Loeffler Serum Medium Base

M1189

Loeffler Serum Medium Base with added bovine serum is used for the cultivation of *Corynebacterium diphtheriae* .

Composition**

Ingredients	Gms / Litre
Heart muscle, infusion from	0.720
Peptic digest of animal tissue	0.710
Sodium chloride	0.360
Dextrose	0.710
Egg powder	7.500
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10 grams in 250 ml distilled water at a temperature of 45°C. Mix well. Add 750 ml of sterile Bovine serum, mix well and dispense into tubes. Coagulate and sterilize by inspissation for 15 minutes at 80 to 90°C or steaming at 100°C for 10-15 minutes.

Principle And Interpretation

Corynebacterium diphtheriae , also called as Klebs-Loeffler bacillus, is a gram-positive, non-encapsulated, non-sporulated, non-motile facultative anaerobe. It causes infection in humans, leading to diseased condition called diphtheria characterized by an inflammatory lesion and membranous exudates on the mucosa of the upper respiratory tract. *C. diphtheriae* may show abundant volutin in films from a moist Loeffler serum slope. Preliminary culture on Loeffler Agar is required to induce the characteristic production of abundant granules in *C. diphtheriae* . 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. It is also used for demonstration of pigmentation and ascospores.

The high serum content helps in determining proteolytic activity of organisms. Peptic digest of animal tissue, heart muscle infusion, and bovine serum provide the amino acids and other complex nitrogenous substances to support growth of *Corynebacterium* . Dextrose is the source of fermentable carbohydrate and energy. Sodium chloride helps in maintaining osmotic balance.

Rub the swabs directly over the surface of medium. Following incubation prepare smears from the surface of slope. For testing proteolysis, 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 *Corynebacterium* (4). Examine cultures and smears stained with Loefflers methylene blue after incubation. Observe for typical cellular morphology of *Corynebacterium* 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 on blood agar, 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.

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 *Corynebacterium* (4).

Quality Control

Appearance
Light yellow to brownish yellow homogeneous

Colour and Clarity
Basal medium : Light amber coloured clear solution After addition of horse serum and coagulation : Off-white coloured opalescent slants forms in tubes

Reaction
Reaction of basal medium (10gms in 250ml distilled water) at 25°C. pH : 7.6±0.2

pH
7.40-7.80

Cultural Response
M1189: Cultural characteristics observed with added sterile Bovine serum after an incubation at 35-37°C for 24-48 hours.

Organism	Growth Cultural Response
Corynebacterium diphtheriae ATCC 11913	fair-good
Corynebacterium diphtheriae type mitis	good-luxuriant
Corynebacterium diphtheriae type gravis	good-luxuriant
Pseudomonas aeruginosa ATCC 10145	good (green colonies with proteolysis)
Staphylococcus aureus ATCC 25923	good (yellow to gold colonies)

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1.Loeffler, 1887, Zentralbl. Bakteriol. 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 J. F., 1985, Media for Isolation-Cultivation-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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