IFU

Instructions for Use

MYCOPLASMA MEDIA

Cat. no. G362	Mycoplasma Agar, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. U407	Mycoplasma Broth, 4oz. Glass Bottle, 100ml	20 bottles/box

INTENDED USE

Hardy Diagnostics Mycoplasma Media are recommended for the cultivation of *Mycoplasma* and for the detection of *Mycoplasma* contamination in biologics and biological processes. These formulations comply with Mycoplasma Agar and Mycoplasma Broth listed in 9 CFR 113.28 and SAM 910.04.[1,2]

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Mycoplasmas are considered commensal or parasitic colonizers of humans, other animals, insects, and plants, with over 100 known species restricted to vertebrate hosts. These microorganisms are small, 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 complex metabolism allows them to survive in many environments and their optimal growth temperature usually falls within the range of their host. However, if their host is unable to regulate its own temperature, many species are capable of growth at ambient temperatures.

Mycoplasma contamination is of concern to many industries, with animal biologics such as live viral products, master cell stocks, and seed viruses being one of the most concerning. *Mycoplasma* contamination can be detected in biologics with the heart infusion test, using heart infusion agar and broth.[1] *Mycoplasma* contamination in cell/tissue cultures is believed to be prominent in 15% of cell cultures in the United States and up to 80% in European countries.[3] Cultures contaminated with *Mycoplasma* typically lead to alterations in cellular metabolism, replication, and ultimately lead to cell death.[3]

Mycoplasma Agar and Mycoplasma Broth are formulated in accordance with 9 CFR 113.28, *Detection of Mycoplasma Contamination* and the *Supplemental Assay Method for Mycoplasma Contamination* (SAM 910.04) for the detection of *Mycoplasma* contamination in animal biologics.[1,2] Mycoplasma Agar is highly nutritious due to the addition of beef heart infusion, peptone supplemented with yeast extract, cysteine, and horse serum. Yeast extract provides diphosphopyridine nucleotides and serum provides cholesterol and a source of protein. Selective agents thallium acetate and penicillin are added to inhibit faster growing contaminants. The concentration of agar is slightly reduced in solid media targeting mycoplasmas in order to encourage growth of larger colonies, since surface colonial growth in this genus is minimal. Mycoplasma Broth is similar in composition, but is lacking in agar. Mycoplasma Agar is used to detect *Mycoplasma* upon direct culture method; whereas, Mycoplasma Broth is used as an enrichment broth before sub-culture to Mycoplasma Agar.

FORMULA

Ingredients per liter of deionized water:*

Mycoplasma Agar	
Heart Infusion Agar	25.0g
Heart Infusion Broth	10.0g
Proteose Peptone #3	10.0g
Horse Serum	126.0ml
Thallium Acetate, 1%	25.0ml
DPN-Cysteine, 1%	21.0ml
Penicillin, 0.5%	5.2ml
Yeast Extract	5.0ml

Mycoplasma Broth	
Heart Infusion Broth	25.0g
Proteose Peptone #3	10.0g
Horse Serum (heat inactivated)	100.0ml
Thallium Acetate, 1%	25.0ml
Tetrazolium Chloride, 1%	5.5ml
Penicillin (100,000 U/ml)	5.0ml
Yeast Extract	5.0ml

Final pH 7.9 +/- 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 for agar media), 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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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

Consult listed references for more information regarding cultivation and isolation of mycoplasmas and for references to procedures in the applicable Standard Requirement.[1-4]

Mycoplasma Agar

1. Inoculate agar with 0.1ml of the sample and tilt each plate in a circular motion to distribute the flow over the surface of the plate. Make a short continuous "Z" streak across the agar surface with a pipette.

2. Keep plates in an upright position with the lid on until the inoculum has been absorbed by the agar prior to inverting for incubation.

3. Apply tape or similar sealant around the vented edge of agar plates to restrict dehydration during incubation.

4. Incubate plates in 4-6% CO_2 at 33 to 37°C for 10-14 days or split incubation of the plates between CO_2 and anaerobic environments per Standard Requirement.[1,2]

5. After incubation, invert plates and examine with a stereoscopic microscope at 35X to 100X or with a standard microscope at 100X magnification.

6. Observe for typical tiny "fried-egg" colonies or finely granular colonies with a berry-like appearance that penetrate the agar surface. Colonies range from 20-300µm in diameter.

Mycoplasma Broth

1. Inoculate the broth with 1.0ml of inoculum and mix thoroughly.

2. Tighten the cap and incubate the broth at 33 to 37°C for a minimum of 14 days.

3. On the 3rd, 7th, 10th, and 14th day of incubation, inoculate 0.1ml of the broth to Mycoplasma Agar using the above procedure and incubate per Standard Requirement. Swirl broth immediately prior to use to evenly distribute the suspension.

INTERPRETATION OF RESULTS

Consult listed references for more information regarding test interpretation.[1,2]

Mycoplasma Agar

Colonies appear transparent, flat, and often resemble a "fried egg" or as finely granular colonies with a berry-like appearance that penetrate the agar surface. Colonies range from 20-300µm in diameter. Plates should be incubated for 7 to 10 days before negative results are reported.[2]

Mycoplasma Broth

The broth should be subcultured to Mycoplasma Agar to confirm the presence of mycoplasmas.

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 these media. Similarities of L-form bacteria and mycoplasma organisms on the agar medium may cause some confusion because they 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 media.

Increased recovery may be enhanced by diluting and plating the sample serially up to 10^{-3}. Diluting the sample minimizes the effects of bacterial inhibitors on the growing mycoplasma.(1)

Consult listed references for more information regarding test interpretation, including control and material tests.(1)

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, swabs, applicator sticks, other culture media, incinerators, incubators, microscopes, 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 bovis ATCC® 25025	K	2-10 days	35°C	CO_2**	Growth; colonies have a "fried egg" and "ground glass" appearance at 100X
Staphylococcus aureus ATCC® 25923	B	24hr	35°C	CO_2**	Partial to complete inhibition
Escherichia coli ATCC® 25922	B	24hr	35°C	CO_2**	Partial to complete inhibition

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

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

***Growth of mycoplasmas in Mycoplasma Broth is confirmed upon subculture to Mycoplasma Agar.

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

Mycoplasma Agar and Mycoplasma Broth should appear clear and pale amber in color.

REFERENCES

1. Title 9, Code of Federal Regulations, Chapter 1, Subchapter E, Part 113.28 - *Detection of Mycoplasma Contamination*. Federal Registrar. Washington, D.C.

2. *Supplemental Assay Method* (SAM), 910.04. Center for Veterinary Biologics, United States Department of Agriculture - Animal and Plant Health Inspection Service. USDA-APHIS. Ames, IA.

3. Chandler, Donna K. F., et al. “Historical Overview of Mycoplasma Testing for Production of Biologics. *American Pharmaceutical Review*, 1 May 2011, www.americanpharmaceuticalreview.com/Featured-Articles/37370-Historical-Overview-of-Mycoplasma-Testing-for-Production-of-Biologics/.

4. Quinn, P.J., et al. 1994. *Clinical Veterinary Microbiology*, Wolfe Publishing, London, England.

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

HDQA 2207F [D]