Reinforced Clostridial Agar

Medium proposed by BARNES and INGRAM (1956) for the cultivation and enumeration of clostridia, other anaerobes and facultative microorganisms in foodstuffs and other materials.

MUNOA and PARES (1988) developed a Bifidobacterium Iodoacetate Medium (BIM-25) on the basis of Clostridial Agar for the selective cultivation and differentiation of Bifidobacterium species.

Mode of Action

This culture medium is free from inhibitors and contains cysteine as a reducing agent. According to HIRSCH and GRINSTED (1954), Polymyxin B can be added to inhibit Gram-negative bacteria.

Typical Composition (g/litre)

Meat extract 10.0; peptone from casein 10.0; yeast extract 3.0; D(+)glucose 5.0; starch 1.0; sodium chloride 5.0; sodium acetate 3.0; L-cysteinium chloride 0.5; agar-agar 12.5.

Preparation

Suspend 50 g/litre, if desired dispense into test tubes, autoclave (15 min at 121 °C). If required, cool to 45-50 °C and add 0.02 g Polymyxin B/litre in form of a filter-sterilized aqueous solution.

pH: 6.8 ± 0.2 at 25 °C.

The medium in the tubes or Petridishes is clear and yellowish-brown.

Experimental Procedure and Evaluation

Prepare stab cultures of the sample material in test tubes or use the pour-plate technique.

Incubation: 24-48 hours at an optimal temperature (e.g. 35 °C) under anaerobic conditions (e.g. Anaerocult® A, Anaerocult® A mini, or Anaerocult® P).

Count the colonies and, if necessary, perform additional tests.

Literature

BARNES, E.M., a. INGRAM, M.: The effect of redox potential on the grown Clostridium welchii strain isolated from horse muscle. – **J. Appl. Bact., 19**; 177-178 (1956).

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

MUNOA, F.J., a. PARES, R.: Selective medium for isolation and enumeration of Bifidobacterium spp. – **Appl. Environm. Microbiol., 54**; 1715-1718 (1988).

Ordering Information

Product	Merck Cat. No.	Pack size
Reinforced Clostridial Agar	1.05410.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Plate basket	1.07040.0001	1 ea
Polymyxin-B-sulfate	EMD Biosciences	

Quality control

Test strains	Growth
Clostridium bifermentans ATCC 19299	good / very good
Clostridium difficile 15	good / very good
Clostridium histolyticum HW-6	good / very good
Clostridium perfringens ATCC 13124	good / very good
Clostridium perfringens ATCC 10543	good / very good
Escherichia coli ATCC 25922	good / very good
Bacillus cereus ATCC 11778	good / very good