IFU

Instructions for Use

CRITERION™ REINFORCED CLOSTRIDIAL MEDIUM

Cat. no. C8720	CRITERION™ Reinforced Clostridial Medium	76gm
Cat. no. C8721	CRITERION™ Reinforced Clostridial Medium	500gm
Cat. no. C8722	CRITERION™ Reinforced Clostridial Medium	2kg
Cat. no. C8723	CRITERION™ Reinforced Clostridial Medium	10kg
Cat. no. C8724	CRITERION™ Reinforced Clostridial Medium	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Reinforced Clostridial Medium is recommended for the cultivation and enumeration of clostridia and other anaerobic and facultative bacteria from foods and clinical specimens. The medium meets the harmonized USP/EP/JP standards for use as an enrichment medium in performing the microbial examination of nonsterile products.

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

CRITERION™ Reinforced Clostridial Medium is a broth medium originally formulated by Hirsch and Grinstead to enhance the growth of clostridia from small inocula.[5] Barnes and Ingram utilized the medium to dilute vegetative cells and Barnes et al. later prepared the medium as a solid version to enumerate clostridia from food.[2,3] The broth medium is highly nutritious and can be used as an enrichment to optimize the growth of clostridia before subculturing to solid agar.

Reinforced Clostridial Medium contains peptones and beef extract, which provide carbon, nitrogen, vitamins, and minerals to support bacterial growth. Dextrose provides an energy source. Sodium chloride helps maintain osmotic balance. Soluble starch, supplied in low concentrations, helps to detoxify metabolic by-products. Cysteine hydrochloride is added as a reducing agent and sodium acetate acts as a buffer. The small quantity of agar in the medium acts to inhibit the dispersion of carbon dioxide, while diffusing oxygen and other reducing substances.

Reinforced Clostridial Medium can be modified for the isolation and differentiation of sulfite-reducing clostridia by adding sodium sulfite and ferric citrate to the medium; the medium can also be made solid by increasing the amount of agar.[4] Differential Reinforced Clostridial Medium is recommended for identifying sulfite-reducing clostridia, such as *Clostridium perfringens*, from food and beverage samples using the most probable number (MPN) method.[4]

FORMULA*

Gram weight per liter:	38.0gm/L

Beef Extract	10.0gm
Casein Peptone	10.0gm
Dextrose	5.0gm
Sodium Chloride	5.0gm
Sodium Acetate	3.0gm
Yeast Extract	3.0gm
Soluble Starch	1.0gm
L-Cysteine HCl	0.25gm
Agar	0.5gm

Final pH 6.8 +/- 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 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 38gm of the dehydrated culture media in one liter of distilled or deionized water. Stir to mix thoroughly.

Note: The medium can be made differential by adding sodium sulfite and ferric citrate or made into a solid medium by increasing the concentration of agar.[4]

2. Heat to boiling to dissolve completely. Do not overheat.

3. Dispense into desired containers.

4. Sterilize in the autoclave at 121ºC. for 15 minutes.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. U172.

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.

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	
Clostridium sporogenes ATCC® 19404**	J	24-48hr	35°C	Aerobic	Turbidity
Clostridium perfringens ATCC® 13124	A	24-48hr	35°C	Aerobic	Turbidity
Bacteroides fragilis ATCC® 23745**	A	24-48hr	35°C	Aerobic	Turbidity

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

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

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™ Reinforced Clostridial Medium powder should appear homogeneous, free-flowing, and beige in color. The prepared medium should appear slightly opalescent to opalescent, and medium amber in color.

REFERENCES

1. 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.

2. Barnes, E.M., J.E. Despaul, and M. Ingram. 1963. The Behaviour of a Food Poisoning Strain of *Clostridium welchii* in Beef. *J. Appl. Microbio.*; 26:415-427.

3. Barnes, E.M. and M. Ingram. 1956. The Effect of Redox Potential on the Growth of *Clostridium welchii* Strains Isolated from Horse Muscle. *J. Appl. Microbio.*; 19:117-127.

4. Gibbs, B.M. and B. Freame. 1965. Methods for the Recovery of Clostridia from Foods. *J. Appl. Microbiol.*; 28:95-111.

5. Hirsch, A. and E. Grinsted. 1954. Methods for the Growth and Enumeration of Anaerobic Spore-formers from Cheese, with Observations on the Effect of Nisin. *J. of Dairy Res.*; 21:101-110.

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

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

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

9. The Official Compendia of Standards. 2008. *USP27-NF22*. United States Pharmacopeial Convention, Rockville, MD.

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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.

HDQA 2207B [D]