

PPLO Broth Base without Crystal Violet (NCM0113)

Intended Use

PPLO Broth Base without Crystal Violet is used with enrichments for the isolation and cultivation of *Mycoplasma* spp. in a laboratory setting. PPLO Broth without Crystal Violet is not intended for the use in the diagnosis of disease or other conditions in humans.

Description

PPLO Broth Base without Crystal Violet 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 μm .

Typical Formulation

Heart Infusion	6.0 g/L
Yeast Enriched Peptone	10.0 g/L
Sodium Chloride	5.0 g/L

Final pH: 7.8 \pm 0.2 at 25°C

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

Supplement

Sterile Serum Supplement, 300 mL

Precaution

Refer to SDS

Preparation

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.

Test Procedure

1. Inoculate organisms into PPLO Broth Base W/O Crystal Violet, following required time and temperature.
2. Subculture onto PPLO Agar.
3. Refer to appropriate references for complete details on the specific procedures for the isolation and identification of *Mycoplasma* spp.

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.

Technical Specification Sheet



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	Approx. Inoculum (CFU)	Expected Results
<i>Mycoplasma bovis</i> ATCC® 25025	10 ³	Growth
<i>Mycoplasma gallinarum</i> ATCC® 19708	10 ³	Growth
<i>Mycoplasma pneumoniae</i> ATCC® 15531	10 ³	Growth
<i>Acholeplasma laidlawii</i> ATCC® 14089	10 ³	Growth

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

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.

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.

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.

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.

