Differential Reinforced Clostridial Broth Base ISO

Intended Use
A medium used for cultivation of Clostridia from water, in compliance with ISO specification ISO 6461- 1: 1986.

Summary
Differential Reinforced Clostridial Broth Base is used for cultivation of Clostridia species from water. It is an enriched, non-selective medium formulated by Hirsch and Grinstead. This medium was developed for the isolation of spore-forming anaerobes, especially *Clostridium* spp. Later, Barnes and Ingram used the medium to develop vegetative cells in assays of *Clostridium perfringens*.

Principle
Tryptose, Yeast extract and meat extract are sources of nitrogen, B-complex vitamins and amino acids. Glucose is a source of complex carbohydrate. Soluble starch detoxifies metabolic by-products. L- Cysteine hydrochloride is the reducing agent and Sodium acetate is the buffering agent. Clostridia have the ability to reduce sulphite to sulphide, forming iron sulphide which changes the colour of the medium to black. As other bacteria can also produce sulphide, vegetative forms must first be removed from the culture by a relevant treatment, e.g., pasteurization. The anaerobic spore-forming microorganisms must then be identified.

Formula*

Ingredients	**g/L**
Tryptose	10.0
Yeast Extract	1.5
Sodium Acetate	5.0
Meat Extract	10.0
Glucose	1.0
L-Cysteine Hydrochloride	0.5
Starch	1.0
Final pH (at 25°C)	7.2 ± 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.

Type of specimen
Dairy samples
Water samples

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 29.00 g of the powder in 1000 mL purified / distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Just before use add 0.5 mL filter sterile solution prepared by mixing equal volumes of 4% w/v solution of sodium sulphite and 7% w/v ferric citrate to 25 mL of single strength medium or 0.4 mL and 2 mL to 10 mL and 50 mL of double strength medium respectively.
5. Mix well.

Quality Control

Dehydrated Appearance: Yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Light yellow to amber coloured, clear solution without any precipitate.
Cultural Response: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 30°C-35°C for 1 week.

Organisms (ATCC)	Growth	H_2S
Clostridium sporogenes (11437)	Good	+
Clostridium perfringens (13124)	Good	+

Key: + = Blackening of the medium

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, A., and E. Grinstead. 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. (1954).
2. Gibbs and Freame. J. Appl. Microbiol. 28:95. (1965).
3. B. Freame and B.W.F. Fitzpatrick, The use of Differential Reinforced Clostridial Medium for the isolation and enumeration of Clostridia from food, Isolation of Anaerobes ed. by D.A. Shapton, R.G. Board, Academic Press, London, New York, 48. (1972).
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201040120100	Dehydrated Culture Media	100 g
201040120500	Dehydrated Culture Media	500 g