INSTRUCTIONS FOR USE

SS AGAR

Dehydrated culture medium

SS Agar:
Salmonella arizonae colonies with large black centre

1- INTENDED USE

In vitro diagnostic. Selective and differential medium for the isolation of Gram-negative enteric pathogens, especially *Salmonella* from clinical specimens.

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

Beef extract	5.000 g
Peptocomplex	5.000 g
Lactose	10.000 g
Bile salts n°3	8.500 g
Sodium thiosulphate	8.500 g
Sodium citrate	8.500 g
Ferric citrate	1.000 g
Neutral red	0.025 g
Agar	13.500 g
Brilliant green	0.330 mg

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In the first half of the twentieth century, several culture media were developed and proposed for the isolation of enteric pathogens from faeces and other materials. SS (Salmonella Shigella) Agar is a modification of desoxycholate medium described by Leifson[1] in 1935, and successfully tested by Catherine Mayfield and Maud Gober[2] in 1941 for the isolation of *Shigella dysenteriae* and *Salmonella* from stool. Several years later, this medium was discovered to be overly selective and some strains of *Shigella* were missed.[3,4] For the isolation of *Shigella* the recommended plating media are Hektoen Enteric Agar or XLD Agar.[5]

SS Agar is a selective and differential medium intended for the isolation of Gram-negative enteric pathogens, especially *Salmonella* from clinical specimens.[5,6]

Peptones provide carbon, nitrogen and trace elements for bacterial growth; the high concentration of bile salts n° 3, sodium citrate and brilliant green inhibit Gram-positive organisms and most of the non-pathogenic coliform flora of the intestinal tract. Since the enteric pathogen *Salmonella* can tolerate these inhibitory substances, they generally grow faster and larger than the coliforms. Lactose is fermented by coliforms, that are able to grow in the presence of the bile salts, with production of acids. The acid condition causes the neutral red indicator to change to a pink-red colour and to bile salts to precipitate over the medium appearing as a hazy zone around the colonies. Ferric citrate is as an indicator of the formation of hydrogen sulphide. *Salmonella* spp. produce thiosulphate reductase that causes the release of a sulphide molecule from the sodium thiosulfate present in the medium. This sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric ammonium citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 60 g in 1000 mL of cold purified water. Heat to boiling stirring constantly. Do not overheat, do not autoclave. Cool to 47-50°C and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-orange, limpid or slightly opalescent
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
SS Agar	Dehydrated medium	4020752	500 g (8,3 L)
		4020754	5 kg (83 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

SS Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swabs[5,6] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[7]

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. 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.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment step in Selenite Broth, followed by sub-culture to SS Agar and to a second less selective plating medium.[5,7]
Incubate inoculated SS Agar plates with the specimen or with specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Do not examine areas of confluent growth as false negative fermentation reactions may occur.
Smooth, opaque colourless colonies with black centres: no fermentation present, H_2S production present: suspect *Salmonella*.
Smooth, opaque colourless colonies without black centre: no fermentation present, H_2S production absent: suspect H_2S negative *Salmonella* or *Shigella* strains that have by-passed the selective system of the medium.
Pink-red colonies: fermentation of lactose: not likely to be *Salmonella*
E.coli grows slightly with red colonies, with intercolonial precipitate, *E.aerogenes* may appear as large, mucoid, opaque pink to cream coloured colonies.
Since H_2S positive *Proteus* spp. may grow with colourless colonies with black or gray-black centre and if *Proteus* colonies are mixed with H_2S positive *Salmonella* colonies, it could be difficult to choose the colonies for further biochemical and serological identification.
It is advised to screen the colonies by flooding the plate with one drop of MUCAP Test reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C_8 esterase enzyme, typical of *Salmonella* spp.[8]

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	35-37°C / 18-24h / A	growth, colourless colonies with black centre
S.flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, colourless colonies
E.faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E.coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, red colonies

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 SS 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, by incubating at 35-37°C for 18-24 hours, with 7 target strains: S.Enteritidis ATCC 13076, S.Typhimurum ATCC 14028, S.Gallinarum, clinical isolate, *S.arizonae*, clinical isolate, *S.flexneri* ATCC 12022, *S.sonnei* ATCC 9290, *S.boydii* ATCC 9207. *Salmonella* colonies are colourless with black centre, *Shigella* colonies are colourless; the amount of growth on the plates is evaluated and shall be comparable in both batches.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of the non-target Gram-positive strain *E.faecalis* ATCC 29212 and with decimal dilutions in saline from 10^{-1} to 10^{-6} of 6 non-target Gram-negative strains: *P.mirabilis* ATCC 10005, *P.vulgaris* ATCC9484, *E.coli* ATCC 25922, *K.pneumoniae* ATCC 27736, *C.freundii* ATCC 8090. The growth of non-target strain *E.faecalis* is inhibited at the dilution 10^{-1} the growth of Gram-negative non-target strains are partially inhibited and the colonies show typical chromatic characteristics, according to the specifications.

13 - LIMITATIONS OF THE METHOD

- Be aware that *Proteus* spp. may or may not be inhibited and colonies may resemble *Salmonella*.[6] Rapid differentiation between very similar colonies may be performed with MUCAP Test.[8]
- Some lactose fermenting *Shigella* and *Salmonella* strains may resemble coliforms and are not recognized on SS Agar.
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, for the isolation of *Salmonella,* additional media with lower selectivity, such as Mac Conkey Agar, should be used. For the isolation of *Shigella* spp. the recommended media are Hektoen Enteric Agar or XLD Agar and a second medium with lower selectivity such as Mac Conkey Agar. Other media for the isolation of other enteric pathogens should be inoculated with the specimen.[5]
- Over time and during the shelf-life, bile salts in SS Agar plates may crystallize and form a precipitate in the medium. This does not affect the performance of the medium.
- 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 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 preparation process of plated 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 +10°C /+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). The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type (plates/bottles), and the storage method applied (temperature and packaging).

16 - REFERENCES

1. Leifson, E. 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. 40:581–599.
2. Mayfield, C. R., and M. Gober. 1941. Comparative efficiency of plating media for the isolation of Shigella dysenteriae. Am. J. Public Health 31:363–368.
3. King, S., and W. I. Metzger. 1968. A new plating medium for the isolation of enteric pathogens: II. Comparison of Hektoen Enteric Agar with S S and E M B Agar. Appl. Microbiol. 16: 579–581.
4. Taylor, W. I. 1965. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. Am. J. Clin. Pathol. 44:471–475.
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. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
8. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526.
9. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

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	2020/05
Revision 2	Modification of “precautions and warnings”, “storage conditions and shelf life”.	2022/01
Revision 3	Removal of obsolete classification	2023/04

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it