

REINFORCED CLOSTRIDIAL MEDIUM (7714)

Intended Use

Reinforced Clostridial Medium is used for the cultivation of anaerobes from food and clinical specimens. Conforms to Harmonized USP/EP/JP Requirements.^{1,2,3}

Product Summary and Explanation

Reinforced Clostridial Medium is an enriched, non-selective medium formulated by Hirsch and Grinstead.⁴ This medium was developed for the isolation of spore-forming anaerobes, especially *Clostridium* spp.⁴ The developers demonstrated the medium was more fertile and enabled growth to be initiated from small inocula more readily than five other media tested.⁴ Hirsch and Grinstead added Polymyxin B, 15 – 20 mg/L, to inhibit the growth of Gram-negative microorganisms.⁴

Reinforced Clostridial Medium conforms to Harmonized United States Pharmacopoeia (USP), European Pharmacopoeia (EU), and Japanese Pharmacopoeia (JP).^{1,2,3}

Principles of the Procedure

Peptone, Beef Extract, and Yeast Extract provide nitrogen, vitamins, and amino acids in Reinforced Clostridial Medium. Dextrose is the fermentable carbohydrate used to enhance recovery and microorganism growth. Sodium Chloride maintains the osmotic balance of the medium. Soluble Starch detoxifies metabolic by-products. Cysteine Hydrochloride is the reducing agent and Sodium Acetate acts as a buffer. The small amount of Agar retards dispersion of CO₂, diffusion of oxygen, and reducing substances.

Formula / Liter

Beef Extract	10 g
Peptone.....	10 g
Sodium Chloride	5 g
Dextrose.....	5 g
Yeast Extract.....	3 g
Sodium Acetate.....	3 g
Soluble Starch.....	1 g
L-Cysteine HCl.....	0.5 g
Agar	0.5 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 38 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige to tan.

Prepared Appearance: Prepared medium is trace to slightly hazy, with or without slight precipitate, and yellow-beige.

Expected Cultural Response and USP/EP/JP Growth Promotion Testing: Cultural response in Reinforced Clostridial Medium inoculated with the organisms listed below. Cultures were incubated anaerobically or under 5 – 10% CO₂ at 35 ± 2°C and examined for growth at 24 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Bacteroides fragilis</i> ATCC® 25285	10 - 100	Growth
<i>Clostridium novyi</i> ATCC® 7659	10 - 100	Growth
<i>Clostridium perfringens</i> ATCC® 13124	10 - 100	Growth
<i>Clostridium sporogenes</i> ATCC® 11437	10 - 100	Growth
<i>Lactobacillus fermentum</i> ATCC® 9338	10 - 100	Growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For *Clostridium* spp. and other anaerobic bacteria refer to specific procedures in appropriate references.^{5,6}

Results

Observe for organism growth. Further tests are required to identify *Clostridium* spp. and other anaerobic bacteria. Refer to appropriate references.^{5,6}

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Clostridium* spp.

Packaging

Reinforced Clostridial Medium	Code No. 7714A	500 g
	7714B	2 kg
	7714C	10 kg

References

1. **United States Pharmacopeial Convention.** 2007. The United States pharmacopeia, Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.
2. **Directorate for the Quality of Medicines of the Council of Europe (EDQM).** 2007. The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France.
3. **Japanese Pharmacopoeia.** 2007. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare.
4. **Hirsch, A., and E. Grinstead.** 1954. Methods for the growth and enumeration of anaerobic spore formers from cheese, with observations on the effect of nisin. J. Dairy Res. **21**:101-110.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.