Bryant-Burkey Broth Base (Modified with Resazurin)

Cat. 1247

For the detection of lactate fermenting Clostridial species in milk and dairy products.

Practical information

Aplications	Categories
Detection	Clostridium

Industry: Dairy products

Principles and uses

Bryant-Burkey Broth Base (Modified with Resazurin) is used for the enumeration of spores of lactate fermenting Clostridia in milk and dairy products, particularly Clostridium tyrobutyricum. This bacterium is the one that causes the swelling of cheeses.

During milking process small numbers of butyric acid fermenting bacteria are introduced into the raw milk. When the contaminated milk is used for producing cheese, the brines become contaminated with heat resistant Clostridia spores. During the ripening of salt brined, semi- and hard cheeses, (for example, Gouda, Edamm, Emmental, Gruyere, and Parmesan) late blowing gasogenic Clostridia ferment lactate into butyric acid, acetic acid and gas (CO_2 and H_2). The gas expands the cheese and causes a defect known as "late blowing" or butyric swelling.

The medium does not contain lactate so it must be added when the medium is prepared. Sodium lactate is fermented under anaerobic conditions by C. tyrobutyricum and other lactate-fermenting Clostridia and uses it as a source of carbon and energy, producing hydrogen and CO_2. Tryptone and beef extract 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. Sodium acetate is the selective agent inhibiting gram-negative bacteria and also promotes the growth of C. tyrobutyricum. L-Cysteine is the reducing agent and resazurin is an oxidation indicator, turning from pink (aerobic) to colorless (under anaerobic conditions).

Formula in g/L

Beef extract	7,5	Resazurin	0,0025
Sodium acetate	5	Tryptone	15
Yeast extract	5	L-Cysteine	0,5

Preparation

Suspend 33 grams of the medium in one liter of distilled water. Add 10 ml of 50 % Sodium lactate. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes of 10 ml and sterilize in autoclave at 121 °C for 15 minutes.

Instructions for use

- Before use, heat tubes and boil for 10 minutes to regenerate anaerobic conditions.
- Allow cooling the tubes at a temperature of 75 ºC. At this temperature, the vegetative cells die and the spores are activated.
- Prepare decimal dilutions of the sample and inoculate into 10 ml of medium in tubes and allow to cool them at room temperature.
- Pour 2 ml of melted paraffin (60 - 65°C) into each tube, previously autoclaved at 121°C for 20 minutes.
- Read results after incubation at 37± 2°C for up to 7 days, considering the tubes with growth and gas production positive.
- To count the spores use the most probable number method (MPN).

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Yellow-pink	5,9±0,2

Microbiological test

Incubation conditions: (37±2 ºC / 7 días).

Microrganisms	Specification	Characteristic reaction
Clostridium perfringens ATCC 10543	Good growth	Gas (+)
Clostridium tryobutyricum EMD 132	Good growth	Gas (+)
Staphylococcus aureus ATCC 25923	Moderate growth	Gas (-)
Pseudomonas aeruginosa ATCC 27853	Null growth	Gas (-)

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

BRYANT M.P. and BURKEY L.A: 1956. The characteristics of lactate fermenting spore forming anaerobes from silage. J. Bact., 43 - 46.
CERF. O. et BERGERE J.L. 1968. Numeration des spores de Clostridium et son application au lait et aux produits laiters. Numeration des différents groupes de Clostridium. Le lait, 48, 501-519.