PPLO Agar Base (Mycoplasma Agar Base)

M266

PPLO Agar Base (Mycoplasma Agar Base) 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
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36 grams in 700 ml distilled water. Heat to boiling 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 Horse serum (RM1239) or 10 vials of Mycoplasma Enrichment Supplement (FD075). Mix well before dispensing. 25% Ascitic fluid can be used instead of Horse serum.

Principle And Interpretation

PPLO Agar was 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. Beef heart infusion, peptic digest of animal tissue and peptone provide nitrogen, vitamins, amino acids and carbon in these media. Sodium chloride maintains the osmotic balance of these formulations. 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. *Mycoplasma* when grow in the agar medium show typical morphology and form colonies below the agar surface and do no grow without serum.

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.

PPLO colonies are round with a dense center and a less dense periphery, resembling a “fried egg” on PPLO Agar. Vacuoles, large bodies characteristic of *Mycoplasma* species are seen in the periphery. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.6% 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 ,1% Horse serum (RM1239) or 10 vials of Mycoplasma Enrichment Supplement(FD075), after an incubation at 22-35°C for 48 hours.

Cultural Response

Organism	**Growth**
Cultural Response	
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 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. Veneral 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.

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