



Bryant Burkley Broth with Resazurine and Lactate

Medium for the detection of the spores
of lactate fermenting Clostridia spp.

Granulated Culture Media – Safer from Merck.



Bryant Burkey Broth with Resazurine and Lactate



Cat. No. 1.01617.0500
(500 g)

Medium for the selective detection of the spores of lactic acid fermenting Clostridia spp. (*Cl. tyrobutyricum*) which are responsible for "late blowing" in brine salted semi-hard and hard cheese

The medium is used to enumerate the spores of lactic acid fermenting Clostridia spp. 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, Edam, Emmental, Gruyere, and Parmesan cheese) "late blowing" gasogenic Clostridia ferment lactate into butyric acid, acetic acid and gas (CO_2 and H_2). 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 *Cl. 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 2 cm of sterile (121°C for 15 min.) melted 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*.

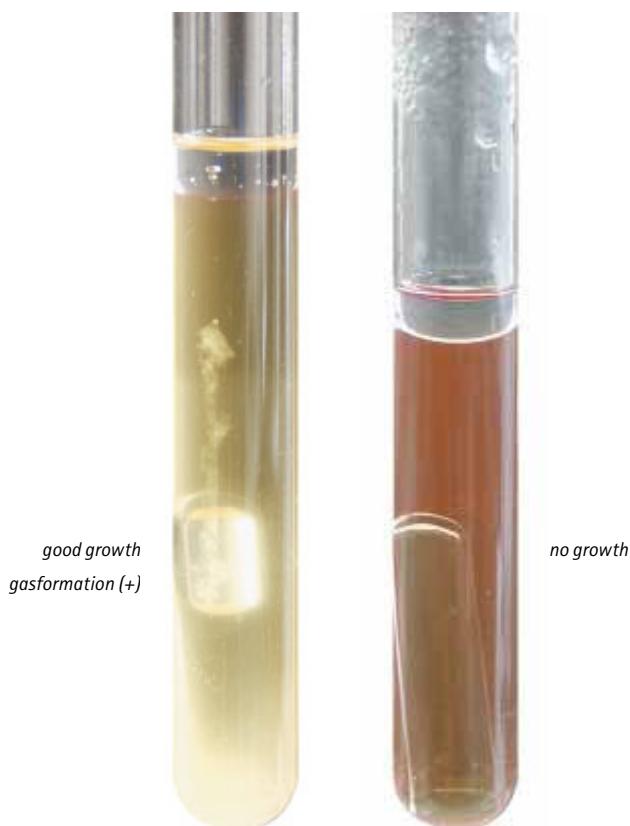
Additives

Merck Cat.No	Product	Pack. size
1.07160.1000	Paraffin viscous	1 kg

Quality control

Test Strains	Growth	Gas formation
<i>Clostridium tyrobutyricum</i> W-7	good/very good	+
<i>Clostridium tyrobutyricum</i> DSM 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	-

Clostridium tyrobutyricum DSM 663



Literature

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- *La technique laiterie* 977, 15-28 (1983)



Further information about the advantages of Merck's Granulated Culture Media you will find in the following promotion materials:

- *Granulated Culture Media* (W.28611.2)
- *TSE - "low risk" products* (W.28612.0)



Merck KGaA
64271 Darmstadt, Germany
Fax +49 (0) 61 51/72 33 80
E-mail: mibio@merck.de
Internet: microbiology.merck.de