Mycoplasma Broth Base w/ CV (PPLO Broth Base w/ CV)

M268

Intended Use:

For isolation and cultivation of *Mycoplasma* species (pleuropneumonia like organisms) from clinical specimens and mixed cultures.

Composition**

Ingredients	g / L
HM infusion B from 250g #	6.000
Peptone	10.000
Sodium chloride	5.000
Crystal violet	0.010
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef heart, infusion from

Directions

Suspend 21.0 grams in 700 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 2.85 ml of PTe 1% Selective Supplement (1 ml per vial) (FD052) along with 300 ml Horse serum (RM1239) or 10 vials of HPYT Enrichment Supplement (FD075). Mix well and dispense aseptically into sterile test tubes. 25% Ascitic Fluid can be used instead of Horse serum.

Principle And Interpretation

PPLO Media were described by Morton, Smith and Leberman (1). It was used in a study of the growth requirements of *Mycoplasma* (2), along with the identification and cultivation of this organism(3-5). Pivotal information regarding *Mycoplasma* has been documented by Sabin (6). Hayflick et al have reported the information regarding the cultivation of *Mycoplasma* (7). For the cultivation of *Mycoplasma* the medium ingredients and all the supplements should be free of any toxic substances even in small amounts. Many *Mycoplasma* require serum for their good growth and also presence of antibiotic is necessary to prevent the growth of contaminating organisms. Crystal violet and potassium tellurite inhibits many gram-negative and gram-positive bacteria. Mostly the *Mycoplasma* species are aerobic or facultatively anaerobic but some are microaerophilic. Few are anaerobic saprophytic Mycoplasma which grow best at 22-35°C while pathogenic strains grow at 35°C.

Mycoplasma when grow in the agar medium show typical morphology, and form colonies below the agar surface and do no grow without serum. After subculture to plates of PPLO Agar, possible broth cultures produce colonies exhibiting the typical morphology, i.e., fried egg appearance. Plates or tubes should be incubated in an atmosphere containing 5-10% carbon dioxide and examined after incubation of 48 hours but they should not be discarded as negative until after incubation for 3 weeks.

Type of specimen

Clinical samples - nasopharyngeal and oropharyngeal swabs

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Since *Mycoplasma* species are aerobic or facultatively anaerobic but some are microaerophilic, proper incubation should be carried out for optimal recovery.
2. Since the medium is highly enriched, care must be taken during preparation and inoculation to avoid contamination.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured may have purple tinge, clear solution in tubes.

Reaction

Reaction of 2.1% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH

7.60-8.00

Cultural Response

Cultural characteristics observed in presence of 10% Carbon dioxide with added PTe 1% Selective Supplement (1 ml per vial) (FD052), 1%Horse serum (RM1239) and HPYT Enrichment Supplement (FD075) after an incubation at 22-35°C for 48 hours.

Organism	Growth
Mycoplasma bovis ATCC 25523	good-luxuriant
Mycoplasma gallinarium ATCC 19708	good-luxuriant
Mycoplasma pneumoniae ATCC 15531	good-luxuriant
Streptococcus pneumoniae ATCC 6303	good-luxuriant

Storage and Shelf Life

Store below 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Morton, Smith and Leberman, 1951, Am. J. Syphilis Gonorrh. Veneral Diseases, 35: 361.
2. Morton and Lecce, 1953. J. Bacteriol., 66:646.
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4. Craven, Wenzel, Calhoun, Hendley, Hamory and Gwaltney, 1976, J. Clin. Microbiol., 4:225.
5. Gregory and Cundy, 1970, Appl. Microbiol., 19:268.
6. Sabin, 1941, Bacteriol. Rev., 5:1, 331.
7. Hayflick and Chanock, 1965, Bacteriol, Rev., 29:185.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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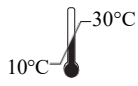
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP

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CE Marking

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