Reinforced Medium for Clostridia (Medium 15) IP

Intended Use
Reinforced Medium for Clostridia is used for the cultivation and enumeration of Clostridia and other anaerobes in compliance with IP.

Summary
Reinforced Medium for Clostridia is formulated by Hirsch and Grinsted. It can be used to initiate growth from small inocula and to obtain the highest viable count of Clostridia. Barnes and Ingrams used the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens*. It can be used in studies of spore forming anaerobes, especially *Clostridium butyrium* in cheese, for enumeration of Clostridia in tube dilution counts or for preparation of plates for isolation. Other spore forming anaerobes, Streptococci and Lactobacilli also grow in this media. This is an enriched but non-selective media.

Principle
Peptone, yeast extract, beef extract, starch, L-cysteine and sodium acetate provide all the necessary nutrients for the growth of Clostridia. Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium.

Formula*
Ingredients	**g/L**
Beef Extract	10.0
Peptone	10.0
Sodium Chloride	5.0
Dextrose Monohydrate	5.0
Sodium Acetate	3.0
Yeast Extract	3.0
Starch soluble	1.0
L-Cysteine Hydrochloride	0.5
Agar	0.5
Final pH (at 25°C)	6.8 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 38.00 g of powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 115°C (10 psi) for 15 minutes as per validated cycle.

Quality Control

Dehydrated Appearance: Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance: Light yellow to amber coloured, clear to slightly opalescent solution with fine settlement of agar gel.

Growth Promotion Test: Growth promotion is carried out in accordance with the method of IP and growth is observed after an incubation at 30°C-35°C for 48 hours under anaerobic conditions Sub-culturing is carried out using Columbia Agar (Harmonized) after enrichment in Reinforced Medium for Clostridia (Harmonized) at 30°C-35°C for 48-72 hours.

Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 48 hours under anaerobic conditions.

Organism (ATCC)	**Growth**
Clostridium sporogenes (11437)	Good
Clostridium sporogenes (19404)	Good

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Hirsch and Grinsted, 1954, J. Dairy Res., 21:101.
2. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
3. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia.
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5. Japanese Pharmacopoeia, 2008.
6. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	**Product description**	**Pack Size**
201180100500	Dehydrated Culture Media	500 g