Technical Data Sheet

NutriSelect® prime

BRYANT BURKEY broth modified by BERGÈRE acc. CNERNA (with resazurin and lactate)

Ordering number: 1.01617.0500 / 1.01617.5000

For the enumeration of the spores of lactic acid-fermenting *Clostridia* spp. in silage, milk and dairy products.

BRYANT BURKEY broth modified by BERGÈRE acc. CNERNA (with resazurin and lactate) is also known as BB broth and BBMB broth.

This culture medium complies with the specifications given by CNERNA (National Centre for the Coordination of Studies and Research on Nutrition and Food, France).

This culture medium is released by the quality control laboratory of Merck KGaA, Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025 for the performance testing of media for microbiology according to DIN EN ISO 11133.

Mode of Action

This medium is used to enumerate the spores of lactic acid fermenting *Clostridium* 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 spores of *Clostridium* spp.

During the ripening of salt brined, semi- and hard cheeses (for example, Gouda, Edamer, Emmentaler, Gruyere, and Parmesan), "late blowing" gasogenic *Clostridium* spp. ferment lactate into butyric acid, acetic acid and gas (CO_2 and H_2).
The butyric acid fermentation (BAF) causes formation of cracks, abnormally shaped or excessively big eyes, or even blowing of the cheese, accompanied by off-flavors after several weeks or months of ripening. Thus, the defect is referred to as "late blowing" or butyric swelling. The blown up cheese has moreover a bad taste.

The medium was originally described by BRYANT and BURKEY and modified by BERGÈRE containing lactate as the sole fermentable carbon source and improved the detection and germination of the

spores. Anaerobiosis is achieved by sealing the tubes with Paraffin. The formation of gas, which lifts the paraffin plug, serves as the diagnostic system.

The main species causing this butyric swelling defect is *Clostridium tyrobutyricum*. Other *Clostridium* spp. belonging to the butyric acid fermenting bacteria (BAB) are *Clostridium butyricum* or *Clostridium sporogenes*. The causative *Clostridia* spp. are anaerobic Gram-positive microorganisms forming heat resistant endospores, which survive pasteurization but not UHT or sterilization of milk.

The method uses the MPN (Most Probable Number) procedure for detecting the presence of spores, but not vegetative cells, since samples are heated to inactivate vegetative cells. Since *C. tyrobutyricum* spores are reported more heat sensitive than those of many other *Clostridium* spp., higher temperatures or longer heat treatments should be avoided.

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 *Clostridium* spp. Lactate acts as the carbon source to allow selective growth of lactate-fermenting spore formers and is the substrate for the gas production of *Clostridium* spp. Sodium acetate facilitates the lactate fermentation by *C. tyrobutyricum*. Sodium acetate also promotes the spore germination, which is activated by the heat treatment of the sample. A strong gas production is visualized by the raising of the paraffin plug.

Typical Composition

Specified by CNERNA		**NutriSelect® prime BRYANT BURKEY broth modified by BERGÈRE acc. CNERNA (with resazurin and lactate)**	
Trypticase	15.0 g/l	Enzymatic digest of casein (Trypticase)	15.0 g/l
Meat extract	7.5 g/l	Meat extract	7.5 g/l
Yeast extract	5.0 g/l	Yeast extract	5.0 g/l
Cysteine HCl	0.5 g/l	Cysteine HCl	0.5 g/l
Sodium acetate	5.0 g/l	Sodium acetate	5.0 g/l
Sodium lactate	5.0 g/l	Sodium lactate	5.0 g/l
Resazurin	0.005 g/l	Resazurin	0.005 g/l
Water	1000 ml	Water	n/a
pH at 25 °C	5.8 ± 0.1	pH at 25 °C	5.8 ± 0.1

Preparation

Dissolve 38.0 g in 1 liter of purified water and mix well. Dispense into tubes or bottles and autoclave (15 minutes at 121 °C).
According to CNERNA, the medium is dispensed by 10 ml into 16 x 160 mm tubes before sterilization.

The dehydrated medium is a powder with yellowish-brown color.
The prepared non-sterilized medium is pink. The freshly sterilized medium is clear to almost clear and yellowish and develops a small pink zone of oxidation on the surface.

The pH value at 25 °C of the prepared sterilized medium is in the range of 5.8 ± 0.1.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Tubes of medium are boiled where appropriate (100 °C for 20 min) to regenerate anaerobiosis and cooled down to 25-30 °C.

According to CNERNA, colourless tubes are inoculated with 1 mL of inoculum and each of its tenfold dilutions using the MPN method with five parallel tubes Than the tubes are overlaid with 2 ml (1.5 to 2 cm) of sterile (121 °C for 20 min) melted (58 -60 °C) paraffin, Cat. No. 76244.

The tubes are heat treated (75 °C ± 1 °C for 10 min) to destroy vegetative cells and activate the germination of spores. The tubes are rapidly cooled down to around 37 °C to solidify the paraffin, e.g. by using an ice-water bath.

The inoculated medium is incubated at 37 °C ± 1 °C for up to 7 days.

Tubes with growth and gas formation indicated by a raised paraffin plug are considered positive. According to CNERNA, for 16 x 160 mm tubes, tubes are considered positive if showing a raise of 1 cm of the paraffin plug.

The MPN index is used to calculate the number of *Clostridium* spp.

The MPN values can be determined as given by CNERNA or with the recommended software according to EN ISO 7218, MPN calculator: http://standards.iso.org/iso/7218/.

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

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

Self-prepared medium can be stored in airtight containers in the dark and protected against evaporation at (5 ± 3 °C), according to CNERNA up to one month.

If after storage the medium is pink (sign of oxidation) for more than 1/3 of its height under the surface, regenerate anaerobic conditions by heating at 100 °C for 20 minutes. Do not repeat this operation more than once.

Microbiological Performance

The performance test is in accordance with the current version of EN ISO 11133.

Test method: Qualitative single tube method (turbidity) for performance testing of liquid media

Test strain	Specification		
	Inoculum	Growth	Gas
Clostridium tyrobutyricum DSM 663	≤ 100 CFU	good to very good	+
Clostridium perfringens ATCC® 10543 [WDCM 00174]	≤ 100 CFU	good to very good	+
Escherichia coli ATCC® 25922 [WDCM 00013]	≤ 100 CFU	good to very good	fair to none
Staphylococcus aureus ATCC® 25923 [WDCM 00034]	≤ 100 CFU	good to very good	-
Pseudomonas aeruginosa ATCC® 27853 [WDCM 00025]	≤ 100 CFU	none	-

Incubation: 5 days at 37 ± 1 °C with paraffin overlay.

Reference medium (inoculum): Blood Agar (Clostridia) and Tryptic Soy Agar (others).

Test method: MPN method

Test strain	Specification
	Passes test
Clostridium tyrobutyricum DSM 663	≤ 100 CFU

Incubation: 5 days at 37 ± 1 °C with paraffin overlay.

The MPN values are determined with the recommended software according to EN ISO 7218,

MPN calculator: http://standards.iso.org/iso/7218/. Five parallel tubes are used for each dilution.

Please refer to the actual batch related Certificate of Analysis.

Literature

CNERNA (Commission « Qualité Bactériologique du lait » du Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation) (1986): Recommandations pour l'estimation de la contamination du lait en spores de Clostridia par la méthode de culture en milieu liquide [Recommendations for estimating the contamination of milk by clostridial spores by a cultural method in liquid media]. Revue Laitière Française, **451**: 39-45.

EN ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations + Amendment 1. EN ISO 7218:2007/Amd1:2013.

EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd1:2018/Amd2:2020.

Bergère, J. L. (1969): La germination de la spore de *Clostridium tyrobutyricum*. I. Action de différents composés sur la phase initiale. Annales de l'institut Pasteur, pp. 179-189.

Bergère, J.L., Gouet, P., Hermier, J., and Mocquot G. (1968): Les *Clostridium* du groupe butyrique dans les produits laitiers. Annales de l'Institut Pasteur. **19**: 41-54.

Brändle, J., Fraberger, V., Schuller, K., Zitz, U., Kneifel, W. and Domig, K.J. (2017): A critical assessment of four most probable number procedures for routine enumeration of cheese-damaging clostridia in milk. International Dairy Journal. **73**: 109-115.

Bryant, M.P., and Burkey, L.A. (1953): Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. Journal of Dairy Science. **36**: 205-217.

Bryant, M.P., and Burkey, L.A. (1956): The characteristics of lactate-fermenting sporeforming anaerobes from silage. Journal of Bacteriology. **71**: 43-46.

Cerf, O., and Bergère, J.L. (1968): La numération des spores de *Clostridium* et son application au lait et aux produits laitiers. Numération des différents groupes de *Clostridium*. Le lait. **48**: 501-519.

Fischer, M., Zhu, S. and de Ree, E. (2012). Culture media for the detection and enumeration of clostridia in food. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 66-89. Royal Society of Chemistry, Cambridge, UK.

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

Ivy, R.A. and Wiedman, M. (2014): *Clostridium tyrobutyricum*. In: Encyclopedia of Food Microbiology, Volume 1, (Batt, C.A. and Tortorello, M.L. eds). Academic Press, Oxford, UK, pp. 468-473.

Jakob E. (2011): Analytik rund um die Buttersäuregärung [Analytics around the topic of butyric acid fermentation]. Agroscope Liebefeld-Posieux ALP Forum. **85**: 1-23.

Touraille, C., and Bergère, J.L. (1974) La germination de la spore de *Clostridium tyrobutyricum*. Biochimie. **56**(3): 404-4212.

Ordering Information

Product	Cat. No.	Pack size
NutriSelect® prime BRYANT BURKEY broth modified by BERGÈRE acc. CNERNA (with resazurin and lactate)	1016170500	500 g
NutriSelect® prime BRYANT BURKEY broth modified by BERGÈRE acc. CNERNA (with resazurin and lactate)	1016175000	5 KG
Paraffin wax	76244	1 KG