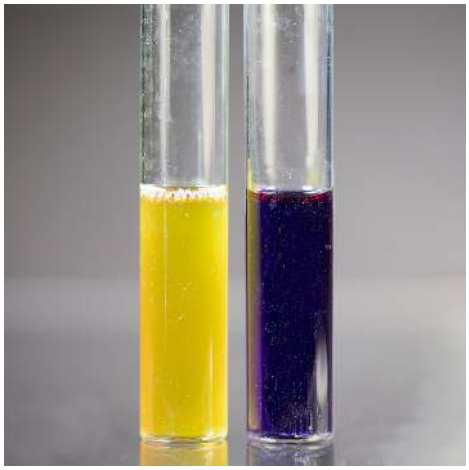
CRONOBACTER SCREENING BROTH BASE
VANCOMYCIN CSB SUPPLEMENT

Dehydrated culture medium, selective supplement and ready to use Tubes



Cronobacter Screening Broth: at left a tube with the growth of *Cronobacter sakazakii*. At right an un-inoculated tube.

1 - INTENDED USE

Cronobacter Screening Broth Base, supplemented with vancomycin, is used for the selective enrichment of *Cronobacter* spp. from the samples of the food chain, according to ISO 22964.

2 - COMPOSITION

CRONOBACTER SCREENING BROTH BASE (4013552)
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Enzymatic digest of animal tissue	10.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Bromocresol purple	0.04 g
Sucrose	10.00 g

VANCOMYCIN CSB SUPPLEMENT (4240057C)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	5 mg

CRONOBACTER SCREENING BROTH (551355)

Enzymatic digest of animal tissue	10.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Bromocresol purple	0.04 g
Sucrose	10.00 g
Vancomycin	10 mg
Purified water	1000 mL

*The formula 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), bacteremia 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]
Cronobacter Screening Broth Base, supplemented with vancomycin, is a selective enrichment medium used for the procedure of determination of the presence or absence of *Cronobacter* spp. in samples from the food supply chain, according to ISO 22964.[5] It must be used combined with Chromogenic Cronobacter Isolation (CCI) Agar.
The enzymatic digest of animal tissues and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Sucrose is a fermentable carbohydrate while bromocresol purple is a pH indicator: sucrose-fermenting bacteria acidify the medium with a colour change of bromocresol purple from purple to yellow. The selective agent of the medium is vancomycin which inhibits the growth of Gram-positive bacteria.

4- DIRECTIONS FOR DEHIDRATED MEDIUM PREPARATION

Suspend 14 g in 500 mL of cold purified water. Heat to dissolve with frequent agitation if necessary. Sterilize by autoclaving at 121°C for 15 minutes. Cool below 47°C and add the contents of one vial of Vancomycin CSB Supplement (REF 4240057C) reconstituted with 5 mL of sterile purified water. Mix well and dispense 10 mL into sterile tubes under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and tubes appearance	purple, clear
Freeze-dried selective supplement appearance	high, soft pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Cronobacter Screening Broth Base	Dehydrated medium Ready to use tubes	4013552 551355	500 g (17.8 L) 20 x 10 mL
Vancomycin CSB Supplement	Freeze-dried supplement	4240057C	10 vials, each for 500 mL of medium base

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

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8 - SPECIMENS

Food products and ingredients intended for human consumption and the feeding of animals; 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.[5]

9 - TEST PROCEDURE

Prepare the test sample in accordance with the relevant part of ISO 6887 dealing with the product concerned.
Add 10 g or 10 mL of sample to 90 mL of Buffered Peptone Water (REF 401278). For inoculum above 10 g preheat the broth to 34-38°C.
Incubate the pre-enrichment broth at 36 ± 2 °C for 18 ± 2 hours.
Transfer 0.1 mL of pre-enriched broth into 10 mL of complete Cronobacter Screening Broth.
Incubate the enrichment broth tubes at 41.5 ± 1 °C for 24 ± 2 hours
Streak a loopful of incubated enrichment broth (about 10 µL) on a plate of CCI Agar and incubate upside down at 41.5 ± 1 °C for 24 ± 2 hours.

10 - READING AND INTERPRETATION

Bacterial growth in Cronobacter Screening Broth is evidenced by the development of turbidity in the broth; *Cronobacter* spp. typically turn the medium to yellow.
After incubation of CCI Agar plates, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical *Cronobacter* colonies are small to medium-sized (1 mm to 3 mm) and blue to blue-green in colour.
Colonies of non-typical Gram-negative bacteria may develop on CCI Agar with the following characteristics: white, with or without a grey or black or green centre; some naturally pigmented colonies of non-*Cronobacter* may appear yellow or red.
Perform the confirmation tests on the typical colonies as reported by ISO 22964.[5]

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.[5]

Control strains	Incubation T°/ t - ATM	Expected results
C. sakazakii ATCC 29544+*S. aureus* ATCC 25923	41.5 °C ± 1°C /24 h ± 2 h / A	growth, the medium turns to yellow
C. muytjensis ATCC 51329+*S. aureus* ATCC 25923	41.5 °C ± 1°C /24 h ± 2 h / A	growth, the medium turns to yellow
S. aureus ATCC 25923	41.5 °C ± 1°C /24 h ± 2 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 Cronobacter Screening Broth Base supplemented with Vamcomycin CSB Supplement are tested for productivity and selectivity with incubation at 41.5°C for 24 hours, 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 41.5°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. muytjensis* ATCC 51329. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Productivity and selectivity are tested together with mixtures of appropriate dilutions of target and non-target strains: *C. sakazakii* ATCC 29544+*S. aureus* ATCC 25923 and *C. muytjensis* ATCC 51329+*S.aureus* ATCC 25923. After incubation of inoculated tubes and sub-culture on CCI Agar plates, the target strains show a predominant growth on plated medium (>10 blue-green colonies).
Selectivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of non-target organisms in test tubes, and recording the highest dilution showing growth after subculture in Tryptic Soy Agar. The growth of non-target tested strains *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *B. subtilis* ATCC 6633 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]
- The use of large sample sizes can compromise the recovery of stressed *Cronobacter* spp. when interfering microflora are present, such as probiotics [5]

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement 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 must be handled with suitable protection. Vancomycin CSB Supplement is classified as dangerous. 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 the screw caps of the tubes and the metal ring of the supplement vial to avoid injury.
- The supplement is sterilized by membrane filtration.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving.

- 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 inoculated medium with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- 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 (tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 22964[5] the medium base in tubes or flasks can be stored at +2°C +8°C for up to six months; the tubes complete with vancomycin may be kept at 5 °C ± 3 °C for 1 day.

16 - REFERENCES

1. Yan QQ, Condell O, Power K, Butler F,Tall BD, Fanning S. Cronobacter species (formerly known as Enterobacter sakazakii) in powdered infant formula: a review of our current understanding of the biology of this bacterium, J App Microbiol 2012; 113:1-15
2. Friedemann, M. Epidemiology of invasive neonatal Cronobacter (Enterobacter sakazakii) infections. Eur J Clin Microbiol Infect Dis 2009; 28:1297–1304.
3. Bowen AB, Braden CR. Invasive Enterobacter sakazakii disease in infants. Emerg Infect Dis 2006;12:1185-89.
4. Healy B, Cooney S, O'Brien S, Iversen C, Whyte P, Nally J, Callanan JJ, Fanning, S (2010) Cronobacter (Enterobacter sakazakii): an opportunistic foodborne pathogen. Foodborne Pathog Dis 2010; 7:339-50.
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 0	First edition	2022/06
Revision 1	Adding of ready to use tubes	2025/02

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