



## Leptospira Medium Base

M1009

### Intended Use:

Recommended for cultivation and maintenance of *Leptospira* species.

### Composition\*\*

Ingredients	g / L
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 45-50°C and aseptically add 100 ml sterile MV Enrichment Supplement (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 stain to show rod forms (5).

### Type of specimen

Clinical samples - urine; Water samples

### Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

In Vitro diagnostic Use. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. 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 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 MV Enrichment Supplement (FD066), after an incubation at 29-30°C for upto 7 days.

Organism	Growth
<i>Leptospira interrogans sero. canicola</i>	good-luxuriant
<i>Leptospira interrogans sero.grippityhosa</i>	good-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 (7,8).

## Reference

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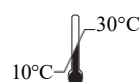
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