



Loeffler Medium Base

M537

Intended Use:

Recommended for cultivation of *Corynebacterium diphtheriae* from clinical specimens and in pure cultures, detection of chromogenesis, proteolysis and the production of ascospores.

Composition**

Ingredients	Gms / Litre
Peptone, special	2.500
HM peptone B #	2.500
Sodium chloride	1.250
Dextrose (Glucose)	2.500
Final pH (at 25°C)	7.3±0.2

Equivalent to Beef extract

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 8.75 grams in 250 ml purified / distilled water. Dissolve the medium completely and sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 50-55°C and aseptically add 750 ml of sterile horse serum (RM1239). Mix well and aseptically dispense into sterile tubes. Sterilize the medium by inspissation at 80-85°C for 2 hours in free flowing steam for at least 3 consecutive days.

Principle And Interpretation

Loeffler Medium was originally devised by Loeffler (1) and was further modified by Perry and Petran (2) and Buck (3). 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.

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

Rub the swabs directly over the surface of medium and after incubation; prepare the smears from surface of slope. For proteolysis testing, inoculate slant and prior to incubation, flood the slant with Brewer Thioglycollate Medium (M019). Incubation should be carried out for 3-4 days or much longer for appearance of proteolysis. Loeffler Medium should be used in parallel with Serum Tellurite Agar for selective isolation of *Corynebacteria* (4).

Type of specimen

Clinical samples- a nasopharyngeal and throat swabs

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines ((2,3)). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets. Use only. Read the label before opening the container. Wear protective gloves/

Limitations :

1. For optimum recovery of *C. diphtheriae*, a nasopharyngeal and throat swab should be obtained while collection of specimen.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Basal: Light amber coloured clear to slightly opalescent solution. After addition of horse serum: Off-white coloured opaque solution

Reaction

Reaction of 3.52% w/v aqueous solution of base at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added 750ml horse serum, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
<i>Corynebacterium diphtheriae</i> ATCC 11913	50-100	fair-good
<i>Pseudomonas aeruginosa</i> ATCC 10145 (00024*)	50-100	good (green colonies with proteolysis)
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	good (yellow to gold colonies)

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area.

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.

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Disclaimer :

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