Enterococcosel™ Agar • Enterococcosel™ Broth

Intended Use

Enterococcosel Agar, a Bile Esculin Agar with Azide, is used for the rapid, selective detection and enumeration of enterococci.[1]

Enterococcosel Broth, a Bile Esculin Broth with Azide, is recommended for use in the differentiation of enterococci and group D streptococci.

Summary and Explanation

Rochaix noted the value of esculin hydrolysis in the identification of enterococci.[2] The enterococci were able to split esculin, but other streptococci could not. Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas the other streptococci could not.[3] Swan used an esculin medium containing 40% bile salts and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.[4] Facklam and Moody preformed a comparative study of tests used to presumptively identify group D streptococci and found that the bile-esculin test provides a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.[5] According to current nomenclature, the group D antigen is considered non-specific since it is produced by the genera *Enterococcus*, *Pediococcus* and by certain streptococci.[6]

Isenberg et al. modified the Bile Esculin Agar formulation by reducing the bile concentration from 40 to 10 g/L and by adding sodium azide.[7] This modification is supplied as **Enterococcosel** Agar. Consult the text for a list of specimens for which this medium is recommended for primary isolation.[8]

Enterococcosel Broth has the same formula as **Enterococcosel** Agar with the agar omitted. Colonies suspected of being *Enterococcus faecalis* can be emulsified in 1 or 2 mL of broth and incubated at 35°C. The combination of esculin and a rather low concentration of bile in the presence of azide permits the selection and differentiation of enterococci by esculin hydrolysis (blackening of the medium) within 2 hours.[7]

Principles of the Procedure

Enterococci and Group D streptococci hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with an iron salt, ferric ammonium citrate, to form a dark brown or black complex.[9] Oxgall is used to inhibit gram-positive bacteria other than enterococci. Sodium azide is inhibitory for gram-negative microorganisms.

User Quality Control

Identity Specifications

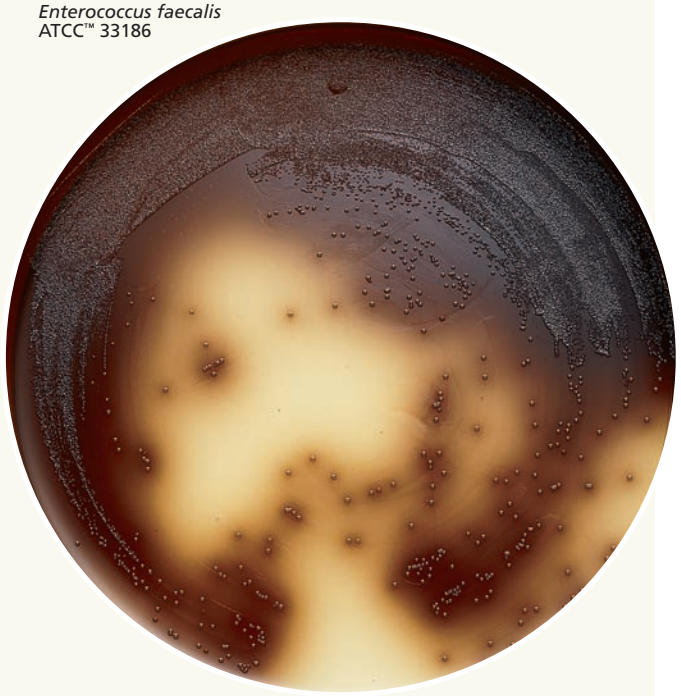
BBL™ Enterococcosel™ Agar

Dehydrated Appearance:	Medium fine, homogeneous, may contain some tan specks.
Solution:	5.6% solution, soluble in purified water upon boiling. Solution is medium, tan with a trace blue cast, clear to moderately hazy.
Prepared Appearance:	Medium, tan with a trace blue cast, clear to moderately hazy.
Reaction of 5.6% Solution at 25°C:	pH 7.1 ± 0.2

BBL™ Enterococcosel™ Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.3% solution, soluble in purified water upon heating. Solution is medium, yellow to tan to yellow green with a bluish cast, clear to hazy.
Prepared Appearance:	Medium, yellow to tan to yellow-green with a bluish cast, clear to hazy.
Reaction of 4.3% Solution at 25°C:	pH 7.1 ± 0.2

Enterococcus faecalis
ATCC™ 33186



Cultural Response

BBL™ Enterococcosel™ Agar or Enterococcosel™ Broth

Prepare the medium per label directions. For agar, inoculate as described below. For broth, inoculate with fresh cultures. Incubate at 35 ± 2°C for 48 hours (agar) or 24 hours (broth).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR	RECOVERY BROTH
Enterococcus faecalis	29212	10^3-10^4	Good, blackening	Good, blackening
Escherichia coli	25922	10^4-10^5	Complete inhibition	Partial to complete inhibition, no blackening
Streptococcus pyogenes	19615	10^4-10^5	Complete inhibition	Partial to complete inhibition, no blackening

Formulae

BBL™ Enterococcosel™ Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Peptic Digest of Animal Tissue	3.0	g
Yeast Extract	5.0	g
Oxgall	10.0	g
Sodium Chloride	5.0	g
Esculin	1.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Azide	0.25	g
Sodium Citrate	1.0	g
Agar	13.5	g

BBL™ Enterococcosel™ Broth

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   BBL™ Enterococcosel™ Agar – 56 g;
   BBL™ Enterococcosel™ Broth – 43 g.
   Mix thoroughly.
2. For agar, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. For broth, heat if necessary to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agar

Use standard procedures to obtain isolated colonies from specimens. Incubate plates 24-48 hours at 35 ± 2°C in and aerobic atmosphere.

Broth

Colonies, from a primary isolation plate, suspected of being enterococci or group D streptococci can be emulsified in 2 mL of **Enterococcosel** Broth and incubated at 35 ± 2°C in an aerobic atmosphere.

Expected Results

After incubation, observe for typical growth and reaction:

Agar

Typical colonial morphology on **Enterococcosel** Agar is as follows:

Streptococci (non-group D)	No growth to trace growth.
Enterococci/group D streptococci	Small, but larger than group A streptococci. Translucent with brownish-black to black zones.
Staphylococci	Large, white, opaque.
Micrococci	Large, white, grayish.
Corynebacteria	Small to large, white to grayish-yellow, smooth and irregular.
Candida	Small to large, white.
Listeria monocytogenes	Small to large, translucent with brownish-black to black zones.
Gram-negative bacteria	No growth to trace growth.

Broth

Enterococci and group D streptococci turn the medium black within 2 hours when a heavy inoculum is used. Other organisms are inhibited or do not turn the medium black.

Limitations of the Procedure

Listeria monocytogenes, *Streptococcus bovis* group, *Pediococcus* and staphylococci may also grow on **Enterococcosel** Agar. However, staphylococci do not produce black zones. Other organisms (e.g., micrococci, *Candida*, corynebacteria and gram-negative bacteria) may appear as small colonies or produce trace growth.

References

1. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Rochaix. 1924. C.R. Soc. Biol. *90*:771.
3. Meyer and Schonfeld. 1926. Zentralbl. Bakteriol. Parasitenk. Infectionskr. Hyg. Abt. Orig. *99*:402.
4. Swan. 1954. J. Clin. Pathol. *7*:160.
5. Facklam and Moody. 1970. Appl. Microbiol. *20*:245.
6. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Isenberg, Goldberg and Sampson. 1970. Appl. Microbiol. *20*:433.
8. Cintron. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
9. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott William & Wilkins, Baltimore, Md.

Availability

BBL™ Enterococcosel™ Agar

CMPH2 ISO MCM9

Cat. No. 212205 Dehydrated – 500 g

United States and Canada

Cat. No. 221492 Prepared Plates – Pkg. of 20*
221493 Prepared Plates – Ctn. of 100*
221381 Prepared Slants – Pkg. of 10*
221382 Prepared Slants – Ctn. of 100*

Europe

Cat. No. 254019 Prepared Plates – Pkg. of 20*

BBL™ Enterococcosel™ Agar//Columbia CNA Agar, Modified with Sheep Blood

Cat. No. 297413 Prepared **I Plate™** Dishes – Ctn. of 100*

BBL™ Enterococcosel™ Broth

Cat. No. 212207 Dehydrated – 500 g
221383 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*