SHIGELLA BROTH BASE
NOVOBIOCIN ANTIMICROBIC SUPPLEMENT
Dehydrated culture medium and supplement

1 - INTENDED USE
With the addition of novobiocin, Shigella Broth Base is used as a selective enrichment broth for the determination (presence or absence) of *Shigella* in samples of the food chain according to ISO 21567 and FDA-BAM.

2 - COMPOSITION
SHIGELLA BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Enzymatic digest of casein	20.0 g
Potassium hydrogen phosphate (anhydrous)	2.0 g
Potassium dihydrogen phosphate (anhydrous)	2.0 g
Sodium chloride	5.0 g
Glucose	1.0 g
Tween® 80	1.5 mL

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

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT - VIAL CONTENT

Novobiocin	10 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Shigella species are Gram-negative, nonmotile, facultatively anaerobic, non-sporeforming, nonmotile rods, belonging to the family of *Enterobacteriaceae*, closely related to *E. coli*. The genus is named after its discovery by Kiyoshi Shiga in 1897. The current classification divides the genus into four species based on serological typing: *S. dysenteriae, S. boydii, S. flexneri* and *S. sonnei*. *Shigella* spp. cause dysentery (shigellosis) in primates, but not in other mammals.[1] Their pathogenic action is due to the marked invasiveness towards the intestinal epithelium of the ileum and the colon and to endotoxins and exotoxins production.[2] Yearly, about 80–165 million cases of *Shigella* diseases are recorded in the world, which lead to between 74,000 and 600,000 states of death, especially in the developing countries.[3]
Shigella Broth is based on the formula developed by Mehlman, Romero and Wentz[4] and is recommended by ISO 21567[5] and FDA-BAM[6] as a selective enrichment broth for the determination of *Shigella* in samples of the food chain.
Essential growth factors are provided by enzymatic digest of casein which is a source of nitrogen, carbon and minerals. Glucose is a source of carbon and energy; sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Phosphates are used as buffering agents to control the pH in the medium. Polysorbate 80 neutralizes preservatives in food products, allowing bacteria to grow. Novobiocin is active mostly against Gram-positive bacteria but also against a few Gram-negative bacteria. Its concentration in the medium recommended by ISO 21567[5] is 0.5 mg/L with anaerobic incubation at 41.5°C, while FDA-BAM[6] recommends 0.5 mg/L for the detection of *S.sonnei* with anaerobic incubation at 44.0°C, and 3 mg/L for the enrichment of other *Shigella* species, with anaerobic incubation at 42°C.

4- DIRECTIONS FOR MEDIA PREPARATION
Suspend 31.5 g in 1000 mL of cold, purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 225 mL in bottles and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature.
Dissolve the content of one vial of Novobiocin Antimicrobic Supplement (REF 4240045) with 4 mL of sterile purified water (novobiocin concentration: 2.5 mg/mL). Add a volume of novobiocin solution to the basic medium to obtain the required antibiotic concentration:
Shigella Broth according to ISO 21567 and FDA-BAM for *S. sonnei*: add 50 µL of solution to 225 mL of Shigella Broth Base (final concentration of 0,5 µg/mL broth after 25 g or 25 mL of sample is added).
Shigella Broth according to FDA-BAM for other *Shigella* species: add 300 µL of solution to 225 mL of Shigella Broth Base (final concentration 3 µg/mL broth after 25 g or 25 mL of sample is added).
The remaining novobiocin solution can be stored at 2-8 ° C for one month.[5]

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greyish, fine, homogeneous, free-flowing powder
Prepared flasks appearance	yellow, slightly opalescent
Freeze-dried selective supplements	low, dense, white tablets; colourless limpid solution after reconstitution
Final pH of complete media (at 20-25°C)	7.0 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Shigella Broth Base	Dehydrated medium	4020402	500 g (15.8 L)
Novobiocin Antimicrobic Supplement	Freeze-dried supplement	4240045	10 vials (10 mg/vial)

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, flasks, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS
Products intended for human consumption and the feeding of animals, and 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.[5,6]

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9 - TEST PROCEDURE

Enrichment of *Shigella* species according to ISO 21567[5] (or *S. sonnei* according to FDA-BAM[6])

The following method is a summary of the technique recommended by

1. In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 g or 225 mL of Shigella Broth containing 0.5 µg/mL of novobiocin, to obtain a tenfold dilution, and homogenize.
2. Incubate under anaerobic conditions with caps and closures loose, or with equipment giving an equivalent effect, so that gas exchange can readily occur without contamination, at 41.5 ± 1 °C (44 °C: FDA-BAM).
3. Transfer a loopful of growth on plates of Mac Conkey Agar REF 401670 (low selectivity), XLD Agar ISO Formulation REF 402208 (moderate selectivity), and Hektoen Enteric Agar REF 401541 (greatest selectivity). FDA-BAM: streak on a Mac Conkey Agar plate.
4. Incubate the plating-out media at 37 °C for 20 h to 24 h. If no typical colonies are seen and the growth of other microorganisms is weak (particularly on the more selective agar), re-incubate the plates for a further 24 h. Examine them again for typical *Shigella* colonies.

Enrichment of other *Shigella* species according to FDA-BAM.[6]

Proceed as above, but use novobiocin at 3.0 µg/mL and incubate anaerobically at 42 °C.

10 - READING AND INTERPRETATION

After incubation of Shigella Broth, growth is evident by the appearance of turbidity.

After subculture on the plating out media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Consult ISO 21567[5], FDA-BAM[6] and the instructions for use of the plated media for a description of *Shigella* colonies on the different selective agars used.

Perform the biochemical and serological confirmation tests on the typical or suspect colonies as reported in the International Standards.[5,6]

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.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Shigella boydii ATCC 9207	41,5°C ± 1°C / 18h ± 2h / AN	Good growth
Shigella flexneri ATCC 12022	41,5°C ± 1°C / 18h ± 2h / AN	Good growth
Shigella sonnei ATCC 9290	41,5°C ± 1°C / 18h ± 2h / AN	Good growth
Shigella dysenteriae ATCC 9721	41,5°C ± 1°C / 18h ± 2h / AN	Good growth
Staphylococcus aureus ATCC 25923	41,5°C ± 1°C / 18h ± 2h / AN	Inhibited

AN: anaerobic 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 Shigella Broth Base supplemented with 0.5 µg/mL of novobiocin (REF 4240045) 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 41.5°C for 24 hours in anaerobic conditions 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. boydii* ATCC 9207, *S. flexneri* ATCC 12022, *S. sonnei* ATCC 9290.. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

Selectivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of the *S.aureus* ATCC 25923 as non-target strain. After incubation the non-target strain is inhibited.

13 - LIMITATIONS OF THE METHOD

- *Shigella* species can form a minority proportion of the total microbial flora when contaminating a food sample or after enrichment. In these circumstances, the direct streaking of the enrichment broth onto one plate per selective agar may fail to allow the detection of *Shigella* colonies. It may therefore be appropriate in some circumstances (e.g., the investigation of foods implicated in illness) to consider the inoculation of either two 90 mm dishes or one large (140 mm) Petri dish to increase the possibility of detection.[5]
- The colonies of some *Enterobacteriaceae* strains are very similar in appearance to those of *Shigella*. Any typical or suspect colonies shall be confirmed as *Shigella* species or not. Also, in some circumstances (e.g., foods implicated in food poisoning), it may be appropriate to investigate more than five colonies from a plate to increase confidence in the absence of *Shigella* in the food sample tested.[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. Novobiocin Antimicrobic Supplement is classified as hazardous. 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 metal ring of the supplement to avoid injury.
- The supplement is sterilized by membrane filtration.
- 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 supplements and the sterilized medium inoculated 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

Dehydrated medium

Upon receipt, store at +10°/+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. 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 and the applied storage conditions (temperature and packaging). According to ISO 21567, Shigella Broth Base prepared in flasks and the remaining novobiocin solution may be stored at 2-8°C for up to 1 month.[5]

16 - REFERENCES

1. Bakera S, Chung H. Thea Recent insights into Shigella: a major contributor to the global diarrhoeal disease burden. Curr Opin Infect Dis. 2018 Oct; 31(5): 449–454.
2. Ryan, Kenneth James; Ray, C. George, eds. (2004). Sherris medical microbiology: an introduction to infectious diseases (4th ed.). McGraw-Hill Professional Med/Tech.
3. Bowen A. Chapter 3: Infectious Diseases Related to Travel". The Yellow Book: Health Information for International Travel. Retrieved 22 June 2016.
4. Mehlman IJ, Romero A, Wentz BA. Improved enrichment for recovery of Shigella sonnei from foods. J Assoc Off Anal Chem 1985 May-Jun; 68(3): 552-5.
5. ISO 21567:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 6: Shigella. Rev. February 2013.

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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	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

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