

**Product :**  
**REINFORCED CLOSTRIDIAL MEDIUM (Eur. Pharm.)****Also known as**

RCM, Reinforced Medium for Clostridia

**Specification**

Liquid medium for the cultivation and enumeration of clostridia by the MPN technique, according to the Pharmacopoeial Harmonized Methods and ISO standards.

**Formula \* in g/L**

|                       |      |
|-----------------------|------|
| Casein peptone .....  | 10.0 |
| Yeast extract .....   | 3.0  |
| Meat extract .....    | 10.0 |
| Dextrose .....        | 5.0  |
| Sodium chloride ..... | 5.0  |
| Sodium acetate .....  | 3.0  |
| Soluble starch .....  | 1.0  |
| Cysteine .....        | 0.5  |
| Agar .....            | 0.5  |

Final pH 6.8 ±0.2 at 25 °C

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

**Directions**

Suspend 38 g of powder in 1 L of distilled water and bring to the boil constantly stirring. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

**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 30-55 °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. If plates are used, they must be incubated in an anaerobic atmosphere.

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

**Quality control****Incubation temperature:** 30-35 °C**Incubation time:** 24-48 h**Inoculum:** Practical range 100±20 CFU. min. 50 CFU (productivity), according to ISO 11133:2014/Amd 1:2018 and Ph. Eur. Anaerobic conditions.

| Microorganism                              | Growth    | Remarks |
|--|-----------|---------|
| <i>Escherichia coli</i> ATCC® 25922        | Good      | -       |
| <i>Pseudomonas aeruginosa</i> ATCC® 27853  | Inhibited | -       |
| <i>Clostridium perfringens</i> ATCC® 13124 | Good      | Gas (+) |
| <i>Clostridium sporogenes</i> ATCC® 19404  | Good      | Gas (D) |
| <i>Clostridium perfringens</i> ATCC® 10543 | Good      | Gas (+) |



Reference : 03-289

**Scharlau Microbiology - Technical data sheet**

**Product :**

**REINFORCED CLOSTRIDIAL MEDIUM (Eur. Pharm.)**

### References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Boca Raton. Fla. USA.
- EUROPEAN PHARMACOPOEIA 10.0 (2020) 10th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- HIRSCH, A. & E. GRINSTEAD (1954) Methods for the Growth and Enumeration of Anaerobic Sporeformers from Cheese, with Observations on the Effect of Nisin.
- INGRAM, M. & E.M BARNES (1956) A simple modification of the deep shake tube for counting anaerobic bacteria. Lab. Practice 5, 4:145.
- ISO 10705-4 Standard (2001) Water Quality - Detection and enumeration of bacteriophages infectin Bacteroides fragilis.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- MUÑOA, F.J. & R. PARÉS-FARRÁS (1988) Selective medium for isolation and enumeration of Bifidobacterium spp. Appl. Environm. Microbiol. 54:1715-1718.
- TARTERA, C., R. ARAUJO, T. MICHEL & J. JOFRE (1992) Culture and decontamination methods affecting enumeration of phages infecting Bacteroides fragilis in sewage. Appl. Environm. Microbiol. 58:8:2670-2673.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

### Storage

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