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. 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). 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.

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.
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

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
Cat. No. 297243 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10* SMWW
*Store at 2-8°C.