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

M267

Intended Use:

For isolation and cultivation of *Mycoplasma* species (pleuropneumonia like organisms).

Composition**

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

**Formula adjusted, standardized to suit performance parameters
- Equivalent to Beef 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 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°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 HPYT 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.

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 (4,5).
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. Few are anaerobic saprophytic *Mycoplasma* which grow best at 22-35°C while pathogenic strains grow at 35°C, hence growth conditions must be maintained.
3. 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 temmperature.

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

7.60-8.00

Cultural Response

Cultural characteristics observed in presence of 10% Carbon dioxide with added 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 in order 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 (4,5).

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.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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**

CE Marking

10°C – 30°C **Storage temperature**

Do not use if package is damaged