# Differential Reinforced Clostridial Agar

**M1603**

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

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Casein enzymic hydrolysate | 5.000 |
| Peptic digest of animal tissue | 5.000 |
| Beef extract | 8.000 |
| Yeast extract | 1.000 |
| Starch | 1.000 |
| Sodium acetate | 5.000 |
| Glucose | 1.000 |
| L-Cysteine hydrochloride | 0.500 |
| Sodium bisulphite | 0.500 |
| Ferric ammonium citrate | 0.500 |
| Resazurin | 0.002 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 7.1±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 42.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

## Principle And Interpretation

Attenborough and Scarr (1) 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* .

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.

Grind the material to be examined in a stomacher and prepare serial 10 fold dilutions in ¼ strength Ringers Solution (M525) or 0.1% Peptone Water (M028). Transfer 1 ml or 0.1 ml of the appropriate dilution (depending upon amount of the initial sample) to the bottom of a molten (45-50°C) Differential Reinforced Clostridial Agar tubes. Prepare duplicate tubes using the same procedure. Tighten the caps of the tubes. Heat one of the duplicate tubes (dilution tubes) to 80 ± 1°C for 10 minutes to kill vegetative cells. Incubate both heat shocked and non-heat shocked tubes at 35 ± 1°C for 5 days. Observe the blackening of tubes for sulphite reduction. Non-heat shocked tubes showing blackening must be subcultured to Differential Reinforced Clostridial Agar for confirmation. Blackening of the medium is presumptive evidence for the presence of sulphite reducing clostridia. Heat shocked tubes showing blackening are confirmed for clostridia.

Alternatively, samples may be inoculated onto the surface of agar plates using streak plate, spread plate or pour plate technique. Medium in agar deeps may be inoculated using stab technique. Differential Reinforced Clostridial Agar may be used to overlay the membrane filter in the filtration technique. Incubate plates and tubes at 35± 2°C for 24-48 hours under anaerobic conditions.

## Quality Control

**Appearance**

Cream to yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Light pink coloured, clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 4.25% w/v aqueous solution at 25°C. pH : 7.1±0.2

**pH**
6.90-7.30

**Cultural Response**
M1603: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 30-35°C for 1 week.

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|---|---|---|---|---|
| *Clostridium perfringens ATCC 13124* | 50-100 | good-luxuriant | >=50% | black |
| *Clostridium sporogenes ATCC 11437* | 50-100 | good-luxuriant | >=50% | black |

## Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Reference
1. Attenborough Sheila J. and Scarr M. Pamela, 1957, J. Appl. Bacteriol., 20:460-466

Revision : 2 / 2015