Leptospira Medium Base, Korthof, Modified (DM1074)

Intended Use
Leptospira Medium Base, Korthof, Modified (DM1074) is recommended for cultivation and maintenance of *Leptospira* species.

Product Summary and Explanation
Leptospirosis is a zoonotic disease, having its reservoir in wild, domestic and peridomestic animals. Infection usually results from direct or indirect exposure to the urine of leptospiruric animals.[1] Indirect exposure through contaminated water and soil accounts for most sporadic cases. Direct exposure occurs in pet owners, veterinarians and persons working with livestock.[2]
Leptospirosis is an acute febrile disease caused by members of the genus *Leptospira*.[3, 4] 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[5, 6] for cultivation and maintenance of *Leptospira* species.

Principles of the Procedure
Leptospira Medium Base, Korthof, Modified contains peptic digest of animal tissue which provides amino acids and other nitrogenous substances necessary to support bacterial growth. Haemoglobin solution and inactivated blood serum provide additional sources of nutrients to the Leptospires. The salts potassium chloride, calcium chloride and sodium bicarbonate supply essential nutrients for the growth of the organisms. Phosphates serve buffering capability. Sodium chloride maintains osmotic equilibrium and also provides essential ions.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	0.80
Sodium chloride	1.40
Sodium bicarbonate	0.02
Potassium chloride	0.04
Calcium chloride	0.04
Monopotassium hydrogen phosphate	0.24
Disodium hydrogen phosphate	0.88
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
a) Preparation of Base:
   1. Suspend 3.42 grams of M457 in 1000 ml distilled water.
   2. Heat if necessary to dissolve the medium completely. Distribute in 100 ml amounts in flasks.
   3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
   4. Cool to 55°C.
b) Preparation of Haemoglobin Solution:
   1. To the rabbit blood clot, after removing serum, add equal volume of distilled water.
   2. Freeze and thaw repeatedly to haemolyse the corpuscles.
   3. Sterilize by Seitz or millipore filtration.
c) Complete Medium:
   1. To 100 ml sterile base, add sterile 8 ml inactivated blood serum and 0.8 ml sterile haemoglobin solution.
   2. Mix thoroughly. Distribute if desired in 2-3 ml amount in sterile screw capped Bijou bottles/tubes.
   3. Test for sterility by incubating at 37°C.

Quality Control Specifications

Dehydrated Appearance	Off-white to yellow homogeneous free flowing powder
Prepared Medium	Yellowish brown coloured, clear to slightly opalescent solution after addition of serum and haemoglobin
Reaction of 0.342% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms	Results to be achieved
		Growth
1.	*Leptospira interrogans sero.grippotyhosa*	good-luxuriant
2.	*Leptospira interrogans sero. Australis*	good-luxuriant
3.	*Leptospira interrogans sero. Canicola*	good-luxuriant

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. All cultures are incubated at room temperature in the dark for up to 6 weeks.
2. The organisms grow below the surface.
3. 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.
4. Letpospires will exhibit corkscrew like motility.

Results

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.
2. The absence of a ringed area of growth doesn't necessarily mean leptospires are not present.
3. Remove a small amount of growth from the disc area and examine microscopically (gram stain is not satisfactory).
4. Microcolonies can be fixed with methanol and stained with Giemsa's stain to show rod forms.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Leptospira Medium Base, Korthof, Modified
Product Code : DM1074
Available Pack sizes : 100gm/ 500gm

References

1. Faine (ed.). 1982. Guidelines for the control of leptospirosis. W. H. O. Offset publication no. 67. World Health Organization, Geneva, Switzerland.
2. Weyant, Bragg and Kaufmann. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Forbes B. A., Sahm A. S., and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
4. 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.
5. Korthof G., 1932, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig., 125:429.
6. Rechcigl M. Jr. (Ed.), 1978, Handbook Series in Nutrition and Food, Vol. III, CRC Press.

Further Information

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