

Fletcher's Media

Fletcher Medium Base • Fletcher's Medium

Fletcher's Medium with 5-FU

Intended Use

Fletcher's Medium is an enriched, semisolid medium used for the cultivation of *Leptospira*.

Fletcher's Medium with 5-FU contains 5-fluorouracil for selective recovery and cultivation of *Leptospira* from clinical specimens.

Summary and Explanation

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. At autopsy, leptospires may be isolated from kidney and liver tissues as well as from blood and urine.

Fletcher developed an enriched medium for the cultivation of *Leptospira* from clinical specimens (urine, blood, kidney and liver tissues).³ Peptone and a rabbit serum enrichment support the growth of leptospires.

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 leptospires.⁴

Principles of the Procedure

Peptone and beef extract provide amino acids and other nitrogenous substances to support bacterial growth. Sodium chloride provides essential ions. A small amount of agar provides a semi-solid consistency, which helps in the detection of motile organisms.

The 5-fluorouracil is a fluorinated pyrimidine analog that inhibits bacterial contaminants without affecting the growth of *Leptospira*.

Formula

Difco™ Fletcher Medium Base

Approximate Formula* Per 920 mL

Peptone	0.3	g
Beef Extract.....	0.2	g
Sodium Chloride	0.5	g
Agar	1.5	g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 2.5 g of the powder in 920 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 80 mL sterile normal rabbit serum at 56°C. Mix well.
5. Determine pH; if necessary, aseptically adjust to pH 7.9 ± 0.1 with 1N HCl or 1N NaOH.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Prepare the medium from Fletcher Medium Base per label directions and aseptically dispense into sterile screw-cap tubes in 5-7 mL amounts. Store at room temperature overnight. Inactivate the whole medium the day following its preparation by placing the tubes in a water bath at 56°C for 1 hour. Allow the medium to cool before inoculation.

Inoculate the medium with one or two drops of blood or urine per tube and distribute throughout the medium. Leptospires 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.

User Quality Control

Identity Specifications

Difco™ Fletcher Medium Base

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 0.25 g/92 mL solution, soluble in purified water upon boiling. Solution is very light amber, clear to very slightly opalescent.

Prepared Appearance: Very light amber, very slightly opalescent.

Reaction of 0.25 g/92 mL
Solution at 25°C: pH 7.9 ± 0.1

Cultural Response

Difco™ Fletcher Medium Base

Prepare the medium per label directions. Inoculate with undiluted cultures and incubate at 30 ± 2°C for up to 5 days.

ORGANISM	ATCC™	INOCULUM	RECOVERY
<i>Leptospira interrogans</i> serotype australis	23605	0.1 mL	Good
<i>Leptospira interrogans</i> serotype canicola	23606	0.1 mL	Good
<i>Leptospira kirschneri</i>	23604	0.1 mL	Good

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.^{1,2}

Incubate tubes in the dark at 25-30°C for up to 6 weeks.

Expected Results

Examine tubes for growth every 5-7 days. Growth occurs as a ringed-area (disk) 1-3 cm below the surface of the medium. The absence of a ringed area of growth does not necessarily mean leptospires are not present. Remove a small amount of growth from the disk area and examine microscopically (the Gram stain is not satisfactory). Microcolonies can be fixed with methanol and stained with Giemsa stain to show rod forms.⁵

Cultures should be held for up to 6 weeks before discarding as negative.

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. 21:265.
4. Johnson and Rogers. 1964. J. Bacteriol. 87:422.
5. Weinman. 1981. In Balows and Hausler (ed.), Diagnostic procedures for bacterial, mycotic and parasitic infections, 6th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Fletcher Medium Base

Cat. No. 298710 Dehydrated – 500 g

BBL™ Fletcher's Medium

Cat. No. 297242 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10*

BBL™ Fletcher's Medium with 5-FU

SMWW

Cat. No. 297243 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10*

*Store at 2-8°C.