



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

M268

PPLO Broth Base w/ CV (Mycoplasma Broth Base w/ CV) with the addition of enrichment, is used for isolation and cultivation of *Mycoplasma* species (pleuropneumonia like organisms) from clinical specimens and mixed cultures.

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from	250.000
Peptic digest of animal tissue	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

Directions

Suspend 21 grams in 700 ml 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°C and aseptically add 2.85 ml of 1% Potassium Tellurite (FD052) along with 300 ml Horse serum (RM1239) or 10 vials of Mycoplasma Enrichment Supplement (FD075). Mix well and dispense 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.

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. 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

M268: Cultural characteristics observed in presence of 10% Carbon dioxide with added 1% Potassium tellurite (FD052), 1% Horse serum (RM1239) and Mycoplasma Enrichment Supplement (FD075) after an incubation at 22-35°C for 48 hours.

Organism	Growth
Cultural Response	
<i>Mycoplasma bovis</i> ATCC 25523	good-luxuriant
<i>Mycoplasma gallinarium</i> ATCC 19708	good-luxuriant
<i>Mycoplasma pneumoniae</i> ATCC 15531	good-luxuriant
<i>Streptococcus pneumoniae</i> ATCC 6303	good-luxuriant

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Morton, Smith and Leberman, 1951, Am. J. Syphilis Gonorrh. Venereal Diseases, 35: 361.
2. Morton and Lecce, 1953. J. Bacteriol., 66:646.
3. Chanock, James, Fox, Turner, Mufso and Hayflick, 1962, Soc. Exp. Biol. Med., 110:884.
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.

Revision : 2 / 2015

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