IFU

Instructions for Use

PPLO (MYCOPLASMA) MEDIA

Cat. no. G04	PPLO Selective Agar, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. G105	PPLO Selective Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. R88	PPLO Selective Broth, 13x100mm Tube, 4ml	20 tubes/box

INTENDED USE

Hardy Diagnostics PPLO Media are recommended for the cultivation and maintenance of *Mycoplasma* spp. and *Ureaplasma* spp.

SUMMARY

PPLO, or pleuropneumonia-like organisms, were first recognized in cattle and many are a major cause of respiratory disease; more still are considered normal flora and potential pathogens of the human urogenital tract.[8] PPLO fall into the class Mollicutes and are recognized as commensal or parasitic colonizers of humans, other animals, insects, and plants. Currently, there are over 100 known species in the genus *Mycoplasma* whose members are restricted to vertebrate hosts. Far fewer *Ureaplasma* spp. have been discovered, but members of this genus are also known to be commensal or potentially pathogenic to their hosts.

Mycoplasma spp. lack a cell wall and are unaffected by penicillins or other beta-lactam antibiotics that target cell wall synthesis. However, they do possess characteristic cell shapes believed to play a role in their ability to thrive in certain hosts. All *Mycoplasma* spp. require sterols, usually in the form of cholesterol, for cytoplasmic membrane stability, making them dependent upon their host's biosynthetic capabilities. Their optimal growth temperature usually falls within the range specific to their host. If their host is unable to regulate its own temperature, many are capable of growth at ambient temperatures.

Ureaplasma spp. also lack a cell wall and require cholesterol for growth. Members of this genus are considered part of the normal flora of the human genital tract, but may cause or play a role in non-specific urethritis, infertility, chorioamnionitis, stillbirth, premature birth and, in the perinatal period, pneumonia, chronic lung disease and meningitis in infants.[9] A defining characteristic of this genus is that members are capable of urea hydrolysis.

PPLO Selective Media are highly nutritious due to the addition of beef heart infusion, peptone supplemented with yeast extract and inactivated horse serum. Yeast extract provides diphosphopyridine nucleotides and serum provides cholesterol and a source of protein. The selective agents amphotericin B, polymyxin B, and penicillin are added to inhibit faster growing contaminants. The concentration of agar is slightly reduced in PPLO solid media in order to encourage growth of larger colonies, since surface colonial growth is minimal.[10] PPLO Selective Broth contains all of the aforementioned ingredients, but is lacking in agar. All PPLO Broth Media contain a phenol red pH indicator to aid in the detection of *Mycoplasma* growth.

FORMULA

Ingredients per liter:*

Beef Heart Infusion	50.0gm
Peptone	10.0gm
Sodium Chloride	5.0gm
Polymyxin B	50.0mg
Amphotericin B	5.0mg
Penicillin	1,000,000U
Deionized Water	680.0ml
Horse Serum	200.0ml
Yeast Extract Solution	100.0ml

In addition,

PPLO Selective Agar also contains:

Noble Agar	9.0gm

PPLO Selective Broth also contains:

Phenol Red	18.0mg/ml

Final pH 7.8 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious

disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Specimen/swab should be placed in a tightly sealed transport container with sufficient transport medium to prevent drying and taken directly to the laboratory. Dacron, polyester, or calcium alginate swabs are acceptable. Samples should be processed as soon as possible after arrival in the laboratory. If there is to be a delay in culturing, specimens should be refrigerated. For long-term storage, or if specimens cannot be cultured within 24 hours, freeze specimens at -70ºC. Do not freeze at temperatures greater than -70ºC.

PPLO Selective Agar: Inoculate and streak the specimen as soon as possible after receiving in the laboratory. If using a liquid inoculum, add 0.1ml of liquid to the agar surface and distribute evenly by rocking the plate back and forth or spreading the inoculum with a sterile bent glass rod. Alternatively, if the material being cultured is directly from a swab, roll the swab over the agar surface and streak for isolation. Increased recovery may be enhanced by diluting and plating the specimen serially up to 10^{-3}. Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.[12] Agar plates should be taped to restrict dehydration. Incubate plates in 5-10% CO_2 at 35ºC. for up to 30 days. Plates may be incubated anaerobically if *Mycoplasma buccale*, *M. faucium*, *M. orale* or *M. salivarium* is suspected.

Microscopic examination at 40-60X of inverted plates reveals the colony morphology of mycoplasmas. Organisms are recognized by typical tiny "fried egg" colonies or finely granular ("ground glass") colonies with a berry-like appearance that penetrate the agar surface. Colonies can range from 20-300µm. Consult listed references for more information regarding cultivation and isolation of mycoplasmas.[6,8,11,12]

PPLO Broth: Inoculate the specimen as soon as possible after it is received in the laboratory. Inoculate broth with 0.1ml of transport media containing a swab. Alternatively broth may be inoculated at a 1:10 ratio with blood or CSF. Tissue specimens may be inoculated by placing several minced fragments directly into the broth. Increased recovery may be enhanced by diluting and plating the specimen serially up to 10^{-3}. Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.[12] Incubate at 35ºC. for up to 30 days.

For PPLO Selective Broth: Examine tubes daily for a change in pH. A pH shift will cause the medium to change from red to yellow. As soon as a pH shift is noted, subculture the broth to an appropriate agar medium. Consult listed references for more information regarding cultivation and isolation of mycoplasmas.[6,8,11,12]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Occasional breakthrough of bacterial growth may occur on medium. Similarities of L-form bacteria and mycoplasma organisms on the agar medium may cause some confusion because both exhibit "fried egg" colonies that penetrate the agar surface. L-form colonies tend to be larger and demonstrate a rougher surface. Many L-forms will revert back to the bacterial form if passed to a penicillin-free medium.

Ureaplasma urealyticum will be inhibited on this medium due to the pH of the medium.
Increased recovery may be enhanced by diluting and plating the specimen serially up to 10^{-3}. Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.[12]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Mycoplasma pneumoniae ATCC® 15531	K	10-21 days	35°C	CO_2**	Color change from red to yellow; growth observed microscopically at 10-21 days
Mycoplasma arginini ATCC® 23838	K	10-21 days	35°C	Anaerobic	Slight turbidity; no color change; growth observed microscopically at 10-21 days
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 25923	B	24hr	35°C	Aerobic	Inhibited
Candida albicans ATCC ATCC®	B	24hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

Note: *Mycoplasma pneumoniae* is inoculated by using a swab to place a single streak on the surface.

** Atmosphere of incubation is enriched with 5-10% CO_2.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

- PPLO Selective Agar media should appear slightly opalescent, and light to medium amber in color.
- PPLO Selective Broth media should appear clear, and red in color.

REFERENCES

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1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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