# Technical Information

## Modified Differential Clostridial Broth

## Product Code: DM 2085

**Application:** - 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-Cystine 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

## Principle & Interpretation

*Clostridium perfringens* is a normal inhabitant of the intestine. Although present in smaller numbers compared to *E. coli ,* its spores can survive & persit in water for long periods even when all other faecal bacteria have died. An examination for their presence in water is suggestive of remote or intermittent pollution and 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 source, essential nutrients and growth factors to the organisms. Starch neutralizes toxic metabolites. This nutritionally rich composition promotes spore germination. Glucose is the fermentable carbohydrate and serves as carbon and energy source. L-cystine hydrochloride acts as reducing agent. Sodium bisulphite and ferric ammonium citrate form indicator system for sulphite reduction. Sulphite reducing clostridia are enumerated as black colonies [(7)]. 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 (DM1443) [(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).]

## Methodology

Suspend 27.5 grams of powder media in 1000 ml distilled water. Shake well and heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

## Quality Control

**Physical 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 Range** 6.90-7.30

**Cultural Response/Characteristics**

DM2085: Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 18-48 hours.

| Cultural Response | Inoculum (CFU) | Growth | Recovery |
|---|---|---|---|
| *Clostridium perfringens ATCC 12924* | 50-100 | luxuriant | Positive reaction blackening of medium |
| *Clostridium sporogenes ATCC 11437* | 50-100 | luxuriant | Positive reaction blackening of medium |

## Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
**Prepared Media:** 2-8$^0$ in sealable plastic bags for 2-5 days.

## Further Reading

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
2. Gibbs B. M. and Freame B., 1965, J. Appl. Bacteriol., 28(1):95.
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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