

Technical Information

Differential Reinforced Clostridial Agar

Product Code: DM 2603

Application: - Differential Reinforced Clostridial Agar is recommended 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

Principle & 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 supply nitrogen source, essential nutrients and growth factors to the organisms. Glucose acts 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 anaerobiosis, in the medium.

Grind the material to be examined in a stomacher and prepare serial 10 fold dilutions in ¼ strength Ringers Solution (DM1525) or 0.1% Peptone Water (DM1028). 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.



Dehydrated Culture Media
Bases / Media Supplements

Methodology

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity

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 Reange

6.90-7.30

Cultural Response

DM2603: 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
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Cultural Response

<i>Clostridium perfringens</i> ATCC 13124	50-100	good-luxuriant	>=50%	black
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<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	>=50%	black
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Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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