Product :
DIFFERENTIAL REINFORCED CLOSTRIDIAL MEDIUM (DRCM)

Specification

Liquid medium used for the enumeration of clostridia in food samples and other products using the MPN technique.

Formula * in g/L

Peptone	10,000	Sodium acetate	5,000
Meat extract	8,000	Sodium bisulfite	0,500
Yeast extract	1,000	Ferric-ammonium citrate	0,500
Starch	1,000	Resazurine	0,002
Glucose	1,000		
L-Cystein	0,500	Final pH 7,0 ±0,2 at 25 ºC	

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 27,5 g of powder in 1 L of distilled water. Bring to the boil, distribute in tubes and sterilize in the autoclave at 121ºC for 15 minutes.

Description

This medium is a modification by Freame and Fitzpatrick of the Gibb's classic medium, used to detect the presence of sulfite reducing clostridia. The modification is, an addition of sodium bisulfite and ferric citrate, which make colonies black and thus more visible. The current version of this medium has no agar in order to facilitate easy observation of the blackened medium. Resazurin, the redox indicator allows the verification of anaerobiosis in the medium in the same assay. L-Cysteine acts as a reducing agent in this medium.

Technique

The sample to be examined is distributed in tubes as per the MPN technique, and covered with paraffin oil to help anaerobiosis. The series of tubes is kept in a boiling water bath at 75ºC for 30 minutes to remove all the dissolved oxygen and vegetative cells. Then, incubate at 30ºC for up to 7 Days.
The spores of sulfate reducing clostridia usually germinate between the second and fourth day, turning the medium black.
The medium can be rendered selective by the addition of 70 IU/mL of polymyxin sulfate.
Prepared tubes without inoculation may be stored for up to 2 weeks provided the resazurin band does not show excessive oxidation (more than a 1/3 of the column).

Quality control

Incubation temperature: 30°C **Incubation time:** 48 h-7days

Inoculum: Practical range 100±20 CFU. min. 50 CFU (productivity)/ 10^4 -10□ CFU (selectivity). Anaerobic conditions.

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Inhibition	w. Polymixin
Clostridium perfringens ATCC® 13124	Good	Black precipitate
Clostridium sporogenes ATCC® 11437	Good	Black precipitate
Clostridium perfringens ATCC® 10543	Good	Black precipitate

Uninoculated tube (Control)

Left: *Clostridium perfringens* ATCC 13124
Center: *Clostridium perfringens* ATCC 10543
Right: *Escherichia coli* ATCC 25922

References

· FREAME, B., FITZPATRICK, B.W.F. (1972) The use of DRCM for the isolation and enumeration of Clostridia form food. In "Isolation of Anaerobes". Ed. Shapton & Board. Academic Press. London.
· GIBBS, B.M., FREAME, B. (1965) Methods for the recovery Clostridia from foods. J. Appl. Bact. 36:23-33.
· MacFADDIN, J.M. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins. Baltimore.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).