Reinforced Clostridial Medium

Intended Use

Reinforced Clostridial Medium is used for cultivating and enumerating clostridia, other anaerobes, and other species of bacteria from foods and clinical specimens.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Reinforced Clostridial Medium is a semisolid medium formulated by Hirsch and Grinstead.[4] Their work demonstrated that the medium outperformed other media in supporting growth of clostridia from small inocula and produced higher viable cell counts.[4] Barnes and Ingram[5] used the medium to dilute vegetative cells of *Clostridium perfringens*. Barnes et al.[6] used a solid (agar) version of the medium to enumerate clostridia in food.

Reinforced Clostridial Medium is a nonselective enrichment medium and grows various anaerobic and facultative bacteria when incubated anaerobically.[7] This medium has been used to detect clostridia, bifidobacteria and other anaerobes in food products[8-11] and fecal samples.[12] Reinforced Clostridial Medium is listed in the *USP* as the recommended medium for the isolation of *Clostridium* sp. from nonsterile pharmaceutical products.[1]

Principles of the Procedure

Reinforced Clostridial Medium contains peptone and beef extract as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium chloride maintains the osmotic balance. In low concentrations, soluble starch detoxifies metabolic by-products. Cysteine HCl is the reducing agent. Sodium acetate acts as a buffer. The small amount of agar makes the medium semisolid.

User Quality Control

Identity Specifications

Difco™ Reinforced Clostridial Medium

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is medium amber, slightly opalescent with dark particles and flocculation when hot.
Prepared Appearance:	Upon cooling, medium amber and becomes more opalescent.
Reaction of 3.8% Solution at 25°C:	pH 6.8 ± 0.2

BBL™ Reinforced Clostridial Medium (prepared)

Appearance:	Light to medium amber and opalescent with particles.
Reaction at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Reinforced Clostridial Medium

Prepare the medium per label directions. Inoculate and incubate tubes with caps tightened at 35 ± 2°C for 18-48 hours. Inoculate 100 mL bottles with *C. sporogenes* cultures and incubate with caps tightened at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	23745	30-300	Good
Clostridium botulinum	3502	30-300	Good
Clostridium perfringens	13124	30-300	Good
Clostridium sporogenes	19404	<100	Growth
Clostridium sporogenes	11437	<100	Growth

BBL™ Reinforced Clostridial Medium (prepared)

Inoculate and incubate bottles with caps tightened at 30-35°C for up to 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Clostridium perfringens	13124	10-100	Fair to good
Clostridium sporogenes	19404	<100	Growth
Clostridium sporogenes	11437	<100	Growth

Formula

Difco™ Reinforced Clostridial Medium

Approximate Formula* Per Liter

Peptone	10.0	g
Beef Extract	10.0	g
Yeast Extract	3.0	g
Dextrose	5.0	g
Sodium Chloride	5.0	g
Soluble Starch	1.0	g
Cysteine HCl	0.5	g
Sodium Acetate	3.0	g
Agar	0.5	g

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

Precautions[13]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing *C. botulinum* or *C. tetani* or their toxins.

2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of these organisms and for activities with a high potential for aerosol or droplet production, and those involving production quantities of toxin.

Directions for Preparation from Dehydrated Product

1. Suspend 38 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Refer to *USP* General Chapter <62> for details on the examination of nonsterile products and the isolation of clostridia using Reinforced Clostridial Medium.[1]

Expected Results

After appropriate incubation time and temperature, subculture each tube or bottle to two Columbia Agar plates. Incubate under both aerobic and anaerobic conditions for 48 hours at 30-35°C to confirm the presence of anaerobic growth. After incubation of these plates, if isolates grow anaerobically only (with or without endospores) and are catalase negative, this indicates the presence of *Clostridium* sp.[1] Perform other confirmatory biochemical testing as necessary.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Hirsch and Grinstead. 1954. J. Dairy Res. *21*:101.
5. Barnes and Ingram. 1956. J. Appl. Bacteriol. *19*:117.
6. Barnes, Despaul and Ingram. 1963. J. Appl. Bacteriol. *26*:415.
7. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
8. Mead. 1995. Principles involved in the detection and enumeration of clostridia in foods. *In* Corry, J.E.L., et al. (eds.), Culture media for food microbiology. Elsevier Science B.V. Amsterdam, The Netherlands.
9. Roy. 2003. Media for the detection and enumeration of bifidobacteria in food products. *In* Corry, J.E.L. et al. (eds.), Handbook of culture media for food microbiology. Elsevier Science B.V. Amsterdam, The Netherlands.
10. Cocolin, Innocente, Biasutti and Giuseppe. 2004. Int. J. Food Microbiol. *90*:83.
11. Health Canada. The compendium of analytical methods, online. Food Directorate, Health Products and food Branch, Health Canada, Ottawa, Ontario Canada.
12. Hartemink and Rombouts. 1999. J. Microbiol. Methods. *36*:181.
13. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Reinforced Clostridial Medium

CCAM EP JP USP

Cat. No. 218081 Dehydrated – 500 g†

BBL™ Reinforced Clostridial Medium

CCAM EP JP USP

Cat. No. 215192 Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10†

Europe

CCAM EP

Cat. No. 254548 Prepared Plates – Pkg. of 20*

** Store at 2-8°C.*
† QC testing performed according to USP/EP/JP performance specifications.