Differential Reinforced Clostridial Agar Base

Intended Use
Differential Reinforced Clostridial Agar Base is used for the enumeration and the cultivation of Clostridia from water.

Summary
Attenborough and Scarr employed Differential Reinforced Clostridial Agar in conjunction with membrane filter for the count of *Clostridium thermosaccharolyticum* in sugar. This medium is also frequently employed for the investigation of intestinal flora, with added blood. It is also used for the total and *Lactobacillus* count of human and animal faeces and for determination of Bacteroides.

Principle
This medium has ingredients like casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract, beef extract, which provide nitrogen source, essential nutrients and growth factors to the organisms. Glucose serves as carbon and energy source. Sodium bisulphite and ferric ammonium citrate forms the indicator system for sulphite reduction, which results in black colour colonies. Resazurin is a redox indicator which helps in detection of anaerobisis, in the medium.

Formula*

Ingredients	**g/L**
Casein enzymic hydrolysate	5.0
Peptic digest of animal tissue	5.0
Beef extract	8.0
Yeast extract	1.0
Starch	1.0
Sodium acetate	5.0
Glucose	1.0
L-Cysteine hydrochloride	0.5
Sodium bisulphite	0.5
Ferric ammonium citrate	0.5
Resazurin	0.002
Agar	15.0
Final pH (at 25°C)	7.1 ± 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
Water samples
Clinical samples – faeces

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 42.50 g of the powder in 1000 mL purified / distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Mix well and dispense as desired.
5. Prepare serial 10-fold dilutions of the sample in 0.1% peptone water.
6. Depending on the amount of the initial sample, transfer 1 mL or 0.1 mL of the appropriate dilution to the bottom of a molten (45°C-50°C) DRCA tube. Prepare a duplicate tube using the same procedure.
7. Heat one of the duplicate DRCA tubes to 80°C ± 1°C for 10 minutes to kill vegetative cells.
8. Incubate both tubes, heat-shocked and non-heats-hocked, at 35°C ± 1°C for 5 days; examine for sulfite reduction.

Quality Control

Dehydrated Appearance: Cream to yellow coloured, homogenous free flowing powder
Prepared Appearance: Light pink coloured, clear to slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 30°C-35°C for 1 week.

Organism (ATCC)	**Growth**
Clostridium sporogenes (11437)	Good
Clostridium perfringens (13124)	Good

Interpretation of results

1. Observe the blackening of tubes for sulphite reduction.
2. Non-heat shocked tubes showing blackening must be subcultured to Differential Reinforced Clostridial Agar for confirmation.
3. Blackening of the medium is presumptive evidence for the presence of sulphite reducing clostridia.
4. Heat shocked tubes showing blackening are confirmed for clostridia.

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. Attenborough Sheila J. and Scarr M. Pamela, 1957, J. Appl. Bacteriol., 20:460-466
2. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	**Product description**	**Pack Size**
201040130100	Dehydrated Culture Media	100 g
201040130500	Dehydrated Culture Media	500 g