INSTRUCTIONS FOR USE

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SELENITE BROTH

Dehydrated culture medium

Selenite Broth – from the left: uninoculated tube and *S.*Typhimurium growth

1 - INTENDED USE

In vitro diagnostic. Enrichment liquid medium for the isolation of *Salmonella* spp. in clinical specimens.

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

Tryptone	5 g
Lactose	4 g
Sodium phosphate bibasic	10 g
Sodium acid selenite	4 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 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 Broth is a selective enrichment medium intended for the isolation of *Salmonella* spp. from clinical specimens, such as faeces and urine.
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, present in faecal specimens, but not for the majority of *Salmonella* spp. 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[4]. 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.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment broth followed by subculture on selective enteric plating media.[5] According to the data of Kelly et al.[6] about 40% of *S.enterica* isolated with an enrichment into Selenite Broth and a subculture onto XLD plates did not grow with a direct inoculation on the primary XLD plates.
Selenite Broth has been demonstrated to be superior to other selective enrichment broths for the isolation of *Salmonella* Typhi from stools.[7]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 23 g in 1000 mL of cold purified water, warm until complete dissolution and distribute into sterile tubes. 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 Broth	Dehydrated medium	4020252 4020254	500 g (21,7) 5 kg (217 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, test tubes, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Selenite Broth may be inoculated with human clinical specimens such as faeces or rectal swab and urine. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.

9 - TEST PROCEDURE

For faeces examination, inoculate test tubes with 1 g of faeces, or 1 mL of faecal suspension obtained suspending 1 g of faeces in 1 mL of saline solution. Rectal swabs received fresh or in transport medium should be rinsed thoroughly in 1 mL of saline.

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For urine examination, centrifuge the specimen and inoculate the sediment.
Incubate the inoculated tubes in aerobic atmosphere at 35-37°C for 16-24 hours.

10 - READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by turbidity and often by a colour change of the medium to pink-orange-red.
Sub-culture by streaking a loopful of broth on selective enteric plating media.
The plating media should be chosen as a combination of greater and lesser inhibitory selective agars. For the isolation of S.Typhi, it is advisable to use Bismuth Sulphite Agar or Chromogenic Salmonella Agar as plating medium.

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
E.coli ATCC 25922	35-37 °C / 16-24h / A	scanty growth
*S.*Typhimurium ATCC 14028	35-37 °C / 16-24h / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Selenite Broth is 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 35-37°C for 16-24 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, S.Enteritidis ATCC 13076, *S.arizonae* ATCC 13314 and S.Gallinarum clinical isolate. 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, S.Enteritidis ATCC 13076+*E.coli* ATCC 25922, S.Enteritidis ATCC 13076+*P.vulgaris* ATCC 9484. After incubation of inoculated tubes at 35-37°C for 16-24 hours and sub-culture on MacConkey Agar and Hektoen Enteric Agar, the target strains will show a predominant growth on plated media.

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.[9]
- Selenite Broth is toxic for *Salmonella* Cholerae-suis and for *Salmonella* Abortus-ovis.[10]
- The value of Selenite Broth as enrichment for *Shigella* spp. has not been clearly established, since some strains of *Shigella*, having similarities with *E. coli*, are inhibited to the same extent as the latter; specimens that might contain organisms inhibited by selective enrichment broth should be plated directly or cultured in a non-selective enrichment broth (e.g. GN Broth).[5]
- Do not incubate the broth over 24 hours. The inhibitory effect diminishes after the first 6-12 hours of incubation.[9]
- The development of *E. coli* and *Proteus* spp. is not indefinitely retarded in Selenite Broth. When the initial proportion of these organisms is high, it is often advantageous to sub-culture onto the solid media after 6 hours as well as after 18 hours.
- After the enrichment in Selenite Broth, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This culture medium is classified as dangerous; before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the preparation process of tubed or bottled media.
- 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.
- 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 plates 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.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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 +2°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 storage method applied (temperature and packaging).

16 – REFERENCES

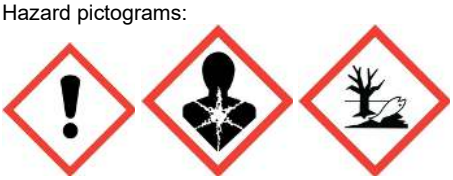
1. Klett A. (1900) Zeitsch. fÎr Hyg. und Infekt. 33. 137-160.
2. Guth F. (1916) Zbl. Bakt. I. Orig. 77. 487-496.
3. Leifson E. New selenite selective enrichment medium for isolation of typhoid and paratyphoid (salmonella) bacilli. A. J Hyg 1936; 24:423
4. Weiss KF, Ayres JC, Kraft AA. Inhibitory action of selenite on Escherichia coli, Proteus vulgaris, and Salmonella Thompson. J Bacteriol 1995; 90:857
5. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.685.
6. Kelly S, Cormican M, Parke L, Feeney GC, Flynn J. Cost-Effective Methods for Isolation of Salmonella enteric in the Clinical Laboratory. J Clin Microbiol 1999; 37:3369
7. Iveson JB, Kovacs N. Comparative trial of Rappaport enrichment medium for the isolation of Salmonellae from faeces J Clin Path 1967; 20: 290
8. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. Smith HW. The evaluation of culture media for the isolation of salmonellae from faeces. J. Hyg 1952; 50:21-36.

402025 SELENITE BROTH
SDS rev 8
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 o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/05
Revision 2	Modification of “precautions and warnings” and “storage conditions and shelf life”.	2022/04
Revision 3	Removal of obsolete classification	2023/04

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

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