Intended Use
PPLO (Mycoplasma) agars and broths, when supplemented with nutritive enrichments, are used for isolating and cultivating Mycoplasma. Mycoplasma Broth Base (Frey) is used for the cultivation of avian mycoplasmas.

Summary and Explanation
Members of the class Mollicutes, Mycoplasma was first recognized from a case of pleuropneumonia in a cow. The organism was designated “pleuropneumonia-like organism,” or PPLO. Although some species are normal human respiratory tract flora, M. pneumoniae is a major cause of respiratory disease (primary atypical pneumonia, sometimes called “walking pneumonia”). M. hominis, M. genitalium and Ureaplasma urealyticum are important colonizers (and possible pathogens) of the human genital tract.

PPLO (Mycoplasma) Agar was described by Morton, Smith and Leberman. It was used in a study of the growth requirements of Mycoplasma, along with the identification and cultivation of this organism. PPLO (Mycoplasma) Broth (without crystal violet) is prepared according to the formula described by Morton and Lecci. Crystal violet is omitted from this formula due to its inhibitory action on some Mycoplasma. It has been used for the cultivation of Mycoplasma for research studies.

PPLO (Mycoplasma) Broth Base (Frey), a modification of other broth media, was developed specifically for the cultivation of avian strains of Mycoplasma.

Mycoplasma Supplement and Mycoplasma Enrichment w/o Penicillin are sterile desiccated enrichments for use in PPLO media as described by Hayflick. The supplements are prepared according to the formulations of Chanock, Hayflick and Barile and Hayflick.

Principles of the Procedure
Meat digests, peptones, beef extract and yeast extract provide the nitrogen, vitamins, amino acids and carbon in these media. Sodium chloride maintains the osmotic balance of these formulations. Agar, the solidifying agent, is used in PPLO (Mycoplasma) Agar at a concentration slightly reduced from usual to ensure formation of the largest possible colonies because the organisms grow into the agar with only slight surface growth.

The base media are supplemented with Mycoplasma Supplement or Mycoplasma Enrichment w/o Penicillin because Mycoplasma spp. are fastidious in their growth requirements. Mycoplasma Supplement contains fresh yeast extract and horse serum. Yeast extract provides the preformed nucleic acid precursors that are required by Mycoplasma spp. Horse serum supplies cholesterol, a growth stimulant.

Mycoplasma Enrichment without Penicillin is a selective enrichment containing the inhibitor thallium acetate, to which a penicillin of choice (penicillin G or a broad-spectrum semisynthetic penicillin) can be added at the time of use to make it selective against gram-positive and gram-negative bacteria.

Formulae

**Difco™ PPLO Agar**

Approximate Formula* Per Liter

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Heart, Infusion from 50 g</td>
<td>6.0 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>14.0 g</td>
</tr>
</tbody>
</table>

**Difco™ PPLO Broth**

Consists of the the same ingredients without the agar.
### Identity Specifications

**Difco™ PPLO Agar (Mycoplasma Agar)**
- Dehydrated Appearance: Beige, homogeneous, free-flowing.
- Solution: 2.1% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
- Prepared Appearance: Light amber, clear to very slightly opalescent.
- Reaction of 2.1% Solution at 25°C: pH 7.8 ± 0.2

**Difco™ Mycoplasma Supplement**
- Desiccated Appearance: Straw-colored, dried button, may be dispersed.
- Rehydrated Appearance: Light to dark straw-colored, clear to slightly opalescent.

**BBL™ Mycoplasma Agar Base (PPLO Agar Base)**
- **Approximate Formula** Per Liter
  - Beef Heart, Infusion from (solids)……………….. 2.0 g
  - Pancreatic Digest of Casein ………………. 7.0 g
  - Beef Extract……………………………………… 3.0 g
  - Yeast Extract …………………………………… 3.0 g
  - Sodium Chloride ………………………………. 5.0 g
  - Agar …………………………………………….. 14.0 g

**BBL™ Mycoplasma Broth Base (PPLO Broth Base)**
- Consists of the same ingredients without the agar.

**BBL™ Mycoplasma Broth Base (Frey)**
- **Approximate Formula** Per Liter
  - Pancreatic Digest of Casein …………………. 7.5 g
  - Papaic Digest of Soybean Meal………………. 2.5 g
  - Yeast Extract ………………………………….. 5.0 g
  - Sodium Chloride ……………………………. 5.0 g
  - Potassium Chloride …………………………. 0.4 g
  - Magnesium Sulfate ………………………… 0.2 g
  - Disodium Phosphate ……………………….. 1.6 g
  - Monopotassium Phosphate ………………….. 0.125 g

### Cultural Response

**Difco™ PPLO Agar or PPLO Broth**
Prepare the medium per label directions. Inoculate agar plates with 0.1 mL of serial dilutions of the test organisms. Incubate plates under 5-10% CO₂ at 35 ± 2°C for up to 7 days. Inoculate tubes of broth with 1.0 mL of serial dilutions of the test organisms and incubate under 5-10% CO₂ at 35 ± 2°C for up to 7 days, then subculture (0.1 mL) to plates of the agar medium and incubate under 5-10% CO₂ at 35 ± 2°C for up to 7 days. Daily examine plates microscopically for growth.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC™</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma arginini</td>
<td>23243</td>
<td>Good</td>
</tr>
<tr>
<td>Mycoplasma bovis</td>
<td>25523</td>
<td>Good</td>
</tr>
<tr>
<td>Mycoplasma gallinarum</td>
<td>19708</td>
<td>Good</td>
</tr>
</tbody>
</table>

**BBL™ Mycoplasma Agar Base (PPLO Agar Base)**
- Solution: 2.25% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.
- Prepared Appearance: Light to medium, yellow to tan, clear to slightly hazy.
- Reaction of 2.25% Solution at 25°C: pH 7.7 ± 0.2

**BBL™ Mycoplasma Enrichment without Penicillin**
Rehydrated Appearance: Dark brown, clear to trace hazy.

### Identity Specifications

**BBL™ Mycoplasma Broth Base (PPLO Broth Base)**
- Dehydrated Appearance: Fine, homogeneous, free of extraneous material.
- Solution: 2.0% solution, soluble in purified water upon warming. Solution is light to medium, yellow to tan, clear to slightly hazy.
- Prepared Appearance: Light to medium, yellow to tan, clear to slightly hazy.
- Reaction of 2.0% Solution at 25°C: pH 7.8 ± 0.2

**BBL™ Mycoplasma Enrichment without Penicillin**
Prepare the medium per label directions (enriched with BBL Mycoplasma Enrichment without Penicillin). Inoculate agar plates with 0.1 mL of serial dilutions of the test organisms. Incubate for 7 days at 35 ± 2°C with 3.5-5% CO₂, for M. pneumoniae and anaerobically for Mycoplasma orale. Incubate tubes of broth with 1.0 mL of serial dilutions of the test organisms and incubate for 7 days at 35 ± 2°C with 3.5-5% CO₂, for M. pneumoniae and anaerobically for M. orale. After 5 days of incubation, subculture tubes (0.1 mL) to Mycoplasma Agar and incubate at 35 ± 2°C for up to 7 days under appropriate atmospheric conditions.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC™</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma orale</td>
<td>23714</td>
<td>Good</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>1553</td>
<td>Good</td>
</tr>
</tbody>
</table>

**BBL™ Mycoplasma Broth Base (Frey)**
Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 3-5% CO₂ for 7 days. Subculture to Mycoplasma Agar plates and incubate aerobically at 35 ± 2°C for 7 days. Examine plates microscopically for growth.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC™</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
</table>
| Escherichia coli | 25922 | 10⁶-10⁷       | Growth in a dilution containing 10⁷ CFU/mL
| Mycoplasma gallisepticum | 19610 | Undiluted    | Good     |
| Mycoplasma synoviae  | 25204 | Undiluted    | Good     |

**User Quality Control**

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both Difco™ and BBL™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.
**Difco™ Mycoplasma Supplement**

Approximate Formula* Per 30 mL Vial
- Yeast Extract ............................................................... 0.09 g
- Horse Serum ............................................................. 22.8 mL

**BBL™ Mycoplasma Enrichment without Penicillin**

Approximate Formula* Per 30 mL Vial
- Horse Serum ............................................................. 20.0 mL
- Yeast Extract (fresh autolysate) .................................. 10.0 mL
- Thallium Acetate ....................................................... 50.0 mg

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

**Directions for Preparation from Dehydrated Product**

**Difco™ PPLO Agar**

1. **PPLO Agar**:
   - Suspend 35 g of the powder in 700 mL of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

2. **PPLO Broth**:
   - Dissolve 21 g of the powder in 700 mL of purified water. Mix thoroughly.

3. **Autoclave at 121°C for 15 minutes.**
4. **Cool medium to 50-60°C.**
5. **Aseptically add 300 mL Difco Mycoplasma Supplement to the medium. Mix well.**
6. **Add selective agents if desired (i.e., thallium acetate or penicillin).**
7. **Test samples of the finished product for performance using stable, typical control cultures.**

**BBL™ Mycoplasma Agar Base**

**BBL™ Mycoplasma Broth Base**

1. **Mycoplasma Agar Base**:
   - Suspend 34 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

2. **Mycoplasma Broth Base**:
   - Suspend 20 g of the powder in 1 L of purified water. Mix thoroughly. Warm slightly to completely dissolve the powder.

3. **Autoclave at 121°C for 15 minutes.**
4. **Cool to 50°C and add enrichment. Recommended enrichments include addition of 20 mL of horse serum and 5 mL of specially prepared yeast extract** to each 75 mL of cooled medium.
5. **Add selective agents if desired (i.e., thallium acetate or penicillin).**
6. **For a selective medium inhibitory to bacteria, add 30 mL of BBL Mycoplasma Enrichment without Penicillin to 70 mL of molten agar medium (50°C) or 70 mL of broth medium and add sterile penicillin G to a final concentration of 500 units/mL.**
7. **Test samples of the finished product for performance using stable, typical control cultures.**

**BBL™ Mycoplasma Broth Base (Frey)**

1. **Dissolve 22.5 g of the powder in 1 L of purified water. Mix thoroughly.**
2. **Autoclave at 121°C for 15 minutes.**
3. **Cool to 50°C and add 100 mL of sterile inactivated horse serum. Mix thoroughly.**

4. **For recovery of *M. synoviae*, add 0.01% (w/v) nicotinamide adenine dinucleotide (NAD) and 0.01% (w/v) L-cysteine HCl. Inactivated swine serum is preferred in place of horse serum.**
5. **Test samples of the finished product for performance using stable, typical control cultures.**

**Difco™ Mycoplasma Supplement**

**BBL™ Mycoplasma Enrichment without Penicillin**

1. **Rehydrate with 30 mL of sterile purified water.**
2. **Rotate gently to dissolve.**
3. **Add 30 mL (the contents of one vial) to 70 mL of sterile medium base.**
4. **Dispense in plates or tubes as desired.**

**Procedure**

**Agar**

Inoculate the surface of plates containing the complete medium by adding drops of liquid inoculum or by a swab-inoculation technique. Incubate plates at 35 ± 2°C for up to 21 days in a moist atmosphere containing 5-10% carbon dioxide or anaerobically if the presence of *M. buccale, M. faucium, M. orale* or *M. salivarium* is suspected.

**Broth**

Test material, either solid or liquid, should be directly inoculated into the broth medium. For preparation of stock organism suspensions, a block of agar culture can be added to the broth. Following incubation at 35 ± 2°C in a moist aerobic atmosphere containing 5-10% carbon dioxide or anaerobically, if appropriate, for various lengths of time, subculture aliquots of the broth to PPLO (Mycoplasma) Agar plates for visualization of typical colonies. The broth usually does not become turbid enough to confirm the presence of growth.

For a complete discussion of the isolation and identification of *Mycoplasma* spp. from clinical specimens, refer to appropriate procedures outlined in the references.

**Expected Results**

**Agar**

PPLO colonies are round with a dense center and a less dense periphery, giving a “fried egg” appearance on PPLO (Mycoplasma) Agar. Vacuoles, large bodies characteristic of *Mycoplasma* spp., are seen in the periphery. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.

**Broth**

After subculture to plates of PPLO (Mycoplasma) Agar, positive broth cultures produce colonies exhibiting the typical morphology; i.e., “fried egg” appearance.

**Limitation of the Procedure**

Thallium acetate can partially inhibit some mycoplasmas.
References


Availability

Difco™ PPLO Agar (Mycoplasma Agar)
Cat. No. 241210 Dehydrated – 500 g

BBL™ Mycoplasma Agar Base (PPLO Agar Base)
Cat. No. 211456 Dehydrated – 500 g

Difco™ PPLO Broth (Mycoplasma Broth)
Cat. No. 211458 Dehydrated – 500 g

BBL™ Mycoplasma Broth Base (PPLO Broth Base)
Cat. No. 211458 Dehydrated – 500 g

BBL™ Mycoplasma Broth Base (Frey)
Cat. No. 212346 Dehydrated – 500 g
Cat. No. 212347 Dehydrated – 5 lb (2.3 kg)

Difco™ Mycoplasma Supplement
Cat. No. 283610 Vial – 6 x 30 mL*

BBL™ Mycoplasma Enrichment w/o Penicillin
Cat. No. 212292 Vial – 10 x 30 mL*

*Store at 2-8°C.