



Fletcher Leptospira Medium Base(Leptospira Medium Base, Fletcher)

M239

Fletcher Leptospira Medium with added serum is used for isolation, cultivation and maintenance of *Leptospira* species.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	0.300
Beef extract	0.200
Sodium chloride	0.500
Agar	1.500
Final pH (at 25°C)	7.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 2.5 grams in 920 ml distilled water (Volume of water should be appropriate to compensate addition of enrichment). Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50-55°C and aseptically add 80 ml sterile Leptospira Enrichment (Rabbit / Horse serum (RM1239) to make final volume to 1 litre.

Principle And Interpretation

The leptospires includes both free-living and parasite forms. Pathogenic species are called *Leptospira interrogans*. The organisms are too small to be observed in wet preparations made from fresh blood. The most reliable method for laboratory diagnosis of leptospirosis is to cultivate the organism from blood or cerebrospinal fluid during the first week of illness or from urine thereafter for several months. A few drops of heparinized or sodium chloride anticoagulated blood are inoculated into Fletchers Medium. Fletcher Leptospira Medium Base is prepared according to the formulation of Fletcher (1) and used for isolation of Leptospira from blood, urine and kidney specimens (2).

All cultures are incubated at room temperature in the dark for up to 6 weeks. The organisms grow below the surface of the medium. Material collected from a few centimeters below the surface of broth cultures should be examined weekly for the presence of growth using a direct wet preparation under dark field illumination. Leptospires will exhibit corkscrew like motility (3).

The medium contains peptic digest of animal tissues and beef extract, which provide the necessary nutrients required for bacterial growth. Sodium chloride provides essential ions. The enrichment supplement provides carbon, vitamins and energy sources required for *Leptospira* growth. A low concentration of agar helps in detecting motility.

Leptospira Medium Base is enriched with addition of rabbit serum. Rabbit serum contains native haemoglobin, which along with thiamine helps in the Leptospiral growth. Examine the tubes for growth every 5-7 days. Growth occurs as a ringed area (disk) 1-3 cm below the surface of the medium. The absences of ringed area of growth doesnt necessarily mean leptospires are not present. Remove a small amount of growth from the disk area and examine microscopically (gram stain is not satisfactory). Microcolonies can be fixed with methanol and stained with Giemsa stain to show rod forms.

Quality Control

Appearance

White to yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.15% Agar gel.

Colour and Clarity of prepared medium

Very light yellow coloured, slightly opalescent gel forms in tubes

Reaction

Reaction of 0.25% w/v aqueous solution at 25°C. pH : 7.9±0.2

pH

7.70-8.10

Cultural Response

M239: Cultural characteristics observed with added sterile Leptospira Enrichment (rabbit/ horse serum(RM1239), after an incubation at 30-32°C for 5 days.

Organism**Growth****Cultural Response**

Leptospira interrogans Sero. luxuriant

Australis

Leptospira interrogans Sero. luxuriant

Canicola

Leptospira

luxuriant

interrogans Sero. grippotyphosa

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. Fletcher, 1927, Trans. Roy. Soc. Trop. Med. and Hyg., 21:265.
2. Galton, Acree, Lewis and Bather, 1956, J. Amer. Vet. Med. Assoc., 128:87.
3. Forbes B. A., Sahm D. F. and Weissfeld A. S., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.

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