

PPLO AGAR

A medium base for the cultivation and maintenance of mycoplasma

Typical formula (g/l)

| | |
|--------------------|----|
| Beef Heart Extract | 5 |
| Tryptone | 10 |
| Sodium Chloride | 5 |
| Agar | 15 |

PPLO ENRICHMENT BROTH

A liquid medium base for the cultivation and maintenance of mycoplasma

Typical formula (g/l)

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|--------------------|-------|
| Beef Heart Extract | 5 |
| Tryptone | 10 |
| Sodium Chloride | 5 |
| Crystal Violet | 10 mg |

DIRECTIONS

Suspend 35g of PPLO Agar or 20g of PPLO Enrichment Broth in 1000ml of cold distilled water. Heat to dissolve, distribute and sterilise at 121°C for 15 minutes. Cool to approximately 60°C and add the required enrichment and selective supplements.

Final pH 7.8 ± 0.2

DESCRIPTION

PPLO Agar and PPLO Enrichment Broth are highly nutritious base media, which should be supplemented with selective agents and enrichments for isolation, cultivation and maintenance of Mycoplasma (PPLO:Pleuropneumonia-like organism). Both media are prepared according to the formulation described by Morton, Smith and Leberman. The heart extract and the enzymatic casein hydrolysate are the base ingredients for the growth of *Mycoplasmataceae*. The pathogenic strains grow on PPLO Agar supplemented with fresh yeast extract (yeast extract solution, 200ml/l) and horse serum. The serum obtained from animal sources, when added to PPLO Agar, show inhibitory effects on a few mycoplasma strains, for this reason addition of gamma-globulin free serum fractions to the base medium is advised.

PPLO Agar may be made selective with incorporation of penicillin (1.000.000 IU/l), which inhibits Gram-positive bacteria, amphotericin B (5mg/l), which inhibits fungal growth and, polymyxin B (50 mg/l). The medium may be supplemented with methylene blue to obtain a selective growth of *M. pneumoniae* and tetrazolium chloride to differentiate *M. pneumoniae* from the other species. PPLO Broth may be supplemented with a pH indicator "phenol red 18 mg/l".

TECHNIQUE

Inoculate and streak the specimen as soon as possible after receipt in the laboratory. Increased recovery may be enhanced by diluting and plating the specimen serially up-to 10⁻³. Diluting the specimen minimises the effect of bacterial inhibitors on the growing mycoplasma. Agar plates should be taped to reduce dehydration. Incubate plates in 5-10% CO₂ at 35°C for up-to 30 days. Plates may be incubated anaerobically if *M. buccale*, *M. faucium*, *M. orale* or *M. salivarium* are suspected.

The colonies when observed with a low magnifying power (40-60 X) often show to be wide and flat with a dark centre ranging between 24 and 100 microns in diameter. These organisms are recognised by typical tiny "fried egg" colonies or finely granular ("ground glass"). The central portion of the colonies grow inside the medium, the peripheral portion grows at the surface. The peripheral parts of the colonies often show the presence of vacuoles, characteristics of pleuropulmonary organisms.

PPLO Enrichment Broth is employed for the selective enrichment of mycoplasmas and the purification of cultures. Inoculate the specimen as soon as possible after receipt in the laboratory. Inoculate with 0.1ml of transport medium containing the swab. Alternatively, PPLO Broth may be inoculated at 1:10 ratio with blood or CSF. Diluting the specimen minimises the effect of bacterial inhibitors on the growing mycoplasma. Incubate at 35°C for up-to 30 days. Examine tubes daily and subculture onto PPLO Agar plates.

STORAGE

Dehydrated medium: 10-30°C

REFERENCES

- APHA (1963) - Diagnostic Procedures and Reagents, 4th edition.
- Morton, H.E., Smith, P.F. & Leberman, P.R. (1951) - Am. J. Syph., 35, 361-369.

PACKAGING

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|----------------|------------------------------|----------------------|
| 4019452 | PPLO Agar | 500g (14.3 l) |
| 4019502 | PPLO Enrichment Broth | 500g (25 l) |