# Leptospira Medium Base

**M1009**

## Intended Use:

Recommended for cultivation and maintenance of *Leptospira* species.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Disodium hydrogen phosphate | 1.000 |
| Potassium dihydrogen 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 purified / 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 Ellinghousen 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 Giemsas stain to show rod forms (5).

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

## Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions :

In Vitro diagnostic 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.

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

| Organism | Inoculum (CFU) | Growth |
|---|---|---|
| *Leptospira interrogans sero. canicola* | good-luxuriant | good-luxuriant |
| *Leptospira interrogans sero.grippotyhosa* | good-luxuriant | good-luxuriant |

## Reference

2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

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 Yolken R. H., (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. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig., 125:429.