



Bryant and Burkey Medium

M1385

Intended Use:

Recommended for detecting and enumerating spores of lactate fermenting *Clostridium* in milk and dairy products.

Composition**

| Ingredients | Gms / Litre |
|---------------------------|-------------|
| Tryptone | 15.000 |
| Yeast extract | 5.000 |
| HM peptone B # | 7.500 |
| Sodium acetate | 5.000 |
| L-Cystine dihydrochloride | 0.500 |
| Resazurin | 0.0025 |
| Final pH (at 25°C) | 5.9±0.2 |

**Formula adjusted, standardized to suit performance parameters

*Equivalent to Beef extract

Directions

Suspend 33.0 grams in 1000 ml purified/distilled water containing 5 grams of Sodium lactate. Heat if necessary to dissolve the medium completely. Mix well and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Bryant and Burkey Medium is used to enumerate the spores of gasogenic clostridia that are responsible for swelling of cheese in dairy industry (1, 2). In normal conditions of use, the medium allows the growth of other microorganisms also which are not directly related to cheese alteration, e.g. *Enquvtfkw o"urqtqigpgu* or *Enquvtfkw o"dwvftkewo*. Germination and growth of butyric acid bacteria (BAB) causes severe defects in cheese with silage being the main source of BAB spores in cheese milk (4). Clostridia spores are heat resistant and therefore can contaminate cheese brines. The gas produced by the growth of clostridia swells the cheese and is responsible for defect known as butyric swelling, resulting in bad taste. The main species causing this butyric swelling defect is *Enquvtfkw o"vftqdwvftkw o0*

Recommended technique for estimation of *Enquvtfkw o"* is to enumerate the spores by the MPN method. Test sample must be previously decontaminated by heating up for 10 minutes at 75°C in order to destroy all the vegetative forms while only leaving the spores alive. The tubes of the medium are then boiled to regenerate anaerobiosis and cooled down to 25-30°C. These tubes are inoculated with the sample and overlaid with 2 cm of sterile paraffin and incubated for upto 7 days at 37°C. Examine the tubes after every 48 hours. A raised paraffin plug indicates gas formation. Tubes will be declared positive if they show clear gas production and MPN index is used to calculate the number of Clostridia.

Resazurin is a redox indicator and monitors the oxygen level turning from pink under aerobic conditions to colourless under anaerobic conditions. The nutrient composition of the basal medium, particularly of tryptone, yeast extract, HM peptone B and L-cysteine HCl help in rapid growth of *Enquvtfkw o* species. Sodium acetate promotes spore germination (3) and improves the selectivity of the medium. Sodium lactate is fermented under anaerobic conditions by *Clostridium tyrobutyricum*. A pink colour indicates the presence of oxygen in the media which turns colourless on boiling.

Type of specimen

Dairy samples

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured, clear solution when hot, becomes pink-red upon cooling

Reaction

Reaction of 3.3% w/v aqueous solution at 25°C. pH : 5.9±0.2

pH

5.70-6.10

Cultural Response

Cultural characteristics observed under anaerobic conditions after an incubation at 35-37°C for 6 days.

| Organism | Growth | Gas production |
|------------------------------------------------|--------|----------------|
| <i>Clostridium tyrobutyricum</i> ATCC 25755 | good | positive |

Reference

1. Bryant M. P. and Burkey L. A., 1956, J. Bacteriol., 71: 43-46.
2. Bergeres J. L. and Sivela S., 1989, Detection and enumeration of Clostridial spores related to cheese quality-Classical and new methods, FIL-IDF Bull. 51:18-23.
3. Touraille C. and Bergere J. L., 1974, Biochimie, 56: 404-422.
4. Vissers M. M. M., Drichuis F., Giffel T., John P. D., Lankveld J. M. G., 2007, J. Dairy Sci., 90:928

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