

TECHNICAL DATA SHEET

Article No. 9749

Reinforced Clostridial Medium (RCM) Ph. Eur.

SYNONYMS

-

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 heat to boiling with constant stirring. Distribute into containers and sterilize by autoclaving 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

Th. Geyer GmbH & Co. KG

Dornierstr. 4 – 6
D-71272 Renningen
Tel.: +49 7159 1637-0
Fax: +49 7159 1637-710
renningen@thgeyer.de
www.thgeyer.de

BW-Bank (Swift/BIC SOLADEST600)
IBAN DE85600501010002036302
Postbank Stuttgart (Swift/BIC PBKDEFFXXX)
IBAN DE3260010070000020708
Deutsche Bank (Swift/BIC DEUTDESSXXX)
IBAN DE06600700700125518100

St.-Nr. 70093/40018 / USt-IdNr. DE147510304
Amtsgericht Stuttgart / HRA-Nr. 254140
Persönlich haftende Gesellschafterin:
Geyer Beteiligungsgesellschaft mbH
Amtsgericht Stuttgart / HRB-Nr. 252035
Geschäftsführer: Lutz-Alexander Geyer / Thomas Roth

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 sulphate 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 ISO 10705-4:2001.

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 and Ph. Eur. Anaerobic conditions.

Microorganism	Growth	Remarks
<i>Escherichia coli</i> ATCC® 25922	Good	None
<i>Pseudomonas aeruginosa</i> ATCC® 27853	Inhibited	None
<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 (+)

REFERENCES

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Boca Raton. Fla. USA.
- EUROPEAN PHARMACOPOEIA 8.0 (2014) 8th 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*.

Th. Geyer GmbH & Co. KG

Dornierstr. 4 – 6
D-71272 Renningen
Tel.: +49 7159 1637-0
Fax: +49 7159 1637-710
renningen@thgeyer.de
www.thgeyer.de

BW-Bank (Swift/BIC SOLADEST600)
IBAN DE85600501010002036302
Postbank Stuttgart (Swift/BIC PBNKDEFFXXX)
IBAN DE32600100700000020708
Deutsche Bank (Swift/BIC DEUTDE33XXX)
IBAN DE06600700700125518100

St.-Nr. 70093/40018 / USt-IdNr. DE147510304
Amtsgericht Stuttgart / HRA-Nr. 254140
Persönlich haftende Gesellschafterin:
Geyer Beteiligungsgesellschaft mbH
Amtsgericht Stuttgart / HRB-Nr. 252035
Geschäftsführer: Lutz-Alexander Geyer / Thomas Roth

- ISO 11133:2014. 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

Keep tightly closed, away from light, in a dry place (4-30 °C).

SHELF LIFE

4 years from date of production.

Th. Geyer GmbH & Co. KG

Dornierstr. 4 – 6
D-71272 Renningen
Tel.: +49 7159 1637-0
Fax: +49 7159 1637-710
renningen@thgeyer.de
www.thgeyer.de

BW-Bank (Swift/BIC SOLADEST600)
IBAN DE85600501010002036302
Postbank Stuttgart (Swift/BIC PBNKDEFFXXX)
IBAN DE32600100700000020708
Deutsche Bank (Swift/BIC DEUTDESSXXX)
IBAN DE06600700700125518100

St.-Nr. 70093/40018 / USt-IdNr. DE147510304
Amtsgericht Stuttgart / HRA-Nr. 254140
Persönlich haftende Gesellschafterin:
Geyer Beteiligungsgesellschaft mbH
Amtsgericht Stuttgart / HRB-Nr. 252035
Geschäftsführer: Lutz-Alexander Geyer / Thomas Roth