

# TECHNICAL DATA SHEET

Article No. 9679

Reinforced Clostridial Medium (RCM), ready-to-use medium

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## SPECIFICATION

Prepared culture medium. Fluid medium for the cultivation and enumeration of clostridia by the MPN Technique according to the EP/USP harm. and ISO 10705.

Colour: Yellow  
pH: 6.8 ± 0.2 at 25 °C

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## COMPOSITION IN G/ L

Peptone from casein	10.00
Yeast extract	3.00
Meat extract	10.00
D(+) Glucose	5.00
Sodium chloride	5.00
Sodium acetate	3.00
Starch	1.00
Cysteine	0.50
Agar	0.50

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## PACKAGE DETAILS

### 9679-10x100ML

Volume	100 ± 3 ml
Bottle size	125 ml
Packaging unit	10 bottles

1 box with 10 x 100 ml in 125-ml-bottles. Injectable cap: Plastic screw inner cap. For the use of syringe needles with a diameter ≤ 0.8 mm.



## 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 most-probable-number (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 on 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 agar is solidified. Inoculate according to final purpose, samples and validated methods (Ph. Eur. and ISO).

## MICROBIOLOGICAL CONTROL

Melt the medium, pour into tubes and inoculate.

Growth Promotion Test 50-100 CFU according to harmonized pharmacopoeial monographs and test methods & ISO 11133:2014/A1:2018

Anaerobiosis. Incubation at 30 – 35 °C, reading at 48 h

Microorganism	Growth
<i>Clostridium sporogenes</i> ATCC® 19404, WDCM 00008	Good - Gas D
<i>Clostridium perfringens</i> ATCC® 13124, WDCM 00007, NCTC® 8237	Good - Gas Positive
<i>Clostridium perfringens</i> ATCC® 10543, WDCM 00174	Good - Gas Positive

### Sterility control:

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

Check at 7 days after incubation in same conditions.



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## 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 infecting *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. PARES-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.

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## STORAGE

8 - 25 °C

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## SHELF LIFE

12 months unopened from date of manufacture

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created: 22.08.2022

