

## 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. 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.<sup>1,2</sup>

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 leptospire 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.<sup>5</sup>

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

## References

1. Forbes, Salm 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

**SMWWW**

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

\*Store at 2-8°C.

## Flo Agar

(See *Pseudomonas* Agars)

## Fluid A • Fluid D

### Intended Use

Fluid A and Fluid D conform with specifications of *The United States Pharmacopeia (USP)*.

Fluid A (peptone water) is used for diluting or rinsing when performing sterility testing. Fluid D (peptone water with polysorbate 80) is used for diluting or rinsing samples containing lecithin or oil when performing sterility testing.

### Summary and Explanation

Pharmaceuticals, biologicals, medical devices or any material claiming to be sterile must be tested for sterility according to the procedures described in the compendia.<sup>1,2</sup> Sterility testing

is performed using the membrane filtration or direct testing methods, depending upon sample type and size.

Fluid A and Fluid D are used for diluting or rinsing when performing sterility testing. These fluids aid in the complete rinsing of the membrane filter apparatus and are not toxic to microorganisms. Fluid D contains polysorbate 80, which acts as a surfactant to break down the lecithin or oils present.

### Principles of the Procedure

Fluid A and Fluid D contain peptic digest of animal tissue, which provides a source of nitrogen for bacteria. Polysorbate 80 in Fluid D is a surfactant.

## Formulae

### Difco™ Fluid A

Approximate Formula\* Per Liter  
 Peptic Digest of Animal Tissue ..... 1.0 g

### Difco™ Fluid D

Approximate Formula\* Per Liter  
 Peptic Digest of Animal Tissue ..... 1.0 g  
 Polysorbate 80 ..... 1.0 mL

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

## User Quality Control

### Identity Specifications

#### Difco™ Fluid A

Appearance: Nearly colorless, clear solution.

Reaction of Solution at 25°C: pH 7.1 ± 0.2

#### Difco™ Fluid D

Appearance: Nearly colorless, clear solution.

Reaction of Solution at 25°C: pH 7.1 ± 0.2

### Toxicity Test

#### Difco™ Fluid A or Fluid D

Perform a toxicity test by inoculating duplicate tubes of Tryptic Soy Broth with the test organisms and adding 1 mL of Fluid A or Fluid D to one tube of each set. Incubate tubes at 20-25°C for up to 5 days. Good recovery in the tubes containing Fluid A or Fluid D indicates that the solution does not have antibacterial or antifungal properties and is suitable for use in appropriate procedures.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Bacillus subtilis</i>	6633	10-10 <sup>2</sup>	Good
<i>Candida albicans</i>	10231	10-10 <sup>2</sup>	Good
<i>Micrococcus luteus</i>	9341	10-10 <sup>2</sup>	Good

## Procedure

Fluid A and Fluid D are provided as prepared, ready-to-use diluents in a variety of bottle sizes and closures. Consult appropriate references for detailed information and recommended procedures.<sup>1,2</sup>

## Expected Results

Consult appropriate references for further information.<sup>1,2</sup>

## References

1. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Council of Europe. 2002. European pharmacopeia, 4th ed. Council of Europe, Strasbourg, France.

## Availability

### Difco™ Fluid A

EP USP

Cat. No. 290651 Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10  
 290821 Prepared Bottles, 100 mL (serum) – Pkg. of 10  
 290652 Prepared Bottles, 300 mL (septum screw cap) – Pkg. of 10

### Difco™ Fluid D

EP USP

Cat. No. 290661 Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10  
 290831 Prepared Bottles, 100 mL (serum) – Pkg. of 10  
 290662 Prepared Bottles, 300 mL (septum screw cap) – Pkg. of 10

# Fluid Sabouraud Medium

(See Sabouraud Media)

# Fluid Thioglycollate Media

(See Thioglycollate Media)

# Folic AOAC Medium

## Intended Use

Folic AOAC Medium is used for determining folic acid concentration by the microbiological assay technique.

## Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;

3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Folic AOAC Medium is prepared for use in the microbiological assay of folic acid according to the procedures of the Folic Acid Assay in the *Official Methods of Analysis of AOAC International*.<sup>1</sup> *Enterococcus hirae* ATCC™ 8043 is the test organism in this assay.