



Leptospira Medium Base

M1009

Leptospira Medium is used for isolation , cultivation and maintenance of *Leptospira* species

Composition**

Ingredients	Gms / Litre
Disodium hydrogen orthophosphate	1.000
Monopotassium phosphate	0.300
Sodium chloride	1.000
Ammonium chloride	0.250
Thiamine	0.005
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 2.56 grams in 900 ml distilled water. Swirl to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to room temperature and aseptically add 100 ml (equivalent to 5 vials) of sterile Leptospira Enrichment (FD066) . Mix well and dispense aseptically in sterile tubes or bottles as desired.

Principle And Interpretation

Leptospirosis is an acute febrile disease caused by members of the genus *Leptospira* (1,2). Direct culture of blood is the most reliable way to detect *Leptospira* during the first week of illness. After the first week of illness and for several months thereafter, leptospires may be isolated by direct culture of undiluted urine specimens. By autopsy, leptospires may be isolated from kidney and liver tissues as well as from blood and urine. The Leptospira Medium Base was originally formulated by Ellinghausen and McCullough (3) and modified by Johnson and Harris (4). Leptospira Medium Base is enriched by the addition of Leptospira Enrichment.

Leptospira Enrichment supplement provides long chain fatty acids as the carbon, energy source and vitamin for the growth of *Leptospira* . The salts supply essential nutrients for the growth of the organisms. Phosphates form buffering system while sodium chloride maintains osmotic equilibrium and also provides essential ions.

Leptospira metabolizes the fatty acids by beta-oxidation and the metabolic end products formed are acetate and carbon dioxide.

All cultures are incubated at room temperature in the dark for up to 6 weeks. The organisms grow below the surface. 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. Letpospires will exhibit corkscrew like motility (1).

Examine the tubes for growth every 5-7 days. Growth occurs as a ringed area (disc) 1-3 cm below the surface of the medium. The absence of a ringed area of growth doesnt necessarily mean leptospires are not present. Remove a small amount of growth from the disc area and examine microscopically (gram stain is not satisfactory). Microcolonies can be fixed with methanol and stained with Giemsa's stain to show rod forms (5).

Quality Control

Appearance

White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

Basal medium : Colourless clear solution; After addition of FD066 : Light yellow coloured clear solution in tubes

Reaction

Reaction of 0.256% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

M1009: Cultural characteristics observed with added sterile *Leptospira* Enrichment (FD066), after an incubation at 29-30°C for upto 7 days.

Organism	Inoculum	Growth
Cultural Response	(CFU)	
<i>Leptospira interrogans sero. canicola</i>	good-luxuriant	good-luxuriant
<i>Leptospira interrogans sero. grippityhosa</i>	good-luxuriant	good-luxuriant

Storage and Shelf Life

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

Reference

1. Forbes B. A., Sahm A. S., and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
2. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. Ellinghausen and McCullough, 1965, Am. J. Vet. Res., 26:39.
4. Johnson and Harris, 1967, J. Bact., 94:27.
5. Korthof G., 1932, Zentralbl. Bakteriell. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig., 125:429.

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