Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Differential Reinforced Clostridial Broth Base

Product Code: DM1549I

Application: - Recommended for cultivation of Clostridia from water. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6461-1:1986.

Composition**

Ingredients	Gms / Litre
Tryptose	10.000
HM extract #	10.000
Yeast extract	1.500
Sodium acetate, hydrated	5.000
Starch	1.000
Dextrose (Glucose)	1.000
L-Cysteine hydrochloride	0.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Meat extract

Principle & Interpretation

Differential Reinforced Clostridial Agar was originally described by Hirsch and Grinstead (4) to initiate the growth from small inoculum and get a higher Clostridial count. Later, Barnes and Ingram (2) used the medium to develop vegetative cells in assays of *Clostridium perfringens*. This medium is developed for the isolation of sulphite-reducing Clostridia from food and for their enumeration in water by multiple tube method. Differential Reinforced Clostridial Broth is used to determine the count of sulphite reducing bacteria by MPN technique (3).
Tryptose, HM extract, yeast extract, starch, and sodium acetate provide essential nutrients for bacterial metabolism. Glucose is the fermentable carbohydrate and serves as carbon and energy source. L-cysteine hydrochloride acts as reducing agent. Sodium sulphite and ferric citrate are added as indicators. Sulphite reducing clostridia produce sulphide from sulphite, which results in the formation of black coloured medium.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further 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.

Methodology

Suspend 29 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. Just before use add 0.5 ml filter sterilized 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. Mix well.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured, clear solution without any precipitate

Reaction
Reaction of 2.9% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed in an anaerobic atmosphere, with added 4% w/v solution of Sodium sulphite and 7% w/v Ferric citrate after an incubation at 30-35°C within 1 week.

Organism	Inoculum (CFU)	Growth	H_2S production
Clostridium perfringens ATCC 13124 (00007*)	50-100	good to luxuriant	positive reaction, blackening of medium
Clostridium perfringens ATCC 12916 (00080*)	50-100	good to luxuriant	positive reaction, blackening of medium
Clostridium sporogenes ATCC 11437	50-100	good to luxuriant	positive reaction, blackening of medium
Escherichia coli ATCC 8739 (00012*)	50-100	good to luxuriant	negative reaction
Escherichia coli ATCC 25922 (00013*)	50-100	good to luxuriant	negative reaction

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Further Reading

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination ofWater and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Barnes E. M. and Ingram M., 1956, J. Appl. Bacteriol., 19(1):117.
3. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology,1996, 14th Edition, Churchill Livingstone
4. Hirsch A. and Grinstead E., 1954, J. Dairy Res. 21:101
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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