PPLO AGAR
PPLO ENRICHMENT BROTH
Dehydrated culture media

1 - INTENDED USE
PPLO Agar and PPLO Enrichment Broth, when supplemented with nutritive enrichments and selective supplements, are used for isolating and cultivating *Mycoplasma*.

2 - COMPOSITION - TYPICAL FORMULAS * (AFTER RECONSTITUTION WITH 1 L OF WATER)

PPLO AGAR	
Beef heart extract	5 g
Tryptone	10 g
Sodium chloride	5 g
Agar	15 g
PPLO ENRICHMENT BROTH	
Beef heart extract	5 g
Tryptone	10 g
Sodium chloride	5 g
Crystal violet	10 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Mycoplasma is a genus of bacteria that, like the other members of the class *Mollicutes*, lack a cell wall around their cell membrane.
Isolation of the first mycoplasma was the bovine pleuropneumonia agent, which was reported initially in 1898 by Nocard and Roux[1]. This bacterium became to know over the next 50 years as "pleuropneumonia-like organisms" or PPLO, in various animals.[2]
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 mycoplasmas. PPLO Agar is prepared according to the formulations described by Morton, Smith and Leberman[3] while PPLO Enrichment Broth is prepared according to the formulation of Morton and Lecce[4]. The beef heart extract and tryptone are the base ingredients for the growth of *Mycoplasmataceae*. Sodium chloride maintains the osmotic equilibrium. Crystal violet in PPLO Enrichment Broth is a selective agent against Gram-positive bacteria. The pathogenic strains grow on PPLO Agar supplemented with fresh yeast extract and horse serum, as reported by Hayflick[5]. The serum obtained from animal sources, when added to PPLO Agar, shows inhibitory effects on a few mycoplasma strains, for this reason addition of gamma-globulin free serum fractions to the base medium is advised.[6]
PPLO Agar may be made selective with incorporation of thallous acetate, penicillin G which inhibits Gram-positive bacteria, and amphotericin B, which inhibits fungal growth.[6] PPLO Agar may be supplemented with 0.2 mL/L of 1% methylene blue solution to obtain a selective growth of *M. pneumoniae* and tetrazolium chloride to differentiate *M. pneumoniae* from the other species. PPLO Enrichment Broth may be supplemented with glucose and a pH indicator such as phenol red for the visualisation of the colour change from violet to yellow of the medium due to glucose fermentation.

4 - DIRECTIONS FOR MEDIUM PREPARATION
PPLO Agar
Suspend 35 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes. Cool to approximately 50-60ºC and add the required enrichment and selective supplements.
PPLO Enrichment Broth
Suspend 20 g in 1000mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and sterilise by autoclaving at 121ºC for 15 minutes. Cool to approximately 50-60ºC and add the required enrichment and selective supplements.
The table below summarizes the enrichments and selective additives to be used.[6] All sterile solutions must be added aseptically to either agar or broth base.

Ingredient	Agar (mL)	Broth (mL)	Final concentration/ mL medium
PPLO Agar	70		
PPLO Enrichment Broth		70	
Horse serum	20	20	
Yeast extract 25% solution	10	10	
Penicillin G solution	1	1	1000
Amphotericin B Solution	0.5	0.5	5 µg
Thallous acetate solution	0.5	0.5	500 µg
D-glucose solution		2	10 µg
Phenol red solution		1	20 µg

5 - PHYSICAL CHARACTERISTICS

PPLO Agar	
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	7.8 ± 0.2
PPLO Enrichment Broth	
Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	violet, limpid
Final pH at 20-25 °C	7.8 ± 0.2

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6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
PPLO Agar	Dehydrated medium	4019452	500 g (14.3 L)
PPLO Enrichment Broth	Dehydrated medium	4019502	500 g (25 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, screw capped tubes, ancillary culture media and reagents.

8 – SPECIMENS

Swabs, sputum, body fluids, macerated tissue and other clinical and non-clinical specimens. For sample collection, storage, transport and preparation, follow good laboratory practice.

9 - TEST PROCEDURE, READING AND INTERPRETATION

PPLO Agar

Prepare a 1:10 and 1:100 dilutions of the specimen in a suitable broth medium, such as PPLO Broth, to reduce potential inhibitory substances that may be present.

Inoculate and streak the specimen as soon as possible after receipt in the laboratory.

Incubate taped plates in 5-10% CO_2 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

PPLO Enrichment Broth may be used for the selective enrichment of mycoplasmas, the purification of cultures or in diphasic cultures.[6] Directly inoculate the specimen or the diluted specimen as soon as possible after receipt in the laboratory into the broth. Diluting the specimen minimises the effect of bacterial inhibitors on the growing mycoplasma.

Incubate at 35°C in 5-10% CO_2 for up-to 30 days. Examine tubes after 2, 5, 10, 15, 25 and 30 days and subculture aliquots of the broth to PPLO plates for visualization of typical colonies.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAIN	INCUBATION T°/ T - ATM	EXPECTED RESULTS
M. hominis ATCC 15488	35-37°C / up to 6 days / CO_2	fried-egg colonies

ATCC is a trademark of American Type Culture Collection

11 – LIMITATIONS OF THE METHOD

- PPLO Enrichment Broth contains crystal violet that inhibits accompanying flora, while the proliferation of most mycoplasma is not affected by crystal violet; exceptions are poultry, T-mycoplasmas and certain pig mycoplasmas. According to Morton and Lecci, CV containing media should be supplemented with ascitic fluid.[6]
- T-strains mycoplasmas are sensitive to thallous acetate, therefore media with this selective agent are not recommended for T-strains (e.g ureaplasmas).[6]
- Kundisin *et al*.[7] recommend the use of both broth and agar medium for initial specimen inoculation.

12 - PRECAUTIONS AND WARNINGS

- These products are for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Complete media with nutrient enrichments and selective supplements should be validated by the user for the intended purposes. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheets.
- These culture media contain raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the products do not contain any transmissible pathogen. Therefore, it is recommended that the culture media be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture media as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

13 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to MacFaddin the base media may be stored at +2°C /+8°C for approximately 6-8 weeks, while the media with additives and selective supplements should be stored at +2°C /+8°C for approximately 3-4 weeks.[6]

14 – REFERENCES

1. Nocard E, Roux ER. Le microbe de la peripneumonie. Ann Inst Pasteur (Paris). 1898; 12:240–262
2. Saraya T.The History of Mycoplasma pneumoniae Pneumonia. Front Microbiol. 2016; 7:364
3. Morton H, Smith PF, Leberman PR. Investigation of the cultivation of pleuropneumonia-like organisms from man. Am J Syph Gonorrhea Vener Dis. 1951: 35:361-9.
4. Morton HE, Lecce JG. Selective action of thallium acetate and crystal violet for pleuropneumonialike organisms of human origin. J Bacteriol 1953 ;66:646-9
5. Hayflick L. Tissue cultures and Mycoplasmas. Texas Rep Biol Med. 1965; 23:285.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Kundsin RB, Parreno A, Poulin S. Significance of appropriate techniques and media for isolation and identification of Ureaplasma urealyticum from clinical specimens. J Clin Microbiol. 1978; 8:445-453.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/12

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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