collection, using either direct inoculation of the specimen by swabs or by means of an inoculating loop. Incubate the tubes with loosened caps for 18-24 hours and up to 4 days at 35 ± 2°C in an aerobic atmosphere. Alternatively, follow recommended procedures for the isolation of *C. diphtheriae*.⁵

Expected Results

Examine cultures and examine smears stained with Loeffler's methylene blue after incubation. Observe for typical cellular morphology of corynebacteria species and for the presence of metachromatic granules which take up the methylene blue dye. Subculture colonies that are catalase positive and exhibit typical morphology onto blood agar to provide growth for identification procedures.

Observe for pigmentation of specific organisms; e.g., *Pseudomonas aeruginosa* (green) and *Staphylococcus aureus* (yellow to gold). Proteolytic activity is evidenced by destruction of the integrity of the coagulated medium.

Limitations of the Procedure

1. Although the production of metachromatic granules on this medium is characteristic of members of the *Corynebacterium* genus, other organisms, such as *Propionibacterium*, some *Actinomyces* and pleomorphic streptococcal strains display stained granules resembling those of the corynebacteria.⁴
2. Loeffler Medium must be used in parallel with a tellurite-containing medium (e.g., Tinsdale Agar or Serum Tellurite Agar) for selective isolation of pathogens, particularly *C. diphtheriae*.⁴
3. Additional culture, biochemical identification and toxigenicity tests must be performed for differentiation and identification.⁴

References

1. Loeffler. 1887. Zentralbl. Bakteriolog. Parasitenkd. 2:105.
2. Perry and Petran. 1939. J. Lab. Clin. Med. 25:71.
3. Buck. 1949. J. Lab. Clin. Med. 34:582.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Sneed. 1992. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Loeffler Blood Serum

Cat. No. 270100 Dehydrated – 500 g*

BBL™ Loeffler Medium

Cat. No. 220906 Prepared Slants – Pkg. of 10*

*Store at 2-8°C.

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 rapidly-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

Hughes⁸ and Dixon and Cuthbert⁹ 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}

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.