INSTRUCTIONS FOR USE

SELENITE BROTH

Ready-to-use flasks

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 *

Tryptone	5 g
Lactose	4 g
Sodium phosphate	10 g
Sodium acid selenite	4 g
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

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 selenite, 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- METHOD OF PREPARATION

The medium is ready to use. If required by the user's procedures, distribute the medium into sterile tubes under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

Medium appearance	very pale yellow, limpid
Final pH at 20-25°C	7.0 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Selenite Broth	Ready-to-use flasks	5120252	6 x 100 mL; 6 glass bottles with flat bottom and aluminium screw-cap; packaging: cardboard box.

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Incubator and laboratory equipment as required, sterile tubes, sterile loops, needles and swabs, 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.
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.

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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 ready-to-use flasks of Selenite Broth and of the raw material used for the production of prepared tubes (dehydrated Selenite Broth REF 402025) 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 and 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.Gallinarum clinical isolate, *S.arizonae* clinical isolate. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is evaluated with dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of non-target organisms in test tubes and 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}). Selectivity is tested with the following non-target strains: *E.coli* ATCC 25922, *P.vulgaris* ATCC 9484. The selectivity 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 subculture on MacConkey Agar and Hektoen Enteric Agar, the target strains will show a predominant growth on plated media.

13 - LIMITATIONS OF THE METHOD

- 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 product is not classified as dangerous according to current European legislation.
- 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 product 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.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- Ready-to-use flasks of Selenite Broth are subject to terminal sterilization by filtration.
- 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.
- 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 flasks in their original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Before use, check the closing and the integrity of the screw cap. Opened flasks must be used immediately for the inoculation or for the preparation of tubed medium Do not use flasks with signs of deterioration (e.g. microbial contamination, abnormal turbidity, large precipitate, atypical colour).

16 - REFERENCES

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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.

TABLE OF APPLICABLE SYMBOLS

REF or 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	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/09
Revision 2	Removal of obsolete classification	2023/04

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

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