Differential Reinforced Clostridium Broth

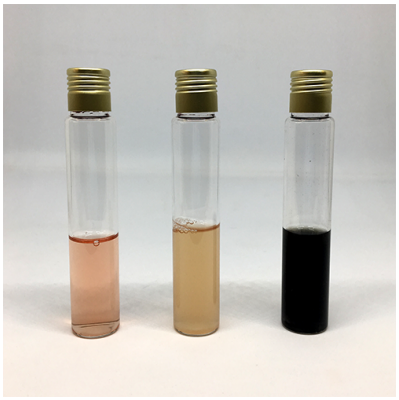
Cat. 1416

For the enumeration of all Clostridium by the MPN method.

Practical information

Aplications	Categories
Non selective enumeration	Clostridium

Industry: Water / Food



Principles and uses

Differential Reinforced Clostridium Broth is used to determine the count of sulfite-reducing bacteria by the MPN technique.

Beef extract, meat peptone and casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Glucose is the fermentable carbohydrate providing carbon and energy. L-Cysteine hydrochloride is the reducing agent. Starch absorbs any toxic metabolites produced. Resazurin is an oxidation indicator, turning from pink (aerobic) to colorless (anaerobic conditions), used as an indicator to monitor anaerobiosis. Ferric ammonium citrate and sodium disulfite are H2S indicators.

Clostridium reduce sulfite to sulfide, the iron sulfide produced 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. To inhibit the growth of most non-spore-forming microorganisms add 70 IU/ ml polymyxin to the broth.

Formula in g/L

Glucose	1	Beef extract	8
Casein peptone	5	Ferric ammonium citrate	0,5
L-Cysteine hydrochloride	0,5	Meat peptone	5
Sodium acetate	5	Starch	1
Yeast extract	1	Sodium disulfite	0,5
Resazurin sodium salt	0,002		

Preparation

Suspend 27,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 ºC for 15 minutes.

Instructions for use

- Inoculate the medium according to the most probable number (MPN) technique.
- Cover with a 3-5 mm layer of sterile paraffin/vaseline to promote anaerobiosis.
- Pasteurize by introducing the medium in a bath at 75 ºC for 30 minutes to remove all the dissolved oxygen and vegetative cells.
- Incubate at 30 ºC for 4-7 days.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
Slightly opalescent	Fine powder	Beige	Reddish brown	7,1±0,2

Microbiological test

Incubation conditions: (30 ºC / 4-7 days).

Microrganisms	Specification	Characteristic reaction
Clostridium perfringens ATCC 10543	Good growth	Black color
Bacillus cereus ATCC 11778	Good growth	-
Clostridium perfringens ATCC 13124	Good growth	Black color
Clostridium sporogenes ATCC 19404	Good growth	Black color
Escherichia coli ATCC 25922	Good growth	-

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

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