KF Streptococcus Broth

Intended Use

KF Streptococcus Broth is used for isolating fecal streptococci.

Summary and Explanation

Kenner et al. developed KF (Kenner Fecal) Streptococcal Broth for the detection and enumeration of enterococci in waters.^{1,2} They found that this formulation was superior to other liquid media in the recovery of enterococci in Most Probable Number (MPN) test systems. The medium is not specific for presumptive identification of group D streptococci. Other tests are required.²⁻⁴

Principles of the Procedure

Peptone provides a source of nitrogen, amino acids and carbon. Yeast extract is a source of trace elements, vitamins and amino acids. Maltose and lactose are the fermentable carbohydrates and carbon sources. Sodium azide is the selective agent. Bromcresol purple is the indicator dye.

The addition of 1% TTC (2,3,5-Triphenyl Tetrazolium Chloride), in the membrane filter procedure, causes the enterococci to have a deep red color as a result of tetrazolium reduction to an acid azo dye.

Enterococcus faecalis ATCC[™] 29212 **User Quality Control** Identity Specifications Difco[™] KF Streptococcus Broth Dehydrated Appearance: Light greenish-beige, free-flowing, homogeneous. Solution: 5.64% solution, soluble in purified water upon boiling. Solution is reddish to light purple, clear to very slightly opalescent. Prepared Appearance: Purple, clear to very slightly opalescent. Reaction of 5.64% Solution at 25°C: pH 7.2 ± 0.2 Cultural Response Difco[™] KF Streptococcus Broth Prepare the medium per label directions. Supplement with TTC Solution 1%. Using the membrane filter technique, inoculate and incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 hours. ORGANISM ATCC™ INOCULUM CFU RECOVERY COLONY COLOR 13048 3×10²-10³ Inhibition Enterobacter aerogenes _

Red Red

Enterococcus faecalis

Enterococcus faecalis

Escherichia coli

19433

29212

25922

30-200

30-200

3×10²-10³

Good

Good

Inhibition

Formula

Difco[™] KF Streptococcus Broth

Approximate Formula* Per Liter		
Proteose Peptone No. 3	10.0	g
Yeast Extract	10.0	g
Sodium Chloride		g
Sodium Glycerophosphate	10.0	g
Maltose		g
Lactose		g
Sodium Azide	0.4	g
Bromcresol Purple	15.0	mg
*Adjusted and/or supplemented as required to meet performance criteria.		5

Directions for Preparation from Dehydrated Product MPN Procedure

1. For an inoculum of 1 mL or less, suspend 56.4 g of the powder in 1 L of purified water. Mix thoroughly.

For an inoculum of 10 mL, suspend 84.6 g of the powder in 1 L of purified water. Mix thoroughly.

- 2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- 3. For an inoculum of 1 mL or less, dispense 10 mL amounts into culture tubes.

For an inoculum of 10 mL, dispense 20 mL amounts into culture tubes.

- 4. Autoclave at 121°C for 10 minutes.
- 5. Test samples of the finished product for performance using stable, typical control cultures.

Membrane Filter Procedure

- 1. Suspend 56.4 g of the powder in 1 L of purified water. Mix thoroughly.
- 2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- 3. Dispense in 100 mL amounts into flasks and autoclave at 121°C for 10 minutes.
- 4. Cool to 60°C and add 1 mL TTC Solution 1% per 100 mL of medium.
- 5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

MPN Procedure

- 1. Inoculate tubes of the KF Streptococcus Broth with the appropriate amount of inoculum.
- 2. Incubate tubes at $35 \pm 1^{\circ}$ C, with loosened caps, for 46-48 hours.

Membrane Filter Procedure

- 1. Place a sterile absorbent pad in each sterile Petri dish.
- 2. Saturate the pads with the sterile medium containing TTC.
- 3. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
- 4. Incubate at $35 \pm 1^{\circ}$ C in an atmosphere saturated with water vapor for 46-48 hours.

Expected Results

MPN Procedure

MPN tubes positive for enterococci are turbid with growth that appears yellow in color and does not produce foaming. When foaming occurs, confirmation for enterococci should be made by Gram staining.

Membrane Filter Procedure

All red or pink colonies visible with $15 \times$ magnification are counted as enterococci colonies.

Limitations of the Procedure

- 1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
- 2. Overheating may lower the pH, resulting in a decrease in productivity of the medium.

References

- Kenner, Clark and Kabler. 1960. Am. J. Public Health 50:1553.
 Kenner, Clark and Kabler. 1961. Appl. Microbiol. 9:15.
 MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.
- Williams & Wilkins, Baltimore, Md.
 Facklam and Moody. 1970. Appl. Microbiol. 20:245.

Availability

Difco[™] KF Streptococcus Broth

Cat. No. 212226 Dehydrated - 500 g

Difco[™] TTC Solution 1%

Cat. No.	231121	Tube – 30 mL
	264310	Bottle – 25 g

