INSTRUCTIONS FOR USE

.

BISMUTH SULPHITE AGAR

Dehydrated culture medium

Bismuth Sulphite Agar:
colonies of *Salmonella* Enteritidis

1 - INTENDED USE

In vitro diagnostic. Highly selective medium for the isolation of micro-organisms belonging to the genus *Salmonella*, especially *Salmonella enterica* subsp. *enterica* serovar Typhi, from clinical and non-clinical specimens.

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

Beef Extract	5.000 g
Tryptone	5.000 g
Peptone	5.000 g
D-glucose	5.000 g
Disodium hydrogen phosphate	4.000 g
Bismuth sulphite indicator	8.000 g
Ferrous sulphate	0.300 g
Brilliant green	0.025 g
Agar	20.000 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

Bismuth Sulphite Agar is a modification of the original formulation devised by Wilson and Blair.[1,2] It is intended for the detection and isolation of *Salmonella enterica* serovar Typhi and other salmonellae from clinical specimens,[3-4] foods,[5,6] water[7] and other samples suspected of containing these pathogens. The medium has a strong inhibitory action and is suitable for heavily contaminated samples. The use of this medium may be particularly useful when lactose fermenting strains of salmonellae are sought.
The freshly precipitated bismuth sulphite in the medium by the heat action, is responsible of the inhibitory properties against coliform bacteria, in the presence of glucose and of a certain excess of sodium sulphite[2]. The selective action of the medium toward Gram-positive organisms is enhanced by the presence of brilliant green.[2] Ferrous sulphate is an indicator of hydrogen sulphide production. The reduction of bismuth sulphite, in the acidic environment created by the fermentation of glucose, results in the production of hydrogen sulphide which, on reacting with the iron salt, precipitates as iron sulphide. This reaction causes a black colony and a brown or black precipitate, while the reduction of bismuth ions to metallic bismuth produces a metallic sheen around the colonies. Blackening of the colony and the formation of the metallic sheen do not occur if the colonies are too small (in the area of the plate where growth is very compact) and if the medium becomes too acidic.[3] Peptones are a source of nitrogen, carbon, vitamins and minerals for bacterial growth; glucose is a source of energy; disodium hydrogen phosphate limits the excess acidity formed during colony development; agar is the solidifying agent.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 52 g in 1000 mL of cold purified water and mix thoroughly. Heat with frequent agitation until boiling and continue to boil for 30 to 60 seconds to dissolve the agar and obtain a uniform suspension (precipitate will not dissolve). Cool to 50-55°C, mix gently to disperse the precipitate evenly and pour into sterile Petri dishes (25 mL medium per plate). Allow the medium to solidify with the dish uncovered. Do not overheat, do not autoclave, do not re-dissolve the medium after preparation. Use the plates no later than 48 hours after preparation, storing them in the dark at room temperature and avoiding excessive drying.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale green, fine, homogeneous, free-flowing powder
Prepared plates appearance	pale green to pale straw, with a smooth cream-like opacity.
Final pH at 20-25 °C	7.7 ± 0.2

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Bismuth Sulphite Agar	Dehydrated medium	40121022	500 g (9.6 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Bismuth Sulphite Agar is intended for the bacteriological processing of clinical specimens such as faeces, rectal swabs, urines and non-clinical samples such as foods and water. The medium can be inoculated with faeces suspended in saline or other liquid transport medium or with the faecal sample enriched in an appropriate selective broth. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[8] For the preparation of non-clinical samples consult the appropriate references.[5,6,7]

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9 - TEST PROCEDURE

Clinical specimens

Inoculate the sample on the surface of the medium. Streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic condition at 35-37°C for 48 hours at 35-37°C. Examine after 24 hours for typical colonies. If plates show little or no growth after 24 hours, incubate an additional 18-24 hours.

Summary of method reported for foods by ISO 6579-1, Annex D

Non-selective pre-enrichment in Buffered Peptone Water, incubation at 34-38 °C for 18 hours.

Additional selective enrichment, in addition to MKTTn and RVS, in Selenite Cystine Broth with incubation at 37°C for 24 and 48h.

Sub-culture on Bismuth Sulphite Agar and XDL Agar plates, incubated at 37°C for 24-48 hours.

Observation of the plates at 24 and 48 hours to detect the presence of typical colonies for confirmation testing.

For a detailed discussion of the method please refer to the current standard.[5]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record each specific morphological and chromatic characteristic of the colonies.

The main characteristics of the colonies on Bismuth Sulphite Agar are given below.

Salmonella Typhi: "rabbit-eye" colonies, round, flat, black, at 18 hours surrounded by a brownish or black zone with or without metallic sheen; after 48 hours the colonies are uniformly black with a marked brown-black halo.

Salmonella Paratyphi A and other salmonellae: variable colony morphology after 18 hours: they may be black, green or clear and mucoid. Uniformly black colonies are observed after 48 hours, often with diffuse staining of the medium and a pronounced metallic sheen.

Shigella spp.: inhibited but some strains (*S.flexneri* and *S.sonnei*) may grow as brownish-green colonies.

Other organisms such as coliforms, *Serratia*, *Proteus*: usually inhibited, but occasional strains give green or brown opaque colonies without metallic sheen or staining of the surrounding medium.

Although *S*.Typhi can grow within 24 hours, the final reading of the colony characteristics must be made after 48 hours of incubation.

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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.Typhimurium ATCC 14028	35-37°C / 24-48 H/ A	brown/ grey/ black colonies, with or without metallic reflections at 24 hours
S.Enteritidis ATCC13076	35-37°C / 24-48 H/ A	brown/ green/ black colonies, with or without metallic reflections at 24 hours
E.coli ATCC 25922	35-37°C / 48 H/ A	partially inhibited, green and brown colonies without metallic sheen
E.faecalis ATCC 29212	35-37°C / 48 H/ A	growth inhibited

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 Bismuth Sulphite Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by semi-quantitative ecometric technique with the target strains *S.enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *S.enterica* subsp. *enterica* serovar Enteritidis ATCC 13076, *S.enterica* subsp. *arizonae* ATCC 13314, *S.enterica* subsp. *enterica* serovar Derby clinical isolated. After incubation at 35-37°C for 24-48 hours, the amount of growth on the plates and colonies' characteristics are evaluated and recorded: they shall be comparable in both batches and according to specifications.

The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *E.coli* ATCC 25922, *P.vulgaris* ATCC 9484, *E.aerogenes* ATCC 13048, *E.faecalis* ATCC 19433. *E.faecalis* is totally inhibited while the growth of the other non-target strains is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- Keep the bottle of powdered medium tightly closed in the dark as the powder tends to deteriorate rapidly when exposed to the atmosphere; deterioration is indicated by the formation of non-friable aggregates and a brownish colour of the powder; once dissolved in water the deteriorated powder forms a brown solution instead of pale green and loses its differential and selectivity characteristics.
- Prolonged heating of the medium during preparation decreases its selectivity.
- Bismuth Sulphite Agar in plates should not be stored for longer than 2 days. After 3 days of storage the medium changes to a green colour with reduction of selectivity, resulting in smaller number of *Salmonella* recovered. Preferably, medium should be used on day prepared and not stored.[3,5-7]
- It is imperative to streak for well isolated colonies; in heavy growth areas *S*.Typhi appears light green and hence would be interpreted as negative for *S*.Typhi growth.[3]
- Atypical colonies may develop if medium is heavy inoculated with organic materials; to prevent this, suspend faecal specimen in sterile saline, centrifuge and use supernatant for inoculation.[3]
- Colonies on Bismuth Sulphite Agar may be contaminated with other viable organisms, and isolated colonies should be subcultured to less selective medium (e.g., MacConkey agar).[3]
- Do not autoclave; heating for period longer than necessary just to dissolve ingredients destroys its selectivity.[3]
- Bismuth Sulphite Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms but should be used in conjunction with other less selective enteric agars (XLD Agar, Brilliant Green Agar, SS Agar, Hektoen Enteric Agar).
- 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.
- Dehydrated media must be handled with suitable protection. 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 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. Apply Good Manufacturing Practice in the preparation process of plated or tubed or bottled media.
- 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 medium should be prepared fresh, just prior to use or stored at room temperature for not more than 2 days. The user is responsible for the manufacturing and quality control processes.

16 - REFERENCES

1. Wilson JW, Blair EM. Use of a glucose bismuth sulphite iron medium for the isolation of B.typhosus and B.proteus.1927; J Hyg 26:374
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3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
5. ISO 6579:2017/ ISO ISO 6579:2017 Amd1:2020. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Content current as of: 03/18/2022
7. ISO 19250:2010 Water quality — Detection of Salmonella spp
8. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology . In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

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	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/04
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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