



SS AGAR

Ready-to-use plates



SS Agar:

Salmonella arizonae colonies with large black centre

1- INTENDED USE

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

2 - COMPOSITION -TYPICAL FORMULA *

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
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

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 deoxycholate medium described by Leifson¹ in 1935, and successfully tested by Catherine Mayfield and Maud Gober² in 1941 for the isolation of *Shigella dysenteriae* and *Salmonella* from stools. 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, it generally grows faster and larger than coliforms. Lactose is fermented by coliforms, that are able to grow in the presence of 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, 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 sulfide molecule from the sodium thiosulfate present in the medium. This sulfide molecule couples with a hydrogen ion to form H₂S gas that reacts with the ferric ammonium citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red-orange, limpid or slightly opalescent
Final pH at 20-25 °C	7.0 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
SS Agar	Ready-to-use plates	542075	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

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

7 - SPECIMENS

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

8 - 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 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.

Maximal recovery of *Salmonella* from faecal specimens is obtained by using the enrichment step in Selenite Broth, followed by subculture 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.





9 - 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, H₂S production present: suspect *Salmonella*.

Smooth, opaque colourless colonies without black centre: no fermentation present, H₂S production absent: suspect H₂S 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 H₂S positive *Proteus* spp. may grow with colourless colonies with black or grey-black centre and if *Proteus* colonies are mixed with H₂S 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 observe after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of C₈ esterase enzyme, typical of *Salmonella* spp.⁸

10 - 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
<i>S. Typhimurium</i>	ATCC	14028	35-37°C / 18-24h / A	growth, colourless colonies with black centre
<i>S. flexneri</i>	ATCC	12022	35-37°C / 18-24h / A	growth, colourless colonies
<i>E. faecalis</i>	ATCC	29212	35-37°C / 18-24h / A	inhibited
<i>E. coli</i>	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, red colonies

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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready to use plates of SS Agar and of the raw material used for the production of prepared plates (dehydrated SS Agar REF 402075) are 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* NCTC 5188, *S. Typhimurium* ATCC 14028, *S. Gallinarum*, clinical isolate, *S. arizonae* ATCC 13314, *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* ATCC 9484, *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.

Accuracy was assessed by reviewing the Quality Control data. The results of 25 batches produced from 9/1/2019 to 05/5/2020 were evaluated. 100% of the batches showed conformity to defined acceptance criteria in terms of productivity and differential properties with target strains and selectivity with non-target strains.

12 - 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 the 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 must 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.
- Growth on the medium depends on the metabolic requirements of each microorganism and on the resistance to the antimicrobials present; some target strains may not be able to grow or may show a delayed growth. A lack of growth or the absence of typical colonies does not preclude the presence of enteric pathogens in the sample.
- Appropriate tests are required for complete identification and epidemiological typing of colonies; if necessary, perform antimicrobial susceptibility tests using recommended methods.
- The device is not intended to diagnose gastro-intestinal infections or to guide the antimicrobial therapy. It is used in a diagnostic set of investigations to provide microbial colonies isolated from clinical samples of patients with suspected *Salmonella* infection.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic device intended for professional use only, is not automated and is not a companion diagnostic tool. It must be used by adequately trained and qualified laboratory personnel, observing 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. Therefore, it is recommended that the ready-to use plates 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 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.





- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal and dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains, in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify the Manufacturer (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- The Manufacturer shall not be held responsible for any loss or damage arising from or related to the use of the product in a manner not in accordance with the instructions provided.















14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - 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

 Catalogue number	 Batch code	 <i>In vitro</i> diagnostic medical device	 Manufacturer	 This way up	 For single use only	 European conformity mark
 Temperature limitations	 Contents sufficient for <n> tests	 Consult electronic instructions for use	 Use by	 Keep away from sunlight	 Fragile, handle with care	 Unique device identifier

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First emission in compliance with IVDR 2017/746	2020/05
Revision 1	Removal of obsolete classification	2023/03
Revision 2	Specimens, performances characteristics, limitations of the method, precautions and warnings, table of applicable symbols.	2026/01

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

