# Mycoplasma Agar Base (PPLO Agar Base) M266

## Intended Use:

Recommended for isolation and cultivation of *Mycoplasma* species (Pleuropneumonia-like organisms - PPLO).

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| HM infusion B from ● | 250.000 |
| Peptone | 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

● - Equivalent to Beef heart, infusion from

## Directions

Suspend 36.0 grams in 700 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°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. HMB infusion from and peptone provide nitrogen and carbon source, long chain amino acids, vitamins, and other essential nutrients. 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.

## Type of specimen

Clinical samples and pharmaceutical samples

## 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. 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 guidleines should be followed while handling clincal specimens. Saftey 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 innoculation 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

**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.

| 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. Use before expiry date on the label.
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).

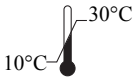
## Reference

1. Morton, Smith and Leberman, 1951, Am. J. Syphilis Gonorrh. Veneral Diseases, 35: 361.
2. Morton and Lecce, 1953. J. Bacteriol., 66:646.
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.
8. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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.

Revision : 02/ 2017

IVD — In vitro diagnostic medical device

CE Marking




Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
B /4-6 , MIDC, Dindori, Nashik MH

www.himedialabs.com

EC REP | CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands,
www.cepartner 4u.eu