Peptone Iron Agar

Intended Use
Peptone Iron Agar is used for detecting hydrogen sulfide production by microorganisms.

Summary and Explanation
Levine and co-workers\(^1\) described a medium containing proteose peptone and ferric citrate for detection of hydrogen sulfide production by coliform bacteria. They demonstrated that such a medium served to differentiate strains that were Voges-Proskauer negative, methyl-red positive and citrate positive from other members of the *Enterobacteriaceae*.

Levine reported that ferric citrate was a much more sensitive indicator of hydrogen sulfide production than lead acetate, producing a medium that gave definite reactions within 12 hours. Peptone Iron Agar is a modification of Levine's original formula in which peptone has been included with proteose peptone and the more soluble ferric ammonium citrate is used in place of ferric citrate.

Tittsler and Sandholzer\(^3\) compared Peptone Iron Agar with lead acetate agar for the detection of hydrogen sulfide and found that Peptone Iron Agar had the advantage of giving earlier reactions and clearer results.

Principles of the Procedure
Peptones are the nitrogen sources in Peptone Iron Agar. Ferric ammonium citrate and sodium thiosulfate are used to detect \(H_2S\) production. Sodium glycerophosphate is a buffering compound. Agar is the solidifying agent.

Formula

**Difco™ Peptone Iron Agar**

<table>
<thead>
<tr>
<th>Approximate Formula* Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone ............................................. 15.0 g</td>
</tr>
<tr>
<td>Proteose Peptone ......................... 5.0 g</td>
</tr>
<tr>
<td>Ferric Ammonium Citrate ............... 0.5 g</td>
</tr>
<tr>
<td>Sodium Glycerophosphate ............ 1.0 g</td>
</tr>
<tr>
<td>Sodium Thiosulfate ...................... 0.08 g</td>
</tr>
<tr>
<td>Agar ................................................. 15.0 g</td>
</tr>
</tbody>
</table>

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

Directions for Preparation from Dehydrated Product

1. Suspend 36 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. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Obtain a pure culture of a test organism. Pick the center of a single colony with an inoculating needle.
2. Inoculate a tube of Peptone Iron Agar by the stab method. Stab the needle to within 1/4 to 1/2 inch of the bottom. Withdraw the needle following the initial line of inoculation.
3. Incubate tubes at 35 ± 2°C for 18-48 hours.
4. Read tubes for growth and hydrogen sulfide production.

Expected Results

Any blackening of the medium along the line of inoculation or throughout the butt indicates hydrogen sulfide production.

For a complete discussion of the identification of coliform bacteria, refer to appropriate references.\(^4\)
## Peptone Water

### Intended Use
Peptone Water is used for cultivating nonfastidious organisms, for studying carbohydrate fermentation patterns and for performing the indole test.

### Summary and Explanation
The formulation of Peptone Water makes it useful for cultivating nonfastidious organisms. This nonselective medium has been used as a basal medium for biochemical tests such as carbohydrate fermentation patterns and production of indole.

### Principles of the Procedure
Peptone Water contains peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium.

### User Quality Control

#### Identity Specifications

**Difco™ Peptone Water**

<table>
<thead>
<tr>
<th>Dehydrated Appearance</th>
<th>Cream-white to light tan, free-flowing, homogeneous.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution:</td>
<td>1.5% solution, soluble in purified water upon warming with frequent agitation. Solution is light amber, clear to very slightly opalescent.</td>
</tr>
<tr>
<td>Prepared Appearance:</td>
<td>Light amber, clear to slightly opalescent.</td>
</tr>
<tr>
<td>Reaction of 1.5% Solution at 25°C:</td>
<td>pH 7.2 ± 0.2</td>
</tr>
</tbody>
</table>

#### Cultural Response

**Difco™ Peptone Water**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>INDOLE REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>10^6 - 10^9</td>
<td>Good</td>
<td>Positive</td>
</tr>
</tbody>
</table>

### For Determining Carbohydrate Fermentation Patterns

1. Add 1.8 mL 1% phenol red solution to 1 liter rehydrated Peptone Water. Mix thoroughly.
2. Dispense into test tubes containing inverted Durham vials.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add sufficient sterile carbohydrate solution to yield a 1% final concentration. Rotate each tube to thoroughly distribute the carbohydrate.

### Procedure

#### For Determining Carbohydrate Fermentation Patterns

1. Inoculate tubes with test organisms.
2. Incubate tubes at 35 ± 2°C for 18-48 hours.
3. Observe for color change.

#### For Performing the Indole Test

1. Inoculate tubes with test organisms.
2. Incubate tubes at 35 ± 2°C for 24 or 48 hours.
3. Using an inoculation loop, spread a loopful of culture over the reaction area of a BBL™ DrySlide™ Indole test slide (Cat. No. 231748).
4. Examine the reaction area for appearance of a pink color within 30 seconds.

### Expected Results

#### For Determining Carbohydrate Fermentation Patterns

Acid is produced when carbohydrates are fermented. This is indicated by a yellow color in the medium. Gas production is indicated by the presence of gas bubbles in the fermentation tube.

#### For Performing the Indole Test

Observe for the formation of a pink color in the DrySlide reaction area, which indicates a positive test for indole production.