

PPLO BROTH w/o CV (7485)

Intended Use

PPLO Broth w/o CV is used with enrichments for the isolation and cultivation of *Mycoplasma* spp.

Product Summary and Explanation

PPLO Broth w/o CV is prepared according to the formula described by Morton and Lecce.¹ Crystal Violet may inhibit the growth of *Mycoplasma*, and therefore was removed from an earlier formula described by Morton, Smith, Williams, and Eikenberg.² *Mycoplasma* was discovered in a case of pleuropneumonia in a cow, and referred to as "pleuropneumonia-like organism" or PPLO.³ *Mycoplasmas* belong to the class of *Mollicutes* "soft skin", which are the smallest free-living organisms.⁴ They are pleomorphic, varying in size from 0.2 to 0.3 micromillimeters.⁴

Mycoplasma pneumoniae is a common cause of mild pneumonia and usually affects people younger than 40.⁵ Studies suggest that it causes 15 – 50% of all pneumonia in adults and an even higher percentage of pneumonias in school-aged children.⁵ The symptoms include headache, fever, cough, chest pain, and sore throat.⁵

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

Supplement

Sterile Serum Supplement, 300 mL

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

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

Directions

1. Dissolve 21 g of the medium in 700 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
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.

Microorganism	Expected Results
<i>Mycoplasma bovis</i> ATCC® 25025	growth
<i>Mycoplasma gallinarum</i> ATCC® 19708	growth
<i>Mycoplasma pneumoniae</i> ATCC® 15531	growth
<i>Acholeplasma laidlawii</i> ATCC® 14089	growth

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

PPLO Broth w/o CV	Code No.	7485A	500 g
		7485B	2 kg
		7485C	10 kg

References

1. **Morton, H. E., and J. G. Lecce.** 1953. Selective action of thallium acetate and crystal violet for pleuropneumonia like organisms of human origin. *J. Bacteriol.* **66**:646-649.
2. **Morton, H. E., P. E. Smith, N. B. Williams, and C. F. Eickenberg.** 1951. Isolation of pleuropneumonia-like organisms from human saliva: A newly detected member of the oral flora. *J. Dent. Res.* **30**:415-422.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. *Bailey & Scott's diagnostic microbiology*, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
4. **Kenny, G. E.** 1985. *Mycoplasmas*. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (eds). *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
5. **Parsons, C.** 2002. Medline Plus, *Mycoplasma pneumoniae*. 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 (410)780-5120 or fax us at (410)780-5470.