Enterococcus Confirmatory HiVeg™ Agar / Broth / MV392 / MV394 / MV419

Enterococcus Presumptive HiVeg™ Broth

Enterococcus Confirmatory HiVeg media are recommended for confirming the presence of Enterococci in water supplies and other sources.

**Composition**:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>MV392 Grams/Litre</th>
<th>MV394 Grams/Litre</th>
<th>MV419 Grams/Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg hydrolysate</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Methylen blue</td>
<td>0.01</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>—</td>
<td>65.00</td>
<td>—</td>
</tr>
<tr>
<td>Bromo thymol blue</td>
<td>—</td>
<td>—</td>
<td>0.032</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C)**: 8.0 ± 0.2

**Recommended for** :

- Confirming the presence of Enterococci in water supplies and other sources.

**Storage** : Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.

**Product Profile**:

- **Vegetable based (Code MV)**: HiVeg hydrolysate
- **Animal based (Code M)**: Casein enzymic hydrolysate

**Recommended for** : Confirming the presence of Enterococci in water supplies and other sources.

**Reconstitution** :

- (MV392) : 30.4 g/l
- (MV394) : 80.4 g/l
- (MV419) : 15.4 g/l

**Quantity on preparation (500g)** :

- (MV392) : 16.44 L
- (MV394) : 6.21 L
- (MV419) : 32.46 L

**pH (25°C)** :

- (MV392) & (MV394) : 8.0 ± 0.2

**Preparation** :

- Suspend 30.4 grams of MV392 or 80.4 grams of MV394 or 15.4 grams of MV419 in 1000 ml distilled water. Boil to dissolve the medium completely. Dispense in 100 ml quantities in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the agar tubes to cool in a slanted position and cool the broth (MV394) to room temperature and add 65 units of Penicillin to each 100 ml of broth prior to use.

**Warning** : Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

**Principle and Interpretation**:

These media are prepared by using HiVeg hydrolysate which is free of BSE/TSE associated risks. These Enterococcus media are the modification of media formulated by Sandholzer and Winter (1) for the detection of Enterococci in water supplies, swimming pools, sewage etc. The enterococci portion of the fecal Streptococcus group is a valuable indicator for determining the extent of fecal contamination of recreational surface waters (2). The Enterococcus Confirmatory HiVeg Broth has same formula as Enterococcus Confirmatory HiVeg Agar except agar, sodium chloride and Penicillin which is used to detect Enterococci from crab meat and oysters etc.

**HiVeg hydrolysate, Yeast extract, Dextrose provide essential growth nutrients for Enterococci. Sodium azide inhibits gram-negative organisms. Penicillin has inhibitory effect on Staphylococci. High concentration of NaCl (6.5%) serves selective enrichment of Enterococci in MV394 (3). The positive presumptive tests are confirmed by inoculating Enterococcus Presumptive HiVeg Broth (MV419) to Enterococcus Confirmatory slant-broth combination prepared with an Azide Agar medium (Enterococcus Confirmatory HiVeg Agar, MV392) overlaid with a Salt Azide Penicillin HiVeg Broth (Enterococcus Confirmatory HiVeg Broth, MV394). A negative catalase test is considered confirmed positive evidence of the presence of Enterococci. Single strength medium can be used for small inoculum. Production of acid and turbidity in an azide presumptive broth when incubated at 45°C is considered positive presumptive evidence for the presence of Enterococci which is confirmed by inoculating in / on Confirmatory Broth/ Agar (MV394, MV392).

**Quality Control**:

**Appearance of Powder**

Yellow coloured, may have slightly greenish tinge, homogeneous, free flowing powder.

**Gelling**

Firm, comparable with 1.5% Agar gel of MV392.

**Colour and Clarity**

Yellow coloured, slightly opalescent gel of MV392 / clear solution of MV394 and MV419 forms in petri plates / tubes which acquires greenish tinge at the surface on standing.

**Reaction**

Reaction of 3.04% w/v of MV392 or 8.04% w/v of MV394 aqueous solution is pH 8.0 ± 0.2 at 25°C. Reaction of 1.54% w/v of MV419 aqueous solution is pH 8.4 ± 0.2 at 25°C.

**Cultural Response**

Cultural characteristics was observed after an incubation at 45°C for 24 hours.

<table>
<thead>
<tr>
<th>Organisms (ATCC)</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis (29212)</td>
<td>20-60</td>
<td>luxuriant</td>
<td>&gt;70%</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli (25922)</td>
<td>10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td>—</td>
</tr>
</tbody>
</table>

Key : + = acid production, yellow colour
- = no acid production, no colour change.

**References**: