MODIFIED LAURYL SULFATE TRYPTOSE (mLST) BROTH BASE
VANCOMYCIN ANTIMICROBIC SUPPLEMENT
MODIFIED LAURYL SULFATE TRYPTOSE (mLST) BROTH

Dehydrated culture medium, selective supplement and ready-to use tubes

1 - INTENDED USE
Enrichment selective broth for the detection of *Cronobacter sakazakii* in milk and milk products.

2 – COMPOSITIONS*
MODIFIED LAURYL SULFATE TRYPTOSE (MLST) BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Enzymatic digest of animal and plant tissue	20.00 g
Lactose	5.00 g
Sodium chloride	34.00 g
Sodium lauryl sulphate	0.10 g
Potassium dihydrogen phosphate	2.75 g
Potassium hydrogen phosphate	2.75 g

VANCOMYCIN ANTIMICROBIC SUPPLEMENT (VIAL CONTENT)

Vancomycin	25 mg

MODIFIED LAURYL SULFATE TRYPTOSE (MLST) BROTH, READY TO USE TUBES

Enzymatic digest of animal and plant tissue	20.00 g
Lactose	5.00 g
Sodium chloride	34.00 g
Sodium lauryl sulphate	0.10 g
Potassium dihydrogen phosphate	2.75 g
Potassium hydrogen phosphate	2.75 g
Vancomycin	10.00 mg
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Cronobacter species (formerly known as *Enterobacter sakazakii*) are Gram-negative rod-shaped, motile pathogenic bacteria of the family *Enterobacteriaceae*. These organisms are regarded as opportunistic pathogens linked with life-threatening infections predominantly in neonates.[1] Clinical syndromes of *Cronobacter* infection include necrotizing enterocolitis (NEC), bacteraemia and meningitis, with case fatality rates ranging from 40-80%.[1,2] The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, water, rice and others.[1,3,4]
Modified Lauryl Sulfate Tryptose (mLST) Broth is a selective broth for the enrichment step of the isolation procedure of *Cronobacter*.
The use of Buffered Peptone Water as a non-selective enrichment, mLST Broth as a selective enrichment and Enterobacter Sakasakii Isolation Agar (ESIA REF 401478) allow the specific detection of *C.sakazakii* in food samples especially in milk powder and powdered infant formula. The above culture media and the work procedure described below are in accordance with the withdrawn standard ISO/TS 22964:2006[4], replaced by ISO Standard 22964:2017.[5]
Essential growth factors are provided by enzymatic digest of animal and plant tissue which is a source of nitrogen, carbon, amino acids and minerals. Lactose is a fermentable carbohydrate and a source of carbon and energy. Phosphates act as buffer system. The high concentration of sodium chloride and the surface-active agent sodium lauryl sulphate act as selective agents in restricting the growth of bacteria other than coliforms, while vancomycin is inhibitory for Gram positive bacteria.

4- DIRECTIONS FOR MEDIUM PREPARATION
Suspend 32.3 g in 500 mL of cold purified water. Heat to dissolve and sterilize by autoclaving at 121°C for 15 minutes. Cool to room temperature and add 1 mL of Vancomycin Antimicrobic Supplement (REF 4240057), reconstituted with 5 mL of sterile purified water. Final vancomycin concentration in the medium: 10 mg/L. Mix well and distribute into sterile tubes (10 mL/tube) under aseptic conditions. The vancomycin solution may be kept at 0 °C to 5 °C for 15 days.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear
Freeze-dried selective supplement	high, soft white, pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	6.8 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Modified Lauryl Sulfate Tryptose (mLST) Broth Base	Dehydrated medium	4014762	500 g (7.7 L)
Vancomycin Antimicrobic Supplement	Freeze-dried supplement	4240057	10 vials (25 mg/vial)
Modified Lauryl Sulfate Tryptose (mLST) Broth	Ready-to-use tubes	551476	20 x 10 mL

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7 - Materials required but not provided

Autoclave, water-bath, sterile inoculation needles and pipettes, incubator and laboratory equipment as required, sterile tubes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - Specimens

Milk powder, powdered infant formula and environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

9 - Test procedure

1. Prepare the initial sample suspension (primary dilution) by adding *x* g of the test sample to 9 times *x* mL of Buffered Peptone Water Casein (REF 401278C): e.g., 25 g + 225 mL of Buffered Peptone Water Casein or 10 g + 90 mL of Buffered Peptone Water Casein.
2. Incubate at 37 ± 1°C for 18 ± 2 hours.
3. After incubation of the inoculated pre-enrichment medium, transfer 0.1 mL of the obtained culture into 10 mL mLST Broth.
4. Incubate at 44 ± 0.5°C for 24 ± 2 hours.
5. After incubation, streak a 10 µ loopful from the mLST broth onto the surface of the ESIA plate (REF 401478) and incubate at 44 ± 1°C for 24 ± 2 hours

10 - Reading and interpretation

The presence of microorganisms in mLST Broth is indicated by a varying degree of turbidity, specks and flocculation.
Subculture onto ESIA plates:
Presumptive positive result for *C. sakasakii*: presence of blue to green colonies, 1 to 3 mm in diameter.
Negative result for *C. sakasakii*: absence of typical blue-green colonies or presence of mauve-violet colonies.
Confirm colonies with biochemical tests recommended by ISO 22964.

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.

Control strains	Incubation T°/ t / ATM	Expected results
C. sakazakii ATCC 29544	44°C / 24 h / A	growth
S. aureus ATCC 25923	44°C / 24 h / A	inhibited

A: aerobic 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 mLST Broth supplemented with vancomycin and ready-to-use tubes are tested for productivity and selectivity, by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 44°C for 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *C. sakazakii* ATCC 29544 and *C. muytjensii* ATCC 51329. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is assesssed with the following non-target strains: *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. After incubation at 44°C for 24 hours, *E. coli* exhibits growth, while the growth of *S. aureus* is totally inhibited.

13 - Limitations of the method

- *Cronobacter* may be present in low numbers in the samples, along with other *Enterobacteriaceae*, such as *E. cloacae*, which may interfere in the determination of the target microorganism.[5]
- Some coliforms grow on ESIA with violet colonies, easily distinguishable from the blue colonies of *C. sakazakii*.

14 - Precautions and warnings

- The medium base, the supplement and the ready-to-use tubes are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Vancomycin Antimicrobic Supplement is classified as hazardous. Before use, consult the Material Safety Data Sheets.
- 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 the 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass. Be careful when opening the metal ring to avoid injury.
- The supplement is sterilized by membrane filtration.
- Each tube of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.

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- The Certificates of Analysis and the Safety Data Sheets 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

Ready-to-use medium in tubes

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

Dehydrated medium

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).

Freeze-dried supplement

Upon receipt, store the product in the original package at +2/ +8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging). According to ISO/TS 22964:2006 the self-prepared tubes of mLST/vancomycin medium may be kept at 0 °C to 5 °C for 1 day and the unused vancomycin solution may be may be kept at 0 °C to 5 °C for 15 days.

16 - REFERENCES

1. Carol Iversen *et al*., The taxonomy of Enterobacter sakazakii: proposal of a new genus Cronobacter gen. nov. and descriptions of Cronobacter sakazakii comb. nov. Cronobacter sakazakii subsp. sakazakii, comb. nov., Cronobacter sakazakii subsp. malonaticus subsp. nov., Cronobacter turicensis sp. nov., Cronobacter muytjensii sp. nov., Cronobacter dublinensis sp. nov. and Cronobacter genomospecies BMC Evol Biol. 2007; 7: 64.
2. Simmons BP et al. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of powdered infant formula. Infect Control Hosp Epidemiol 1989; 10: 398.
3. Van Acker J et al. Outbreack of necrotizing enterocolitis associated with *E.sakazakii* in powdered milk formula. J Clin Microbiol 2001; 39:293-297.
4. ISO/TS 22964:2006. Milk and milk products – Detection of *Enterobacter sakazakii*
5. ISO 22964:2017. Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/09

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

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