Technical Information

Leptospira Medium Base

Product Code: DM 2009

Application: - 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

Principle & Interpretation

Leptospirosis is an acute febrile disease caused by members of the genus *Leptospira* [1, 2]. Direct culture of blood is the most reliable method 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 devised by Ellinghousen and McCullough [3] and later 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 is indicated as a ringed area (disc) 1-3 cm below the surface of the medium. The absence of a ringed area of growth doesnt necessarily means 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].

Methodology

Suspend 2.56 grams powder media 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 (MS2066). Mix well and dispense aseptically in sterile tubes or bottles as desired.

Quality Control

Physical Appearance

White to cream homogeneous free flowing powder.

Colour and Clarity of prepared medium

Basal medium: Colourless; After addition of MS2066: 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 Range 7.30-7.70

Cultural Response/ characteristics

DM 2009: Cultural characteristics observed with added sterile Leptospira Enrichment (MS2066), after an incubation at 29-30°C for upto 7 days.

Organism	Growth
Leptospira interrogans sero. Canicola	good-luxuriant
Leptospira interrogans sero.grippotyhosa	good-luxuriant

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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.

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