Differential Reinforced Clostridial Agar

M1603

Intended Use:

Recommended for the enumeration and cultivation of *Clostridia* from water and clinical samples.

Composition**

Ingredients	g / L
Tryptone	5.000
Peptone	5.000
HM peptone B #	8.000
Yeast extract	1.000
Starch	1.000
Sodium acetate	5.000
Dextrose (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
Equivalent to Beef extract

Directions

Suspend 42.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50 °C. 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 tryptone, peptone and yeast extract, HM peptone B, 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.

Type of specimen

Water samples; Clinical sample- faeces;

Specimen Collection and Handling:

For water samples follow appropriate techniques for handling specimens as per established guidelines (2).

For clinical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. (3,4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological testing is required for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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 (00007*)	50-100	good-luxuriant	>=50%	black
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%	black

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

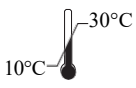
Reference

1. Attenborough Sheila J. and Scarr M. Pamela, 1957, J. Appl. Bacteriol., 20:460-466
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015)Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.