



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

M267

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

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from	250.000
Peptic digest of animal tissue	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.0 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 300 ml of Horse serum (RM1239). If crystal violet is used as the selective agent, enrich with Ascitic fluid instead of Horse serum (RM1239). If desired add 10 vials of Mycoplasma Enrichment Supplement (FD075) instead of horse serum . Mix well and dispense aseptically into sterile tubes.

Principle And Interpretation

PPLO Broth without crystal violet is prepared according to the formula described by Morton and Lecce (1). 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 (2, 3).

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. 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.

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.

After subculture to plates of PPLO Agar, possible broth cultures produce colonies exhibiting the typical morphology, i.e., fried egg appearance.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured clear solution in tubes

Reaction

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

pH

7.60-8.00

Cultural Response

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

Cultural Response

Organism	Growth
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Cultural Response	
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<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 and Lecce, 1953. J. Bacteriol., 66:646.
2. Adler and Da Massa, 1967, Appl. Microbiol., 15:245.
3. Leland, Lapworth, Jones and French, 1982, J. Clin. Microbiol., 16:709.

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