



Modified Differential Clostridial Broth

M1085

Modified Differential Clostridial Broth is recommended for enumeration of Clostridia from foodstuffs and other samples by MPN technique.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Meat peptone	5.000
Meat extract	8.000
Yeast extract	1.000
Starch	1.000
Dextrose	1.000
L-Cysteine hydrochloride	0.500
Sodium acetate	5.000
Sodium bisulphite	0.500
Ammonium ferric citrate	0.500
Resazurin sodium	0.002
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.5 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Clostridium perfringens is a normal inhabitant of the intestine. Although present in smaller numbers compared to *E.coli*, its spores can survive in water for long periods and persist when all other faecal bacteria have died. An examination for their presence in water is valuable in demonstrating remote or intermittent pollution and in confirming the presence of faecal contamination when only coliforms other than *E. coli* are cultured from water (1).

Modified Differential Clostridial Broth was developed for the enumeration of spores of sulphate reducing Clostridia in dried foods (2-4). This medium is a modification of the media devised by Hirsch and Grinsted (5) and Gibbs and Hirsch (6) and is used for the enumeration of Clostridia from food stuffs and other samples by MPN technique. It is differential by means of detecting the ability of organisms to reduce sulphite to sulphide and thereby form iron sulphide, a black coloured complex. This medium is non-selective as other bacteria can also produce sulphide. Therefore, to enumerate Clostridia, pasteurization must be done to remove the vegetative cells.

Modified Differential Clostridial Broth has ingredients like casein enzymic hydrolysate and yeast extract, meat extract, meat peptone and starch which provide nitrogen, carbohydrate sources, essential nutrients and growth factors to the organisms. Starch also neutralizes toxic metabolites. This nutritionally rich composition promotes spore germination. Dextrose is the fermentable carbohydrate and serves as carbon and energy source. L-Cysteine hydrochloride acts as reducing agent. Sodium bisulphite and ferric ammonium citrate form indicator system for sulphite reduction. Resazurin is a redox indicator, which helps in detection of anaerobiosis, in the medium. Antibiotics like polymyxin B can be added to the medium to inhibit non-spore formers (2). Clostridia can be identified directly in this broth as they produce a black colouration, which is unlikely in Reinforced Clostridial Medium (M443) (8).

In order to facilitate spore germination, a heat treatment of the spores/ samples at 80°C for 10 minutes may be given before inoculation (7).

Quality Control

Please refer disclaimer Overleaf.

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent solution with upper one third or less portion pink on standing

Reaction

Reaction of 2.75% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 18-48 hours.

Cultural Response

Organism	Growth	Inoculum (CFU)	H ₂ S production
Cultural Response <i>Clostridium perfringens</i> ATCC 12924	luxuriant	50-100	positive reaction, blackening of medium
<i>Clostridium sporogenes</i> ATCC 11437	luxuriant	50-100	positive reaction, blackening of medium

Storage and Shelf Life

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

Reference

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
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3. Weenk G., Fitzmaurice E., and Mossel, D. A. A., 1991, J. Appl. Bacteriol., 70, 135-143.
4. Weenk G. H., Van den Brink J. A., Struijk C. B. and Mossel, D. A. A., 1995, Int. J. Food Microbiol., In press, 27:185.
5. Hirsch A. and Grinsted E., 1954, J. Dairy Res., 21 : 101.
6. Gibbs B. M. and Hirsch A., 1956, J. Appl. Bacteriol., 19:129.
7. Corry J. E. L., Curtis G. D. W., and Baird R. M., Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, 1995, Elsevier, Amsterdam.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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