Differential Reinforced Clostridium Broth (DRCM) ISO

Cat. 1416

For the enumeration of all Clostridium by the MPN method.

Practical information

Aplications	Categories
Non selective enumeration	Clostridium

Industry: Water / Food

Regulations: ISO 6461

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	10
Ferric ammonium citrate	0,7	L-Cystine	0,5
Sodium acetate	5	Sodium sulfite	0,4
Starch	1	Tryptone	10
Yeast extract	1,5		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 30,1 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

For detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) according to ISO 6461-1 (UNE-EN 26461-1):
- Heat the sample in a water bath at 75±5 ºC for 15 min.
- Add 50 ml of sample to a 100 ml bottle containing 50 ml of the double strength medium.
- Add 10 ml of sample to a series of five 25 ml bottle containing 10 ml of the double strength medium.
- Add 1 ml of sample to a series of five 25 ml bottle containing 25 ml of the single strength medium.
- Incubate the inoculated bottles at 36±1 ºC for 44±4 h.
- Bottles in which blackening is observed shall be regarded as positive.
- Express the results as the most probable number of sulfite-reducing anaerobes (clostridia).

Quality control

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

Microbiological test

Incubation conditions: (36±1 ºC / 44±4 h).

Microorganisms	Specification	Characteristic reaction
Clostridium perfringens ATCC 12916	Turbidity (1-2)	Black color
Clostridium perfringens ATCC 13124	Turbidity (1-2)	Black color
Escherichia coli ATCC 25922	Turbidity (0-1)	-

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).
UNE-EN 26461_1 Detección y recuento de los esporos de microorganismos anerobios sulfito-reductores (clostridia) Parte 1: Método por enriquecimiento en un medio líquido.