Differential Reinforced Clostridial Broth (DRCM)

Medium proposed by GIBBS and FREAME (1965) for the enumeration of all clostridia by the MPN method in foodstuffs and other materials.

This culture medium was successfully utilized by FREAME and FITZPATRICK (1971) and GIBBS (1973) to isolate and count clostridia. The Institute for Food Technology and Packing of the Technical University of Munich (Institut für Lebensmitteltechnologie und Verpackung der TU München) (1976) recommends this medium for the examination of packing materials. It complies with the requirements of the DIN Norm 38411 for the examination of water.

Mode of Action

Differential Reinforced Clostridial Broth represents a development of the Reinforced Clostridial Media proposed by HIRSCH and GRINDSTED (1954) and GIBBS and HIRSCH (1956). The redox indicator resazurin is used to monitor anaerobiosis. Clostridia reduce sulfite to sulfide, the formed iron sulfide causes the culture medium to turn black. As other bacteria can also produce sulfide, vegetative forms must first be removed from the culture by a relevant treatment (e.g. pasteurization), and the anaerobic spore-forming microorganisms must then be identified. GIBBS and FREAME (1956) inhibited the growth of most non-spore-forming microorganisms by adding polymyxin (70 IU/ml) to the broth.

Typical Composition (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; meat extract 8.0; yeast extract 1.0; starch 1.0; D(+)glucose 1.0; L-cysteinium chloride 0.5; sodium acetate 5.0; sodium di-sulfite 0.5; ammonium iron(III) citrate 0.5; sodium resazurin 0.002.

Preparation

Suspend 27.5 g/litre, dispense into test tubes, autoclave (15 min at 121 °C).

pH: 7.1 ± 0.2 at 25 °C.

The ready-to-use broth in the tube is clear and reddish-brown.

- **The prepared culture medium can be stored for up to 2 weeks.**

Experimental Procedure and Evaluation

Inoculate the culture medium, cover with a 3 to 5 mm layer of sterilized paraffin viscous and pasteurize (30 min at 75 °C in a water bath!).

Incubation: at least 7 days at 30 °C.

Microbial growth can usually be seen after 3-4 days. The cultures should be observed for up to 4 weeks as occasionally some time is required for spore germination to start. The cultures should be checked for a black colouration. Further tests should be performed to identify the clostridia.

Note for testing milk samples:
Milk can curdle or coagulate in the presence of strongly acid- and gas-forming microorganisms. This curding prevents blackening of the broth. If milk coagulation and strong gas formation occur in testing a milk sample, this may be suspected to contain Clostridium perfringens. Further tests should follow for a more precise identification.

Literature

Arbeitsgruppe des Instituts für Lebensmitteltechnologie und Verpackung an der Technischen Universität München: Merkblätter für die Prüfung von Packmitteln. Merkblatt 28. "Bestimmung von Clostridiensporen in Papier, Karton, Vollpappe und Wellpappe." - **Verpackungs-Rdsch., 27/20**; Techn. wiss. Beilage, 82-84 (1976).

DIN Deutsches Institut für Normung e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Mikrobiologische Verfahren (Gruppe K). Nachweis von sulfitreduzierenden sporenbildenden Anaerobiern (K 7). - **DIN 38411**.

FREAME, B., a. FITZPATRICK, B.W.F.: The use of Differential Reinforced Clostridial Medium for the isolation and enumeration of Clostridia from food. - In "Isolation of Anaerobes" ed. by SHAPTON, D.A., a. BOARD, R.G., Academic Press, London, New York, 48-55 (1972).

GIBBS, B.M.: The detection of Clostridium welchii in the Differential Clostridial Medium technique. - **J. Appl. Bact., 36**; 23-33 (1973).

GIBBS, B.M., a. FREAME, B.: Methods for the recovery of clostridia from foods. - **J. Appl. Bact., 28**; 95-111 (1956).

GIBBS, B.M., a. HIRSCH, A.: Spore formation by Clostridium species in an artificial medium - **J. Appl. Bact., 19**; 129-141 (1956).

HIRSCH, A., a. GRINSTED, E.: Methods for the growth and enumeration of anaerobic spore-formers from cheese, with observations on the effect on nisin. - **J. Dairy Res., 21**; 101-110 (1954).

Ordering Information

Product	Merck Cat. No.	Pack size
Differential Reinforced Clostridial Broth (DRCM)	1.11699.0500	500 g
Paraffin viscous	1.07160.1000	1 l
Polymyxin-B-sulfate	EMD Biosciences	

Quality control

Test strains	Growth	Blacking
Escherichia coli ATCC 25922	good / very good	-
Bacillus cereus ATCC 11778	fair / good	-
Pseudomonas aeruginosa ATCC 27853	poor / fair	-
Clostridium bifermentans ATCC 19299	good / very good	+
Clostridium perfringens ATCC 10543	good / very good	+
Clostridium perfringens ATCC 13124	good / very good	+
Clostridium sporogenes ATCC 11437	good / very good	+
Clostridium sporogenes ATCC 19404	good / very good	+