**Directions for Preparation from Dehydrated Product**

1. Suspend 18 g of the powder in 1 L of purified water. Mix thoroughly.
2. Add 10 mL of Andrade’s indicator.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45-50°C in a water bath.
6. Aseptically add 0.5% or 1% of sterile carbohydrate (see table).

<table>
<thead>
<tr>
<th>CARBOHYDRATE</th>
<th>FINAL CONCENTRATION</th>
<th>ADD BEFORE AUTOCLAVING</th>
<th>ADD AFTER AUTOCLAVING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adonitol</td>
<td>0.5%</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.5%</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>0.5%</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>1%</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>0.5%</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol*</td>
<td>0.5%</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Inositol</td>
<td>0.5%</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Lactose</td>
<td>1%</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Mannitol</td>
<td>1%</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>0.5%</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1%</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.5%</td>
<td>–</td>
<td>X</td>
</tr>
</tbody>
</table>

*Medium containing glycerol should be autoclaved for 10 minutes at 15 lbs pressure (121°C).

7. Dispense 9 mL amounts into test tubes containing inverted vials (Durham tubes).
8. Test samples of the finished product for performance using stable, typical control cultures.

**Procedure**

For a complete discussion on identification of *Enterobacteriaceae*, refer to the appropriate procedures outlined in the references.1-4,6

**Expected Results**

A positive result for gas includes production in at least 3% of the volume of the fermentation tube. A positive reaction for acid is a change in color from light amber to dark pink or red.

**Limitation of the Procedure**

Negative tubes remain colorless and should be observed regularly for a total of 30 days.

**References**


**Availability**

Difco™ Enteric Fermentation Base
Cat. No. 218281 Dehydrated – 500 g

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**Enterococcusel™ Agar • Enterococcusel™ Broth**

**Intended Use**

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

*Enterococcus* 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 *Enterococcusel* Agar. Consult the text for a list of specimens for which this medium is recommended for primary isolation.8

*Enterococcusel* Broth has the same formula as *Enterococcusel* 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.8 Oxgall is used to inhibit gram-positive bacteria other than enterococci. Sodium azide is inhibitory for gram-negative microorganisms.
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.

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 yellow-green with a bluish cast, clear to hazy.
- **Reaction of 4.3% Solution at 25°C**: pH 7.1 ± 0.2

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).

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC™</th>
<th>INOCULUM CFU</th>
<th>RECOVERY AGAR</th>
<th>RECOVERY BROTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>29212</td>
<td>10⁴-10⁵</td>
<td>Good, blackening</td>
<td>Good, blackening</td>
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<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>10⁴-10⁵</td>
<td>Complete inhibition</td>
<td>Partial to complete inhibition, no blackening</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>19615</td>
<td>10⁴-10⁵</td>
<td>Complete inhibition</td>
<td>Partial to complete inhibition, no blackening</td>
</tr>
</tbody>
</table>

Enterococcosel Agar, cont.
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

Enterococcus, *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


Availability

BBL™ Enterococcosel™ Agar

<table>
<thead>
<tr>
<th>CMPH</th>
<th>MCM7</th>
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<tbody>
<tr>
<td>Cat. No.</td>
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**United States and Canada**

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<th>Cat. No.</th>
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<tbody>
<tr>
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<tr>
<td>221493</td>
<td>Prepared Plates – Ctn. of 100*</td>
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<tr>
<td>221381</td>
<td>Prepared Slants – Pkg. of 10*</td>
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<tr>
<td>221382</td>
<td>Prepared Slants – Ctn. of 100*</td>
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**Europe**

<table>
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<tr>
<th>Cat. No.</th>
<th>Prepared Plates – Pkg. of 20*</th>
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<tbody>
<tr>
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</table>

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

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Prepared Plates – Pkg. of 20*</th>
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</thead>
<tbody>
<tr>
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</table>

**BBL™ Enterococcosel™ Broth**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Prepared Tubes – Pkg. of 10*</th>
</tr>
</thead>
<tbody>
<tr>
<td>212207</td>
<td>Dehydrated – 500 g</td>
</tr>
<tr>
<td>221383</td>
<td></td>
</tr>
</tbody>
</table>

*Store at 2-8°C.*

**Enterococcus faecalis**

ATCC™ 51299

Intended Use

Enterococcosel Agar with Vancomycin, 8 µg/mL, is used for primary screening of asymptomatic gastrointestinal carriage of vancomycin-resistant enterococci (VRE).1

Summary and Explanation

Enterococci are known to cause a wide variety of infections. Most commonly they infect the urinary tract, abdomen, bloodstream, endocardium, biliary tract, burn wounds and indwelling catheters.2 Enterococcus faecalis causes 80 to 90% of infections, while *E. faecium* causes the remainder.3 Today the enterococci are the fourth leading cause of nosocomial infection and the third leading cause of bacteremia in the United States.4 The case/fatality rates for enterococcal bacteria range from 12 to 68% with death due to sepsis in 4 to 50% of the cases.5

Because the potential exists for vancomycin-resistant genes to be transferred to other gram-positive organisms and because the treatment options for VRE infections are limited, the CDC issued infection control guidelines for hospitals and long-term care facilities.6 Guidelines include stool and rectal swab culture surveys of asymptomatic patients who may be carrying VRE.