

# Instructions for Use

## MYCOPLASMA MEDIA

<a href="#">Cat. no. G102</a>	Mycoplasma Agar, 15x100mm Plate, 18ml	10 plates/bag
<a href="#">Cat. no. G212</a>	Mycoplasma Agar with Cefoperazone, 15x100mm Plate, 18ml	10 plates/bag
<a href="#">Cat. no. R102</a>	Mycoplasma Broth, 13x100mm Tube, 4ml	20 tubes/box

## INTENDED USE

Hardy Diagnostics Mycoplasma Media is recommended for use in isolation of *Mycoplasma* species associated with bovine mastitis.

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

## SUMMARY

Mastitis, or inflammation of the mammary gland, is the most common and most expensive disease of dairy cattle throughout most of the world. Mycoplasmal mastitis was first reported in the U.S. in 1961.<sup>(4)</sup>

Several species of *Mycoplasma* can cause mastitis in dairy cows (*M. bovis*, *M. californium*, *M. canadense*, *M. arginini*, etc.), with *M. bovis* being the most frequent and pathogenic bovine mycoplasma in the U.S.<sup>(4,8)</sup> *Mycoplasma* spp. are highly contagious and can be spread from cow-to-cow during milking by means of fomites such as milking machines and teat cups, contaminated hands and airborne transmission in poorly ventilated barns.<sup>(5,8)</sup> Mastitis-causing mycoplasma, *M. bovis* in particular, are commonly found in the mucous membranes of the respiratory and urogenital tracts of healthy cows, and transfer of the microorganisms from the lungs to the mammary gland can occur.<sup>(6-8)</sup> *M. bovis* is a frequent cause of mastitis, arthritis and, less often, of genital infections.<sup>(6)</sup>

There is no effective treatment for mycoplasmal mastitis. Control of the disease relies on identification of infected cows through culturing milk samples from all cows in the herd and through segregation of infected animals.<sup>(7,8)</sup> Although mycoplasmal mastitis can be clinically severe, there is rarely any systemic involvement. However, a mastitis problem within a dairy herd can cause major economic loss: deaths due to peracute forms of mastitis, loss of cows through premature culling, and loss of milk production.<sup>(4)</sup>

Most species of *Mycoplasma* use either glucose or arginine as their major source of energy and require cholesterol or related sterols for growth. Therefore, special or complex media are required for successful isolation of *Mycoplasma* spp.<sup>(4,6)</sup> Hardy Diagnostics' Mycoplasma Media contain inactivated horse serum to provide cholesterol and a source of protein. Thallium acetate and ampicillin, or cefoperazone are also added to inhibit bacterial growth. Mycoplasma Agar is used to detect mycoplasma upon direct culture method, whereas, Mycoplasma Broth is used as an enrichment before subculturing onto Mycoplasma Agar.

## FORMULA

Ingredients per liter of deionized water:\*

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<b>Mycoplasma Broth:</b>	
Pancreatic Digest of Casein	7.0gm
Sodium Chloride	5.0gm
Beef Extract	3.0gm
Yeast Extract	3.0gm
Beef Heart Infusion	2.0gm
Thallium Acetate, 10% Solution	5.0ml
Ampicillin	0.25gm
DNA, 0.2% Solution	10.0ml
Horse Serum, Heat Inactivated	150.0ml

**In addition,**

<b>Mycoplasma Agar contains:</b>	
Noble Agar	15.0gm
Cefoperazone (replaces Ampicillin) - <b>G212</b>	0.07gm

Final pH 7.8 +/- 0.3 at 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C., 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 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

**Sample Collection:** All *Mycoplasma* are sensitive to pH changes in milk. The best recovery rates are achieved when fresh milk samples are plated soon after collection and delivery to the laboratory. Samples can be kept refrigerated for three days or frozen for longer periods before culturing onto Mycoplasma Medium.<sup>(8)</sup>

**Mycoplasma Agar:** For direct culture, composite or quarter milk samples are usually streaked over one-half of the media surface, while bulk tank milk samples are streaked over the entire plate surface.<sup>(8)</sup> Mycoplasma Agar is then incubated in 5-10% CO<sub>2</sub>, at 35-37°C., for at least 24-36 hours. Plates are held for up to seven days before being reported as negative.

**Mycoplasma Broth:** For the enrichment broth method, milk samples are directly inoculated into the broth and incubated aerobically with tight caps, at 35-37°C., for 48 hours. After incubation, the broth is subcultured onto Mycoplasma Agar (Cat. no. G102 or G212) as described above.<sup>(5)</sup>

## INTERPRETATION OF RESULTS

After incubation, Mycoplasma Agar are examined for colonies under low power on a standard microscope or, more effectively, under a stereomicroscope or dissecting microscope. Growth may be visible after three days of incubation, however, 5 to 7 days of incubation are needed for the full development of mycoplasma colonies. Colonies appear transparent, flat, and often resemble a fried egg. Plates should be incubated for 7 to 10 days before negative results are reported.<sup>(5,6,8)</sup>

## 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.

Most *Mycoplasma* isolated from bulk tank milk and cow milk samples are pathogenic, but some milk samples may contain *Acholeplasma laidlawii*, a common non-pathogenic saprophytic contaminant often found in the dairy environment and on teat skin. Therefore, species identification (accomplished by immunofluorescence or an indirect immunoperoxidase test) of *Mycoplasma*-like colonies is recommended.<sup>(8)</sup>

There is no published research for the comparison between direct culture and enrichment culture for bulk tank milk. It can only be assumed that enrichment culture will detect *Mycoplasma* in small numbers more effectively than direct culture. However, enrichment culture of bulk tank milk may also detect nonpathogenic *Mycoplasma* in small numbers, especially during rainy weather.<sup>(5)</sup>

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:

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Test Organisms	Results
<i>Mycoplasma bovis</i> ATCC® 25025*	Growth
<i>Staphylococcus aureus</i> ATCC® 25923	Partial to complete inhibition
<i>Escherichia coli</i> ATCC® 25922*	Partial to complete inhibition

\* Recommended QC strains for User Quality Control according to the CLSI document M22, when applicable.

## 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. Mycoplasma Agar and Mycoplasma Broth may form a slight precipitate at refrigerated storage that clears upon incubation.



Microscopic image of *Mycoplasma bovis* (ATCC® 25025) colonies growing on Mycoplasma Agar (Cat. no. G102). Incubated in CO<sub>2</sub> for 72 hours at 35°C.

*Mycoplasma* .

## REFERENCES

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3. National Mastitis Council, *Laboratory Handbook on Bovine Mastitis* . 1999. NMC Inc., Madison, WI.
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5. UC Davis Veterinary Medicine Extension, *Culturing for Mycoplasma* .
6. Thurmond M.C., Tyler J.W., Luiz D.M., Holmberg C.A. and Picanso J.P. 1989. The effect of pre-enrichment on recovery of *Streptococcus agalactiae* , *Staphylococcus aureus* and *Mycoplasma* from bovine milk. *Epidem Inf.* 103:465-474.
7. *A Practical Look at Contagious Mastitis* . Internet: [www.nmconline.org/contmast](http://www.nmconline.org/contmast), 04/18/02.
8. Gonzalez, R.N. *Mycoplasma Mastitis in Dairy Cattle: If Ignored, it Can Be a Costly Drain on the Milk Producer*. Internet: [www.nmconline.org/articles/mycoplasma](http://www.nmconline.org/articles/mycoplasma). Quality Milk Promotion Services, College of Veterinary Medicine, Cornell University, Ithaca, New York.

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