SELENITE CYSTINE BROTH

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Selective enrichment liquid medium 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
Sodium acid selenite	4.00 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.
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

Suspend 23 g in 1000 mL of cold purified water, warm to dissolve completely and distribute into sterile tubes or flasks. Do not overheat or autoclave.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing 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	Dehydrated medium	4020262 4020264	500 g (21,7) 5 kg (217 L)
Selenite Cystine Broth	Ready-to-use tubes	552026 552026A	20 x 10 mL 20 x 9 mL
Selenite Cystine Broth	Ready-to-use flasks	5120262 512026A2	6 x 100 mL 6 x 90 mL

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.

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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, 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.[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 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 characteristic 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. Selenite Cystine Broth 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization 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 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 Sheets 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

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, large amounts of a red precipitate, atypical colour).

Ready-to-use flasks

After receipt, store in the original packaging at +2°C / +8°C away from light. Under these conditions, the vials are valid until the expiration date indicated on the label. Do not use beyond the expiration date. Flasks removed from secondary packaging can be used until the expiration date. Opened flasks should be used immediately. Check the closure and integrity of the screw cap before use. Discard flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, atypical color).

Dehydrated medium

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.[9]

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.
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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.
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10. ISO 19250:2010 Water quality — Detection of Salmonella spp.
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12. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
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402025 SELENITE CYSTINE BROTH

Regulation (EU) 2020/878

Classification

Acute toxicity, category 4	H302 Harmful if swallowed.
Acute toxicity, category 4	H332 Harmful if inhaled.
Specific target organ toxicity - repeated exposure, category 2	H373 May cause damage to organs through prolonged or repeated exposure.
Hazardous to the aquatic environment, chronic toxicity, category 2	H411 Toxic to aquatic life with long lasting effects.

Labelling

Hazard pictograms:

Signal words: Warning

Hazard statements:

H302+H332 Harmful if swallowed or if inhaled.
H373 May cause damage to organs through prolonged or repeated exposure.
H411 Toxic to aquatic life with long lasting effects.

Precautionary statements:

P273 Avoid release to the environment.
P391 Collect spillage.
P261 Avoid breathing dust / fume / gas / mist / vapours / spray.

P312 Call a POISON CENTRE / doctor / . . . if you
P264 Wash . . . thoroughly after handling.

Contains: Sodium acid selenite (synonyms: sodium hydrogen selenite, sodium biselenite)

TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	**LOT** Batch code	Manufacturer	This side up	Store in a dry place	Fragile
emperature nitation	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 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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