



PRODUCT SPECIFICATION SHEET

Fletcher Leptospira Medium Base (Leptospira Medium Base, Fletcher) (DM364)

Intended Use

Fletcher Leptospira Medium Base (Leptospira Medium Base, Fletcher) (DM364) is recommended for isolation, cultivation and maintenance of *Leptospira* species.

Product Summary and Explanation

Leptospirosis is an acute, febrile disease caused by members of the genus *Leptospira*. The leptospire includes both free-living and parasite forms. Pathogenic species are called *Leptospira interrogans*. The organisms are too small to be observed in wet preparations made from fresh blood. The most reliable method for laboratory diagnosis of leptospirosis is to cultivate the organism from blood or cerebrospinal fluid during the first week of illness or from urine thereafter for several months. At autopsy, Leptospire may be isolated from kidney and liver tissues as well as from blood and urine.^(1, 2) A few drops of heparinized or sodium chloride anticoagulated blood are inoculated into Fletcher's Medium. Fletcher developed an enriched medium for the cultivation of *Leptospira* from clinical specimens (urine, blood, kidney and liver tissues).^(3, 4) All cultures are incubated at room temperature in the dark for up to 6 weeks. The organisms grow below the surface of the medium. 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. Leptospire will exhibit corkscrew like motility.⁽¹⁾

Principles of the Procedure

Fletcher Leptospira Medium Base contains peptic digest of animal tissues and beef extract, which provide the necessary nutrients required for bacterial growth. Sodium chloride provides essential ions. The enrichment supplement provides carbon, vitamins and energy sources required for *Leptospira* growth. A low concentration of agar helps in detecting motility. Leptospira Medium Base is enriched with addition of rabbit serum. Rabbit serum contains native haemoglobin, which along with thiamine helps in the Leptospiral growth. A small amount of agar provides a semi-solid consistency, which helps in the detection of motile organisms. When supplemented with 5-fluorouracil, the medium is recommended for urine and other specimens containing mixed microbial flora to provide selective inhibition of bacterial contaminants without inhibiting the growth of leptospire.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	0.30
Beef extract	0.20
Sodium chloride	0.50
Agar	1.50
Final pH: 7.9 ± 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

1. Suspend 2.5 grams of the medium in 920 ml of distilled water, (Volume of water should be appropriate to compensate addition of enrichment).
2. Heat if necessary, to dissolve the medium completely.





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- Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
- Cool to 50-55°C and aseptically add 80 ml sterile Horse serum (DM084) to make final volume to one litre.

Quality Control Specifications

Dehydrated Appearance	White to yellow homogeneous free flowing powder
Prepared Medium	Very light yellow coloured, slightly opalescent gel forms in tubes
Reaction of 0.25% Solution	pH : 7.9 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 0.15% Agar gel

Expected Cultural Response: Cultural characteristics observed with added sterile Horse serum (DM084), after an incubation at 30-32°C for 5 days.

Sr. No.	Organisms	Results to be achieved
		Growth
1.	<i>Leptospira interrogans sero.Australis</i>	good-luxuriant
2.	<i>Leptospira interrogans sero.Canicola</i>	good-luxuriant
3.	<i>Leptospira interrogans serotype grippoty</i>	good-luxuriant

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

Test Procedure

- Dispense the prepared medium aseptically into sterile screw-cap tubes in 5-7 mL amounts. Store overnight at room temperature. Inactivate the whole medium the day following its preparation by placing the tubes in a water bath at 56°C for 1 hour. Before inoculation allow the medium to cool.
- Inoculate the medium with one or two drops of blood or urine sample per tube and distribute throughout the medium. Leptospire are most likely to be isolated from blood during the first week of illness. Thereafter, they are more likely to be isolated from urine.
- Both undiluted and 10-fold diluted urine specimens should be cultured because the undiluted urine may contain growth-inhibiting substances. Repeat the inoculation procedures to obtain optimal recovery of *Leptospira*, since they may be shed sporadically.
- Leptospira* may also be cultured from liver and kidney tissues. Aseptically macerate tissue specimens and inoculate using 1:1, 1:10 and 1:100 dilutions.
- Consult appropriate texts for detailed information about the processing and inoculation of tissues and other specimens.
- Incubate tubes in the dark at 25-30°C for up to 6 weeks.

Results

- Examine the tubes for growth every 5-7 days. Growth is observed as a ringed area (disk) 1-3 cm below the surface of the medium.
- The absence of ringed area of growth does not necessarily indicate leptospire are not present.
- For further confirmation remove a small amount of growth from the disk area and examine microscopically (gram stain is not satisfactory).
- Microcolonies can be fixed with methanol and stained with Giemsa stain to show rod forms.
- Cultures should be held for up to 6 weeks before discarding as negative.





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Storage

Store the sealed bottle containing the dehydrated medium at 10 - 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 : Fletcher Leptospira Medium Base (Leptospira Medium Base, Fletcher)

Product Code : DM364

Available Pack sizes : 100gm / 500gm

References

1. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
2. Weyant, Bragg and Kaufmann. 1999. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Fletcher. 1927-28. Trans. Roy. Soc. Trop. Med. & Hyg. 27:265.
4. Galton, Acree, Lewis and Bather, 1956, J. Amer. Vet. Med. Assoc.,128:87.

Further Information

For further information please contact your local MICROMASTER Representative.



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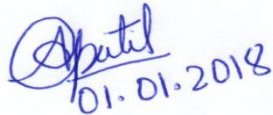
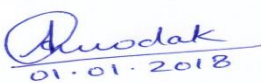

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