Loeffler Medium Base

Intended Use
Loeffler Medium Base with added horse serum, used for cultivation of *Corynebacterium diphtheriae* from clinical specimens and in pure cultures, detection of chromogenesis, proteolysis and the production of ascospores.

Summary
Loeffler Medium was originally devised by Loeffler and was further modified by Perry and Petran and Buck. Loeffler medium enhances primary and secondary isolation and cultivation of fastidious pathogenic microorganisms especially from nose and throat. It also restores virulence and other identifying properties (microscopic and colonial) after they have been lost due to prolonged incubation or repeated subculturing.

Principle
The high serum content helps in determining proteolytic activity of organisms. It is also used demonstration of pigmentation and ascospores. Peptone special and beef extract provide essential growth nutrients. Dextrose is the source of fermentable carbohydrate and energy.

Formula*
Ingredients	**g/L**
Peptone Special	2.5
Beef Extract	2.5
Sodium Chloride	1.25
Dextrose	2.5
Final pH (at 25°C)	7.3 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 8.75 g of the powder in 250 mL purified / distilled water.
2. Dissolve the powder completely and sterilize by autoclaving at 115°C (10 psi) for 20 minutes as per validated cycle.
3. Cool to 50°C-55°C and aseptically add 750 mL of sterile horse serum (204080110100).
4. Mix well and aseptically dispense into sterile tubes.
5. Sterilize the medium by inspissation at 80°C-85°C for 2 hours in free-flowing steam for at least 3 consecutive days.

Quality Control
Dehydrated Appearance: Yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Basal medium - Light amber coloured, clear solution.
With added serum and after coagulation - opalescent slant.
Cultural Response: Cultural characteristics observed after an incubation of 3-4 days at 30°C-35°C.

Organism (ATCC)	Growth	Colour of Colony
Corynebacterium diphtheriae (11913)	Good	-
Pseudomonas aeruginosa (10145)	Good	Green
Staphylococcus aureus subsp. *aureus* (25923)	Good	Yellow

Interpretation of Results

1. Examine the cultures and smears with Loeffler's methylene blue after inoculation.
2. Observe for typical cellular morphology of *Corynebacterium* species and for the presence of metachromatic granules, which take up the methylene blue dye.
3. Colonies that are catalase positive and exhibit typical morphology are subcultured on to blood agar plates to provide growth for identification procedures.
4. Observe for pigmentation of specific organisms; e.g. *Pseudomonas aeruginosa* (green) and *Staphylococcus aureus* (yellow to gold).
5. Proteolytic activity is observed by destruction of the integrity of the coagulated medium.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. Though the production of metachromatic granules on this medium is characteristic of Corynebacterium genus, other organisms such as Propionibacterium, some Actinomyces and pleomorphic Streptococcal strains display stained granules resembling those of Corynebacteria.
2. Loeffler Medium Base must be used in parallel with a tellurite-containing medium for the selective isolation of pathogens, particularly *C. diphtheria*.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Loeffler F., 1887, Zentralb. Bakteriol. Parasitenkd., 2:102.
2. Perry and Petran, 1939, J. Lab. Clin. Med., 25:71.
3. Buck, 1949, J. Lab. Clin. Med., 34:582.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201120260100	Dehydrated Culture Media	100 g
201120260500	Dehydrated Culture Media	500 g