

Instructions for Use

REINFORCED CLOSTRIDIAL MEDIUM, USP

Cat. no. U172	Reinforced Clostridial Medium, USP, 4oz Glass Bottle, 100ml	16 bottles/box
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INTENDED USE

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

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

SUMMARY

Reinforced Clostridial Medium Is A Broth Medium Originally Formulated By Hirsch And Grinstead To Enhance The Growth Of Clostridia From Small Inocula.⁽⁴⁾ 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 Also 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.

FORMULA

Ingredients per liter of deionized water:*

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 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration, 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 on the specific methods and procedures needed to isolate clostridia.^(2-6,8)

1. Transfer 10ml of the sample into 100ml of Reinforced Clostridial Medium.
2. Incubate containers with tight caps at 35°C. for 48 hours.
3. After incubation, growth is evident by the appearance of turbidity in the medium. Subculture the medium to a nonselective solid agar, such as Columbia Blood Agar (Cat. no. A16), and incubate specimen under anaerobic conditions at 35°C. for 48 hours for the detection of clostridia.

INTERPRETATION OF RESULTS

The anaerobic growth of gram-positive rods, with or without endospores, yielding a negative catalase reaction indicates the presence of clostridia.

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.

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

The media may need to be pre-reduced. This can be achieved by boiling the media in a hot water bath before use to drive oxygen out that may have been introduced from agitation during shipment.

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, 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	
<i>Clostridium sporogenes</i> ATCC® 19404**	J	48hrs	30-35°C	Aerobic***	Turbidity
<i>Clostridium sporogenes</i> ATCC® 11437**	J	48hrs	30-35°C	Aerobic***	Turbidity

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

** Tested in accordance with USP <62>. ⁽⁸⁾

***Bottles are incubated in an aerobic incubator with tight caps to create an atmosphere of low oxygen tension.

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

Reinforced Clostridial Medium should appear slightly opalescent to opalescent, and medium amber in color.

REFERENCES

1. Anderson, N.L., et al. 2005. *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. 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.
5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
6. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media* , M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. The Official Compendia of Standards. *USP-NF* . United States Pharmacopeial Convention, Rockville, MD.

ATCC is a registered trademark of the American Type Culture Collection.

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