IFU

Instructions for Use

CRITERION™ MYCOPLASMA BROTH BASE

Cat. no. C8000	CRITERION™ Mycoplasma Broth Base	50gm
Cat. no. C8001	CRITERION™ Mycoplasma Broth Base	500gm
Cat. no. C8002	CRITERION™ Mycoplasma Broth Base	2kg
Cat. no. C8003	CRITERION™ Mycoplasma Broth Base	10kg
Cat. no. C8004	CRITERION™ Mycoplasma Broth Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Mycoplasma Broth Base is recommended for use as an enrichment medium for cultivating *Mycoplasma* spp. prior to subculturing specimens to plated culture media.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

Mycoplasma belong to the class of Mollicutes, or "soft skin," and were first isolated from a pleuropneumonia infection in cattle.[1] *Mycoplasma* spp. are believed to be the smallest, free-living organisms; they are pleomorphic and vary in size from 0.2 to 0.3 nanometers.[2]

Since their initial discovery, several species of *Mycoplasma* have been isolated from human respiratory and genital tracts. *M. pneumoniae* is known to cause primary atypical pneumonia or "walking pneumonia", whereas *M. hominis*, *M. genitalium* and *Ureaplasma urealyticum* are important colonizers and potential pathogens of the human genital tract.[1,3,6] *Mycoplasma pneumoniae* usually infects people younger than 40 years of age, causing between 15 and 50 percent of all pneumonia cases in adults, and causes an even higher percentage of pneumonia in school-aged children.[2] The resulting symptoms from infection by *M. pneumoniae* include headache, fever, cough, chest pain, and sore throat.

Morton, Smith and Leberman first described the use of pleuropneumonia-like organism, or PPLO, media for identifying and cultivating *Mycoplasma*.[3] 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 the successful isolation of *Mycoplasma* spp.[4,6]

Hardy Diagnostics CRITERION™ Mycoplasma Broth Base, when supplemented, contains horse serum to provide cholesterol and a source of protein and yeast extract for essential vitamins and amino acids. If desired, thallium acetate can be added, in addition to penicillin or ampicillin, to inhibit accompanying microbial flora. Alternatively, cefoperazone may be added when researching *Mycoplasma* spp. from human patient specimens. CRITERION™ Mycoplasma Broth Base is available for use as an enrichment medium prior to subculturing specimens onto a suitable plated medium for the culture of isolated colonies.

FORMULA*

Gram weight per liter:	25gm/L
Casein Peptone	7.0gm
Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Beef Extract	3.0gm
Disodium Phosphate	2.2gm
Monopotassium Phosphate	2.0gm
Beef Heart Infusion	2.0gm

Final pH 7.8 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light beige.

Store the prepared culture media at 2-8ºC.

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 25gm of the dehydrated culture media in 750ml of distilled or deionized water. Stir to mix thoroughly.

2. Heat as necessary to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 10 minutes.

4. Cool media to 45-50ºC.

5. Aseptically add 200ml of horse serum and 50ml of filter sterilized yeast extract to the cooled medium. Alternatively, Mycoplasma Supplement (Cat. no. 283610) can be used. Refer to the manufacturer's package insert for supplement preparation instructions. Also, add antibiotic supplements as desired.

6. Aseptically pour desired volume into sterile tubes.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Due to nutritional variation, some strains may grow poorly or fail to grow at all on this medium.

There is little research for the comparison of direct culture versus enrichment culture. It can be assumed that enrichment culture will detect *Mycoplasma* spp. in smaller numbers more effectively than direct culture. However, enrichment culture may also detect nonpathogenic *Mycoplasma* in small numbers.[4]

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., 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® 29085	10 to 100 CFU	7-14days	35°C	CO_2**	Growth upon subculture to SP4 Agar w/Glucose
*Escherichia coli**** ATCC® 25922	B	24-48hrs	35°C	Aerobic	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.

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

**** Recommended negative QC strain for User Quality Control according to the CLSI document M22 if medium is prepared with selective agents.

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Mycoplasma Broth Base powder should appear homogeneous, free-flowing, and light beige in color. The prepared media should appear clear, and light amber in color.

REFERENCES

1. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

2. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

3. Morton, Smith and Leberman. 1951. Venereal diseases. *Am. J. Syphylis Gonorrh.*; 35:361.

4. UC Davis Veterinary Medicine Extension, *Culturing for Mycoplasma*.

5. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

6. *Cumitech 19; Laboratory Diagnosis of Chlamydial and Mycoplasmal Infections*. American Society for Microbiology, Washington D.C., August, 1984.

7. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

8. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

9. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

HDQA 2207B [D]