QUALITY CONTROL PROCEDURES

I INTRODUCTION

Loeffler Medium is a culture medium for the detection and propagation of corynebacteria.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Streak-inoculate the slant surfaces with a 0.01 mL calibrated loop using 10^{-1} dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
   b. Incubate with loosened caps at 35 ± 2°C in an aerobic atmosphere.
2. Examine tubes for up to 4 days for amount of growth, pigmentation and proteolysis. After the 4-day reading, prepare slides of the *Corynebacterium* strain and stain with Loeffler's methylene blue. Examine stained smears for the typical morphology of corynebacteria and for the presence of metachromatic granules.
3. Expected Results

Organisms	ATCC™	Recovery	Microscopic Examination
**Corynebacterium diphtheriae*	51696	Fair to good growth	Metachromatic granules and banding, nonsporeforming rods showing club-shaped swelling
Corynebacterium diphtheriae	9675	Fair to good growth	Metachromatic granules and banding, nonsporeforming rods showing club-shaped swelling
Corynebacterium pseudodiphtheriticum	10700	Fair to good growth	Metachromatic granules and banding, nonsporeforming rods showing club-shaped swelling
**Pseudomonas aeruginosa*	10145	Growth of brown-green colonies with proteolysis	N/A
Staphylococcus aureus	25923	Growth of colonies with cream to gold pigmentation with or without proteolysis	N/A
Streptococcus pyogenes	19615	Fair to heavy growth; nonproteolytic	N/A

*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 7.6 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Loeffler Medium is used for the cultivation of corynebacteria.

V 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] Perry and Petran suggested modification of the original formulation.[2] Buck, in 1949, described a modified Loeffler's medium for cultivating *Corynebacterium diphtheriae*.[3] The current formulation incorporated these later modifications.

This medium has a variety of uses in microbiological investigations.

1. The primary value of Loeffler Medium is in the growth and morphological characterization of members of the genus, *Corynebacterium*. This formulation enhances the formation of metachromatic granules within the cells of the organisms.
2. Due to its serum content, Loeffler Medium can be used for the determination of proteolytic activities of microorganisms.
3. The gray-white surface of the medium provides an excellent background for the detection and observation of colonial pigmentation.
4. If all extraneous moisture is removed aseptically from the slants and the upper part of the slant is heated until the slant ruptures, this medium can be used for the detection of ascospores.

VI PRINCIPLES OF THE PROCEDURE

Heart muscle and animal tissue peptone 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. The eggs and beef serum cause the medium to coagulate during the sterilization process and are sources of protein which are utilized for metabolism of the corynebacteria and other organisms.

VII REAGENTS

Loeffler Medium

Approximate Formula* Per Liter Purified Water

Beef Serum	70.0 g
Heart Muscle, Infusion from (solids)	0.72 g
Peptic Digest of Animal Tissue	0.71 g
Sodium Chloride	0.36 g
Dextrose	0.71 g
Egg (whole, dried)	7.5 g

*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"[4-7] 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.

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. Allow the medium to warm to room temperature before inoculation.

After storage in an upright position, tubes of coagulated egg media may appear to have a large amount of water at the bottom of the slants. The fluid will be reabsorbed into the medium if the tubes are placed on their sides so that the fluid covers the surface of the slant. Storage in this position will result in virtually complete reabsorption of excess fluid.

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.[8,9] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Loeffler Medium Slants

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

Test 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 up to 4 days at 35 ± 2°C in an aerobic atmosphere.

Alternatively, follow recommended procedures for the isolation of *C. diphtheriae*.[10]

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 media. The tip of the electrode should be positioned in the central portion of the agar mass in solid media.

X RESULTS

After incubation, examine cultures and examine smears stained with Loeffler's methylene blue stain. Observe for typical cellular morphology of corynebacteria species and for the presence of metachromatic granules which take up the methylene blue dye. 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.

XI 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.[5]
2. Loeffler Medium must be used in parallel with Serum Tellurite Agar for selective isolation of pathogens, particularly *C. diphtheriae*.[11]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Loeffler Medium slants are tested for performance characteristics. Samples are tested with cell suspensions of *Corynebacterium diphtheriae* ATCC 51696 and ATCC 9675, *C. pseudodiphtheriticum* ATCC 10700, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* ATCC 19615, inoculated by streaking the slants with normal saline suspensions diluted to yield 1 x 10^3 to 1 x 10^4 CFU. Inoculated tubes are incubated with loose caps at 35–37°C for up to 4 days in an aerobic atmosphere. After incubation, slides are prepared from the growth of *C. diphtheriae* and *C. pseudodiphtheriticum* and stained with methylene blue. Metachromatic granules and banding within nonsporeforming rods showing club-shaped swelling typical of corynebacteria is observed microscopically on all lots. Growth of brown-green colonies with proteolysis is observed with *P. aeruginosa*. Fair to heavy growth without proteolysis is observed with *S. pyogenes*. Growth of colonies with cream to gold pigmentation with or without proteolysis is observed with *S. aureus*.

XIII AVAILABILITY

Cat. No.	Description
220906	**BBL™** Loeffler Medium Slants, Pkg. of 10 size K tubes
220907	**BBL™** Loeffler Medium Slants, Ctn. of 100 size K tubes

XIV REFERENCES

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