

Bryant Burkey Broth with Resazurine and Lactate

Medium for the selective enrichment of lactate fermenting *Clostridia* spp. (*Cl. tyrobutyricum*) which are responsible for "late blowing" in brine salted semi-hard cheese

The medium is used to enumerate the spores of lactic acid fermenting *Clostridia* spores in silage, milk and dairy products. During milking process low numbers of butyric acid fermenting bacteria (BAB) originating from silage are introduced into the raw milk. When the contaminated milk is used for cheese production, cheese brines become contaminated with heat resistant *Clostridia* spores. During the ripening of salt brined, semi- and hard cheeses (for example, Gouda, Edammer, Emmentaler, Gruyere, and Parmesan) "late blowing gasogenic *Clostridia* ferment lactate into butyric acid, acetic acid and gas (CO₂ and H₂). The gas swells the cheese and is responsible for a defect termed "late blowing" or butyric swelling. The blown up cheese has moreover a bad taste. The main species causing this butyric swelling defect is *Co. tyrobutyricum*. Other *Clostridia* belonging to the butyric acid fermenting bacteria (BAB) are *Cl. butyricum* or *Cl. sporogenes*. The causative *Clostridia* spp. are anaerobic Gram-positive microorganisms forming heat resistant endospores, which survive pasteurisation but not UHT or sterilisation of milk.

Mode of Action

Vegetative cells are killed by a heat treatment (75 °C for 10 min.). Resazurin is a redox indicator and monitors the oxygen level. The nutrient composition of the basal medium, particularly the high quality of the peptones creates the conditions for a rapid growth of lactate fermenting *Clostridia* spp. Sodium acetate promotes the spore germination, which is activated by the heat treatment of the sample. Lactate is the substrate for the *Clostridia* spp. producing gas. A strong gas production is visualised by the raising of the paraffin plug.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 5.0; meat extract 7.5; sodium acetate 5.0; cysteine HCl 0.5; resazurin 0.0025; calcium lactate 5.0.

Preparation

Dissolve 38 g in 1000 ml of demin. water

Autoclave (15 min. at 121°C).

Cool to 45-50 °C and dispense in bottles or tubes.

pH: 5.9 ± 0.1 at 25 °C.

The prepared non-boiled broth is pink and the boiled broth is colourless. A pink colour indicates the presence of oxygen.

Experimental Procedure and Evaluation

The MPN method is employed in the examination on lactate fermenting *Clostridia* spp. Tubes of medium are boiled where appropriate (100°C for 10 min.) to regenerate anaerobiosis and cooled down to 25-30 °C. Colourless tubes are inoculated with sample or sample dilutions and overlaid with 2cm of sterile (121°C for 20 min.) melted (58-60°C) paraffin. The tubes are heat treated (75°C for 10 min.) to kill vegetative microorganisms and cooled down to 37°C to solidify the paraffin.

The inoculated medium is incubated at 37 °C for up to 7 days. The tubes are evaluated every 48 h. Tubes with growth and gas formation indicated by a raised paraffin plug are considered positive. The MPN index is used to calculate the number of *Clostridia*.

Further biochemical identification verifies the presence of *Cl. tyrobutyricum*.

Literature

Bergere, J.L. 1979 Development de l'ensilage. Ses consequences sur la qualite du lait et des produits laitiers. Revue laiterie Francaise.

Bergere, J.L. et al. 1968 Les Clostridium du group butyrique dans les produits laitiers. Ann. Institut Pasteur Lille **19**, 41-54.

Bryant, M.P. & Burkey, L.A.: 1953 Cultural methods and some characteristics of some more numerous groups of bacteria with bovine rumen. J Dairy Science **36**, 205-217.

Cerf, O. & Bergere, J.L. 1968 La numeration des spores de Clostridium et son application au lait et aux produits laitiers. Numeration des differents groupes de Clostridium. Le Lait **48**, 501-509.

Franknet, J & de Carheil, M. 1983 Les tests de controle des germes butyriques. La technique laiterie **977**, 15-28.

Ordering Information

Product	Merck Cat. No.	Pack size
Bryant Burkey Broth with Resazurine and Lactate	1.01617.0500	500 g
Paraffin	1.07158.1000	1 kg



good growth
gasformation (+)



no growth

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Quality control

Test strains	Growth	Gas Formation
<i>Clostridium tyrobutyricum</i> W 7	good / very good	+
<i>Clostridium tyrobutyricum</i> DSMZ 663	good / very good	+
<i>Clostridium perfringens</i> ATCC 10543	good / very good	+
<i>Escherichia coli</i> ATCC 25922	good / very good	- (poor)
<i>Staphylococcus aureus</i> ATCC 25923	good / very good	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	none	-