

**Leptospira Medium Base****M1009**

Leptospira Medium Base is used for isolation , cultivation 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 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. Leptospires 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 doesn't 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 ; 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

**Cultural Response**

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

Organism	Growth					
<i>Leptospira interrogans sero. canicola</i>	good-luxuriant					
<i>Leptospira interrogans sero. grippityhosa</i>	good-luxuriant					

**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., Jorgensen J. H. 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.

**Storage and Shelf Life**

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