BRYANT BURKEY BROTH BASE WITH RESAZURIN

Dehydrated culture medium

1 - INTENDED USE

Medium for the detection and enumeration of spores of lactate fermenting clostridia.

2 - COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	15.0 g
Yeast extract	5.0 g
Beef extract	7.5 g
Sodium acetate	5.0 g
Cysteine HCl	0.5 g
Resazurin	2.5 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Bryant and Burkey Medium is based on the lactate fermentation media described by Rosenberger[1] and Bryant and Burkey[2], as modified by Bergère[3]. Bryant Burkey Broth Base with Resazurin is prepared according to the formulation recommended by CNERNA[4].
It is suitable for the enumeration of spores of lactate fermenting *Clostridia* spp. in silage, milk and dairy products and in particular for the detection of *Clostridium tyrobutyricum* responsible for the "late blowing" in brine salted semi-hard and hard cheese. The gas produced by the growth of clostridia swells the cheese and is responsible for defect known as butyric swelling, resulting in bad taste.
Tryptone, yeast extract and beef extract provide nitrogen, carbon, vitamins, minerals and amino acids for microbial growth. L-cysteine is the reducing agent and resazurin is a redox indicator and monitors the oxygen level. Sodium acetate promotes the spore germination, which is activated by the heat treatment of the sample and improves the selectivity of the medium. Sodium lactate is not included in the medium so it must be added; sodium lactate, in the presence of sodium acetate, is fermented under anaerobic conditions by *C.tyrobutyricum* and other lactate-fermenting clostridia into butyric acid, acetic acid and gases (CO_2 and H_2). Gas production is demonstrated by an upward movement of a paraffin plug which is overlaid on the medium.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 33 g in 1000 mL of cold purified water and add 10 g of 50% sodium lactate solution. Heat to dissolve stirring constantly. Distribute 10 mL in 16x160 mm tubes. Sterilize by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	light brown to reddish, limpid
Final pH at 20-25 °C	5.9 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Bryant Burkey Broth Base with Resazurin	Dehydrated medium	4012692	500 g (15.1 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, test tubes, Erlenmeyer flasks, ancillary culture media and reagents, 50% sodium lactate solution.

8 - SPECIMENS

Dairy samples decontaminated by heating for 10 minutes at 75°C in order to destroy all the vegetative forms. For detailed information on sample collection and handling procedures, consult appropriate texts.[4]

9 - TEST PROCEDURE

1. Cool the tubes to 25°C after autoclaving or regenerate the anaerobic conditions by heating the tubes to 100°C for 10 minutes if the medium is pink for more than 1/3 of its height. Do not repeat the operation more than once.
2. Inoculate colourless tubes with 1 mL of sample and 1 mL of its tenfold dilutions. using MPN method with five tubes.
3. Overlay the medium with 2 mL (1.5-2 cm) of paraffin autoclaved at 121°C for 15 minutes and cooled to 58-60°C.
4. Heat the tubes at 75°C for 10 minutes to destroy vegetative cells and activate the germination of spores.
5. Cool the tubes rapidly in an ice-water bath to solidify the paraffin.
6. Incubate the inoculated tubes at 37°C for up to 7 days. The tubes are evaluated every 48 hours.

10 - READING AND INTERPRETATION

After incubation observe the presence of growth (turbidity of the medium) and gas formation.
Tubes with growth and gas formation indicated by a 1 cm raise of the paraffin plug are considered positive.[4]
Calculate the number of *Clostridium* spp by MPN tables/software.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

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CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. tyrobutyricum ATCC 25755	35-37° / 44-48H /AN	growth with gas

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated Bryant Burkey Broth Base with Resazurin supplemented with 50% sodium lactate solution, are tested for productivity and selectivity by comparing the results with previously approved Reference Batches.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 35-37°C for 44-48 hours and recording the highest dilution showing growth and gas production in Reference Batch (GrRB) and in Test Batch (GrTB). Productivity is tested with the following strains: *C.tyrobutyricum* ATCC 25755, *C.perfringens* ATCC 13124 and *C.sporogenes* ATCC 3584. The productivity index GrRB-GrTB for each test strain shall be ≤ 1. *C. tyrobutyricum* and *C.perfringens* exhibit growth with gas production while *C.sporogenes* grows without gas production.

13 - LIMITATIONS OF THE METHOD

- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging).

16 - REFERENCES

1. Rosenberger KF. The development of methods for the study of obligate anaerobes in silage. Proc Soc Appl Bacteriol1951;14:161-164
2. Bryant MP, Burkey LA. The characteristics of lactate-fermenting sporeforming anaerobes from silage. J Bacteriol 1956; 71: 43-46
3. Bergère JL, Gouet P, Hermier J, Mocquot G. Les Clostridium du groupe butyrique dans les produits laitiers. Ann Inst Pasteur 1968;19: 41-54
4. CNERNA (Commission « Qualité Bactériologique du lait » du Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation): Recommandations pour l'estimation de la contamination du lait en spores de Clostridia par la méthode de culture en milieu liquide. Revue Laitière Française 1986; 451:39-45.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/05

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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