SELENITE CYSTINE BROTH BASE
SODIUM BISELENITE
Dehydrated culture medium and additive

1 - INTENDED USE
Selective enrichment medium base used in procedures for the detection of *Salmonella* spp. in food and water samples.

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

Tryptone	5.00 g
Lactose	4.00 g
Sodium phosphate bibasic	10.00 g
L-cystine	0.01 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Selenite cystine broth is based on early works by Klett[1] and Guth[2] who demonstrated the selective inhibitory effects of selenite and used it for the culture of typhoid organisms. Twenty years later, Leifson[3] utilized this information to fully investigate selenite activity, to formulate the liquid medium selenite broth and to promote its wide use as an enrichment medium for the isolation of *Salmonella* spp.
Selenite cystine broth is based on a modification made in 1953 by North and Bartram[4] of the original Leifson's formula, differing only in the addition of L-cystine which is considered to enhance *Salmonella* growth by reduction of toxicity.[5]
Selenite cystine broth is recommended by FDA-BAM[6] and AOAC[7] methods for selective enrichment of guar gum and foods suspected to be contaminated with *Salmonella* Typhi. It is included as selective enrichment in ISO 6579-1 Annex D[8-9] and in ISO 19250[10] for the detection of *Salmonella* Typhi and Paratyphi.
To minimise any possible risk of teratogenicity for laboratory operators, sodium acid selenite is not included in the dehydrated medium Selenite Cystine Broth Base, but must be prepared separately as a solution with the supplied raw material REF 4123651 and added to the medium base.
Tryptone provides carbon, nitrogen and trace elements for bacterial growth. Sodium acid selenite (synonyms: sodium hydrogen selenite, sodium biselenite), at neutral pH, is inhibitory for coliforms and certain other microbial species, such as faecal streptococci and other Gram-positive bacteria, but not for the majority of *Salmonella* spp., including *Salmonella* Typhi and *Salmonella* Paratyphi. It is believed that, in part, the toxicity of selenite for microorganisms may be attributable to the incorporation of selenium analogues of sulphur-containing amino acids into proteins[11]. The phosphate buffer lessens the toxicity of selenite and tends to minimise the alkalinising effects induced by the reduction of sodium selenite; these alkalinising effects would notably diminish the selective properties of the medium. The acids produced by the microorganisms from lactose also contribute to neutralise alkaline reactions of the medium. L-cystine enhance *Salmonella* growth by again reducing the toxicity of the culture medium.

4- DIRECTIONS FOR MEDIUM PREPARATION
Dissolve 4 g of Sodium Biselenite (REF 4123651) in 1 litre of cold purified water and then add 19 g of Selenite Cystine Broth Base. Warm to dissolve completely and distribute into sterile tubes or flasks. Do not overheat or autoclave.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium base appearance	white, fine, homogeneous, free-flowing powder
Sodium biselenite appearance	white powder
Medium appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.1

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Selenite Cystine Broth Base	Dehydrated medium	402026B2 402026B4	500 g (21.7L) 5 kg (217 L)
Sodium Biselenite	Raw material	4123651	100 g (25 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile tubes, ancillary culture media and reagents.

8 - SPECIMENS
Food, feed, food chain and water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[5-10]

9 - TEST PROCEDURE
The detection of *Salmonella* in foods necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in two selective liquid media, plating out and recognition, confirmation.
Pre-enrichment
Salmonella organisms in foods and water are often present in low numbers and may be sub-lethally injured. Through pre-enrichment *Salmonella* cells grow to a detectable level. US methods[5-7] suggest different pre-enrichment media depending on the sample to be analysed, while ISO Standards[8-10] recommend a sole medium (Buffered Peptone Water).
Selective enrichment
FDA-BAM method[6] for guar gum and foods suspected to be contaminated with serovar Typhi:

- Transfer 1 mL of pre-enrichment broth to 10 mL of Selenite Cystine Broth and another 1 mL in 10 mL of Tetrathionate Broth.
- Incubate 24 ± 2 h at 35°C.

ISO 6579-1 method for the detection of Salmonella Typhi and Paratyphi[8,9]
10 mL of Selenite Cystine Broth are inoculated with 1 mL of the pre-enrichment culture (in addition to inoculation of RVS broth or MSRV agar and MKTTn broth) and incubated between 34 °C and 38 °C for 24 h and 48 h.

Plating out

US methods[6,7]: vortex the enrichment culture tubes and streak a 10 µL onto Bismuth Sulphite Agar, Hektoen Enteric Agar and XLD Agar and incubate at 35°C for 22-26 hours.
ISO 6578 (Annex D)[8,9]: inoculate by means of a 10 µl loop the surface of an XLD plate so that well-isolated colonies will be obtained. Proceed in the same way with Bismuth Sulphite Agar.
Incubate the plates of both media between 34 °C and 38 °C and examined after 24 h, and again, if necessary, after 48 h.

Confirmation

Perform confirmation tests of colonies obtained on plated media in accordance with the method of analysis in use.

10 - READING AND INTERPRETATION

After incubation, the growth of organisms in Selenite Cystine Broth is indicated by turbidity and often by a colour change of the medium to pink-orange-red.

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, it is responsibility of the end-user to perform Quality Control testing 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.[9]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028 + *E. coli* ATCC 25922 + *E. faecalis* ATCC 29212	34-38 °C / 24 h ± 3 h / A	>10 characteristic colonies on XLD agar or other medium of choice
E. coli ATCC 25922	34-38 °C / 24 h ± 3 h / A	Partial inhibition, ≤100 colonies on TSA
E. faecalis ATCC 29212	34-38 °C / 24 h ± 3 h / A	Partial inhibition, <10 colonies on TSA

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 and ready-to use Selenite Cystine Broth Base supplemented with sodium biselenite 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 37°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: S. Typhimurium ATCC 14028 and S. Enteritidis ATCC 13076. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Productivity and selectivity are tested also together with mixtures of appropriate dilutions of target and non-target strains: S. Typhimurium ATCC 14028+*E. coli* ATCC 25922+*P. aeruginosa* ATCC 27853. After incubation of inoculated tubes at 37°C for 24 hours and sub-culture on Brilliant Green Agar Modified, the target strains will show a predominant growth on plated media with more than 10 colonies.
Moreover, selectivity is evaluated by inoculating approximately 10,000 CFU/tube of non-target organisms by dilution to extinction method, and incubating at 37°C for 24 hours and sub-culturing on Tryptic Soy Agar plates. Selectivity is tested with the following strains: *E. coli* ATCC 25922 and *E. faecalis* ATCC 29212. CFUs of *E.coli* shall be ≤100 on the sub-cultured plates of Tryptic Soy Agar; CFUs of *E. faecalis* shall be less than 10 on the sub-cultured plates of Tryptic Soy Agar.

13 - LIMITATIONS OF THE METHOD

- After a long storage period of the dehydrated medium, the colour of the prepared broth might change to reddish/red. The microbiological performance however is not affected. Discard the tubes if selenite oxidizes and forms large amounts of a red precipitate.[12]
- Selenite Broth is toxic for *Salmonella* Cholerae-suis and for *Salmonella* Abortus-ovis.[13]
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

14 - PRECAUTIONS AND WARNINGS

- This culture medium 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. Sodium biselenite is classified as dangerous by the current legislation. 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 medium powder 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 products 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 +12°C /+8°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 the validation of their shelf life, according to the type (tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 6579 the self-prepared tubes can be stored at +2°C +8°C in the dark until a red precipitate occurs.[8]

16 – REFERENCES

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5. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev March 2022.
7. Association of Official Analytical Chemists. Official Methods of Analysis, 19th ed. 2012. AOAC, Arlington, VA
8. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.
9. ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC.
10. ISO 19250:2010 Water quality — Detection of Salmonella spp.
11. Weiss KF, Ayres JC, Kraft AA. Inhibitory action of selenite on Escherichia coli, Proteus vulgaris, and Salmonella Thompson. J Bacteriol 1995; 90:857
12. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
13. Smith HW. The evaluation of culture media for the isolation of salmonellae from faeces. J. Hyg 1952; 50:21-36.

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 2	Updated layout and content	2022/09

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

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