Fletcher Leptospira Medium Base (Leptospira Medium Base, Fletcher)

M239

Intended Use:

Recommended for isolation, cultivation and maintenance of *Leptospira* species.

Composition**

Ingredients	g / L
Peptone	0.300
HM peptone B #	0.200
Sodium chloride	0.500
Agar	1.500
Final pH (at 25°C)	7.9±0.2

Equivalent to Beef extract
**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 2.5 grams in 920 ml purified/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 45-50°C and aseptically add 80 ml sterile Leptospira enrichment (Rabbit / Horse serum (RM1239)) to make final volume to 1 litre. Dispense as desired.

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 peptone and HM peptone B, 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 doesn't 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.

Type of specimen

Clinical samples - blood or cerebrospinal fluid (if first week of illness), urine

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional 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.

Limitations :

1. Successive specimens cultured at least 1 day apart increase the likelihood of positive culture, since *Leptospira* may be shed sporadically (6).
2.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

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

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

Organism	Growth
Leptospira interrogans Sero. Australis	luxuriant
Leptospira interrogans Sero. Canicola	luxuriant
Leptospira interrogans Sero.grippotyphosa	luxuriant

Storage and Shelf Life

Store between 10-30°C 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 protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

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.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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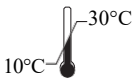

Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

Do not use if package is damaged

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