Intended Use
PPLO Broth W/O CV is used with enrichments for the isolation and cultivation of Mycoplasma spp. in a laboratory setting. PPLO Broth W/O CV is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation
PPLO Broth W/O CV is prepared according to the formula described by Morton and Lecce. Crystal Violet may inhibit the growth of Mycoplasma, and therefore was removed from an earlier formula described by Morton, Smith, Williams, and Eikenberg. Mycoplasma was discovered in a case of pleuropneumonia in a cow, and referred to as “pleuropneumonia-like organism” or PPLO. Mycoplasmas belong to the class of Mollicutes “soft skin”, which are the smallest free-living organisms. They are pleomorphic, varying in size from 0.2 to 0.3 micromillimeters.

Principles of the Procedure
Heart Infusion and Yeast Enriched Peptone are the nitrogen, carbon, vitamin, and mineral sources in PPLO Broth W/O CV. Sodium Chloride maintains the osmotic balance of this medium. Sterile serum is used as a growth supplement.

Formula / Liter
Heart Infusion.................................................................6 g
Yeast Enriched Peptone ..................................................10 g
Sodium Chloride ..............................................................5 g
Final pH: 7.8 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions
1. For Laboratory Use Only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Dissolve 21 g of the medium in 700 mL of purified water.
2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 300 mL of the appropriate sterile serum supplement.
5. Mix thoroughly.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.
Prepared Appearance: Prepared medium is trace to light hazy with none to trace precipitate and yellow.

Expected Cultural Response: Cultures were inoculated into PPLO Broth W/O CV and incubated at 35 ± 2°C aerobically. After 3, 4, and 5 days of incubation the test organisms were subcultured onto PPLO Agar. PPLO Agar plates were incubated at 35 ± 2°C under CO₂ enrichment for 3 – 8 days and examined for the presence of Mycoplasma colonies.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Approx. Inoculum (CFU)</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma bovis ATCC® 25025</td>
<td>10³</td>
<td>Growth</td>
</tr>
<tr>
<td>Mycoplasma gallinarum ATCC®19708</td>
<td>10³</td>
<td>Growth</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae ATCC® 15531</td>
<td>10³</td>
<td>Growth</td>
</tr>
<tr>
<td>Acholeplasma laidlawii ATCC® 14089</td>
<td>10³</td>
<td>Growth</td>
</tr>
</tbody>
</table>

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Inoculate organisms into PPLO Broth W/O CV, following required time and temperature. Subculture onto PPLO Agar. Refer to appropriate references for complete details on the specific procedures for the isolation and identification of Mycoplasma spp.

**Results**
Examine PPLO Agar after 4 to 7 days of incubation. Mycoplasma colonies are round, 0.01 to 0.5 mm in diameter with a dense center and a less dense periphery producing a “fried egg” appearance.

**Storage**
Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

**Expiration**
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

**Limitation of the Procedure**
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

**Packaging**

<table>
<thead>
<tr>
<th>PPLO Broth W/O CV</th>
<th>Code No.</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7485A</td>
<td>500 g</td>
</tr>
<tr>
<td></td>
<td>7485B</td>
<td>2 kg</td>
</tr>
<tr>
<td></td>
<td>7485C</td>
<td>10 kg</td>
</tr>
</tbody>
</table>

**References**
5. Parsons, C. 2002. Medline Plus, Mycoplasma pneumonia. Department of Internal Medicine, Division of Infectious Diseases, University of Virginia, Charlottesville, VA.

**Technical Information**
Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.