

## Specification

Fluid medium for the cultivation and enumeration of clostridia by the MPN Technique according to the Pharmacopoeial Harmonised Method.

## Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottle Bottle 125 ml with: 100 ± 3 ml	1 box with 10 bottles 125 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.	12 months	8-25 °C

## Composition

Composition (g/l):	
Peptone from casein.....	10.0
Yeast extract.....	3.00
Meat extract.....	10.0
D(+) Glucose.....	5.00
Sodium chloride.....	5.00
Sodium acetate.....	3.00
Starch.....	1.00
Cysteine.....	0.50
Agar.....	0.50

## Description /Technique

### Description

Reinforced Clostridial Agar was originally described by Hirsch and Grinstead to enhance the growth of small numbers and achieve a higher clostridial count. Later, Barnes and Ingram used the medium to develop vegetative cells in assays of *Clostridium perfringens*. Barnes also used this medium to count clostridia in food; moreover other authors used this medium in enumeration assays of *C. thermoscharolyticum* in sugar, the study of intestinal flora, and for bacterial counts in human or animal faeces, etc.

For enumeration by the MPN method, the liquid version is the preferred one.

Muñoz and Parés added a filter sterilized solution of nalidixic acid 0,02 g/L, polymyxin 0,025 g/L, kanamycin sulfate 0,05 g/L, sodium iodine-acetate 0,025 g/L and triphenyl-tetrazolium HCl 0,025 g/L to obtain a selective and differential medium for bifidobacteria in water and wastewater. Tartera *et al.* use it with the addition of antibiotics (BPRM Broth) for the isolation and enumeration of bacteriophages from bacteroides. This technique was adopted in the 10705-4:2001 ISO standard.

### Technique

Material to be examined is grinded in a mill or Stomacher®, and a decimal dilution bank prepared. From each of the dilutions, an aliquot is added to a Petri dish or tube and molten medium at 50 °C is poured over each sample.

Let the medium solidify and incubate at 30-35 °C (depending on the suspected microorganism) for 1-10 days. An anaerobic environment can be achieved in tubes by covering with oil immediately after the Reinforced Clostridial Medium is solidified.

Inoculate according to final purpose, samples and validated methods (Ph. Eur. and ISO).

**Quality control****Physical/Chemical control**

Color : yellow

pH: 6.8 ± 0.2 at 25°C

**Microbiological control**

Melt the medium pour into tube and inoculate.

Growth Promotion Test 50-100 CFU according to harmonized Pharmacopoeia monographs (EP) and test methods &amp; ISO 11133:2014/A1:2018

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Anaerobiosi. Incubation at 30 - 35°C reading at 48 h

**Microorganism***Clostridium sporogenes* ATCC® 19404, WDCM 00008*Clostridium perfringens* ATCC® 13124, WDCM 00007, NCTC® 8237*Clostridium perfringens* ATCC® 10543, WDCM 00174**Growth**

Good - Gas D

Good - Gas Positive

Good - Gas Positive

**Sterility Control**

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

**Bibliography**

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