Loeffler Medium Base M537

Loeffler Medium Base with added horse serum is used for the cultivation of *Corynebacterium diphtheriae* from clinical specimens and in pure cultures. .

Composition***

Ingredients	Gms / Litre
Peptone, special	2.500
Beef extract	2.500
Sodium chloride	1.250
Dextrose	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 8.75 grams in 250 ml distilled water. Dissolve the medium completely and sterilize by autoclaving at 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

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. With special double stains, e.g. Alberts stain, the metabolic granules stand out as purple black against the lightly green counter stained cytoplasm. This helps to distinguish them from most short, non-pathogenic diptheroides, which lack these granules. *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. Peptone special and beef extract 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 Base 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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Basal medium : Light amber clear to slightly opalescent solution; After addition of horse serum: Off-white coloured opalescent slant forms in tubes

Reaction

Reaction of 0.88 % w/v aqueous solution of base at 25°C. pH : 7.3±0.2

Cultural Response

M537: Cultural characteristics observed with added 750ml horse serum, after an incubation at 35-37°C for 24-48 hours.

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

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.

Storage and Shelf Life

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.