BRYANT BURKEY BROTH BASE (MODIFIED WITH RESAZURIN)

CAT Nº: 1247

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

FORMULA IN g/l

Tryptone	15.00	Sodium Acetate	5.00
Beef Extract	7.50	L-Cysteine	0.50
Yeast Extract	5.00	Resazurin	0.0025

Final pH 5.9 ± 0.2 at 25ºC

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. The prepared medium should be stored at 2-8°C. If the medium presents a pink color in more than 1/3 under the surface when it is going to be used, regenerate the anerobic conditions by heating at 100ºC for 10 minutes. The color is yellow-pink.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BRYANT-BURKEY BROTH BASE 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 from silage are introduced into the raw milk. When the contaminated milk is used for producing chesse, the 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 (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. The 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).

Before use, heat tubes and boil for 10 minutes to regenerate anaerobic conditions. Prepare decimal dilutions of the sample and inoculate into 10 ml of medium in tubes. Pour 2 ml of melted paraffin (60 - 65°C) into each tube, previously autoclaved at 121°C for 20 minutes.

Heat tubes at 75°C for 15 minutes to kill vegetative cells and active spores. Allow to cool to room temperature.
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).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37± 2ºC and observed after for up to 7 days

Microorganisms	Growth	Gas production
Clostridium tryobutyricum EMD 132	Good	+
Clostridium perfringens ATCC 10543	Good	+
Staphylococcus aureus ATCC 25923	Moderate	-
Pseudomonas aeruginosa ATCC 27853	Null	-

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.

STORAGE

Once opened keep powdered medium closed to avoid hydration.