Differential Reinforced Clostridial Broth Base M549I

Intended Use:

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**

As per ISO 6461-1:1986

Ingredients	g/ L
Peptone tryptic digest of meat	10.000
Meat Extract	10.000
Yeast extract	1.500
Hydrated sodium acetate	5.000
Starch	1.000
Glucose	1.000
L-Cysteine hydrochloride	0.500
Final pH (at 25°C)	7.10-7.20

Differential Reinforced Clostridial Broth Base M549I

Ingredients	g/ L
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 Peptone tryptic digest of meat

Equivalent to Meat extract

Directions

Suspend 29.0 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.

Principle And Interpretation

Differential Reinforced Clostridial Agar was originally described by Hirsch and Grinstead (1) 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). The composition and performance criteria of this medium are as per the specifications laid down in ISO 6461-1:1986 (4).

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:

ISO 6461/1-1986 (4)

Add 50ml of water sample to 50ml of the double strength complete medium or 1ml of sample to 25ml of single strength complete medium. Incubate the inoculated bottles at 37±1°C for 44±4 hours.

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 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

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

Productivity : Cultural response was observed after an incubation (anaerobic atmosphere) at 36 ± 1°C for 44 ± 4 hours, with added 4% w/v solution of Sodium sulphite and 7% w/v Ferric citrate.

Specificity : Cultural response was observed after an incubation (anaerobic atmosphere) at 36 ± 1°C for 44 ± 4 hours, with added 4% w/v solution of Sodium sulphite and 7% w/v Ferric citrate.

Organism	Inoculum (CFU)	Growth	H_2S production
Productivity			
Clostridium perfringens ATCC 13124 (00007*)	50-100	weak to good	positive reaction, blackening of medium
Clostridium perfringens ATCC 12916 (00080*)	50-100	weak to good	positive reaction, blackening of medium
Specificity			
Escherichia coli ATCC 8739 (00012*)	10^3 - 10^4	none to weak	negative reaction
Escherichia coli ATCC 25922 (00013*)	10^3 - 10^4	none to weak	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 15-30°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Hirsch A. and Grinstead E., 1954, J. Dairy Res. 21:101
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. Water quality - Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 1 : Method by enrichment in a liquid medium, ISO 6461/1:1986.
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.

Revision : 05/2024