Technical Information

Leptospira Medium Base, Korthof, Modified

Product Code: DM 1457

Application: - Leptospira Medium is recommended for isolation, cultivation and maintenance of *Leptospira* species.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	0.800
Sodium chloride	1.400
Sodium bicarbonate	0.020
Potassium chloride	0.040
Calcium chloride	0.040
Monopotassium hydrogen phosphate	0.240
Disodium hydrogen phosphate	0.880
Final pH (at 25°C)	7.2±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 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. Leptospira Medium Base, Korthof, Modified is formulated as described by Korthof (3, 4) for cultivation and maintenance of *Leptospira* species.

Peptic digest of animal tissue supply amino acids and other nitrogenous substances to support bacterial growth. Haemoglobin solution and inactivated blood serum supply additional sources of nutrients to the Leptospires. The salts provide essential nutrients for the growth of the organisms. Phosphates form buffering system while sodium chloride helps to maintain osmotic equilibrium and also supplies essential ions.

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 (3).

Methodology

1) Preparation of Base

Suspend 3.42 grams of DM 1457 in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Distribute in 100 ml amounts in flasks. Sterilize by autoclaving at 115°C for 15 minutes. Cool to 55°C.

2) Preparation of Haemoglobin Solution:

To the rabbit blood clot, after removing serum, add equal volume of distilled water. Freeze and thaw repeatedly to haemolyse the corpuscles. Sterilize by Seitz or millipore filtration.

3) Complete Medium:

To 100 ml sterile base, add sterile 8 ml inactivated blood serum and 0.8 ml sterile haemoglobin solution. Mix thoroughly. Distribute if desired in 2-3 ml amount in sterile screw capped Bijou bottles/tubes. Test for sterility by incubating at 37°C.

Quality Control

Appearance
Off-white to yellow homogeneous free flowing powder

Colour and Clarity
Yellowish brown coloured, clear to slightly opalescent solution after addition of serum and haemogloin

Reaction
Reaction of 0.342% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range
7.00-7.40

Cultural Response
DM 1457: Cultural characteristics observed with added inactivated blood serum and sterile haemoglobin solution, after an incubation at 30°C for upto 2-7days.

Organism	Growth
Leptospira interrogans sero.grippotyhosa	luxuriant
Leptospira interrogans sero Australis	luxuriant
Leptospira interrogans sero. Canicola	luxuriant

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period 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. Korthof G., 1932, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig., 125:429.
4. Rechcigl M. Jr. (Ed.), 1978, Handbook Series in Nutrition and Food, Vol. III, CRC Press.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
- Central Drug House Pvt. Ltd. reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents. Do not use the products if it fails to meet specification for identity and performance parameters.