

INSTRUCTIONS FOR USE
ChromArt

CHROMOGENIC SALMONELLA AGAR BASE

SALMONELLA SELECTIVE SUPPLEMENT

Dehydrated culture medium and supplements



CSA . At left: *Salmonella* sp. colonies (magenta-red); at right *E.aerogenes* colonies (blue-green)

1-INTENDED USE

In vitro diagnostic. Selective and chromogenic medium base, used with selective supplements, for the isolation and differentiation of *Salmonella* spp., from clinical and non-clinical specimens.

2 - COMPOSITIONS
CHROMOGENIC SALMONELLA AGAR BASE
Typical formula after reconstitution with 1 L of water*

| | |
|---------------------|--------|
| Peptones | 10.0 g |
| Selective compounds | 12.0 g |
| Chromogenic mixture | 0.9 g |
| Agar | 15.0 g |

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

SALMONELLA SELECTIVE SUPPLEMENTS
Salmonella Selective Supplement Vial A
Vial contents for 500 ml of medium

| | |
|--------------------|--------|
| Emulsifying agents | 5.7 mL |
|--------------------|--------|

Salmonella Selective Supplement Vial B
Vial contents for 500 ml of medium

| | |
|------------|--------|
| Cefsulodin | 2.5 mg |
|------------|--------|

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Salmonella spp. remain one of the most important causes of foodborne gastroenteritis. Fluorogenic and chromogenic tests and media designed for the specific detection of *Salmonella* spp. have been available for at least 30 years. In 1987 a rapid fluorogenic screening reagent (MUCAP Test) for the identification of *Salmonella* colonies has been developed and proposed by Biolife Italiana, based on the detection of a specific *Salmonella* enzyme, C_{8} esterase, by using a fluorogenic 4-methylumbelliferone-conjugated substrate.¹ Some years later, the same principle of detection of C_{8} esterase enzyme has been exploited for the development of a chromogenic plating medium, Chromogenic Salmonella Agar, that demonstrated very high specificity and sensitivity for the detection of *Salmonella* spp.^{2,3} Chromogenic Salmonella Agar Base with Salmonella Selective Supplements is a selective and diagnostic medium, with a clear background, useful for the isolation of *Salmonella* spp. from clinical and non-clinical specimens and for the presumptive identification of the colonies. Chromogenic Salmonella Agar is included by ISTISAN Report⁴ in the plating media range for the detection of *Salmonella* spp. and chromogenic media are included as the second plating medium in ISO Standards for detection of *Salmonella* in food and water.^{5,6}

Peptones provide carbon, nitrogen, vitamins and trace elements for bacterial growth. The selective compounds incorporated in the medium are the following: cefsulodin, a third generation cephalosporin antibiotic that has very specific activity against *P. aeruginosa* and *S.aureus*, sodium desoxycholate that suppresses the growth of Gram-positive and some Gram-negative bacteria and Tergitol 4, active mainly against the growth of *Proteus* spp.

Differentiation of *Salmonella* from other organisms that grow is achieved by:

- a chromogenic substrate for C_{8} esterase enzyme, that is cleaved by *Salmonella* spp. with the release of an insoluble magenta-red chromophore.
- a chromogenic glucopyranoside derivative which is cleaved by β -glucosidase with the release of an insoluble blue-green chromophore. Some *Enterobacteriaceae*, including *Klebsiella* and *Enterobacter*, but not *Salmonella*, are β -glucosidase positive and if growing will form blue-green or dark blue colonies, even if they are esterase positive, which make them easy to differentiate from magenta-red *Salmonella* colonies. The chromogenic and selective compounds of the medium also allow the detection of the rare lactose-positive *Salmonella* strains, missed on traditional media based on lactose fermentation. Chromogenic Salmonella Agar is useful also for the detection of *S.Typhi* and *S.Paratyphi*. Because in the formulation is omitted the opaque compound, the prepared plates have a transparent background.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 19 g in 500 mL of cold purified water; add the content of one vial of Salmonella Selective Supplement Vial A, heat to boiling stirring constantly and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of Salmonella Selective Supplement Vial B, reconstituted with 2 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS
CHROMOGENIC SALMONELLA AGAR BASE

| | |
|---|---|
| Dehydrated medium appearance | beige, fine, homogeneous, free-flowing powder |
| Solution and prepared medium appearance | light yellowish, limpid |
| Final pH at 20-25 °C | 7.2 ± 0.2 |

SALMONELLA SELECTIVE SUPPLEMENT VIAL A

| | |
|------------------------|---------------------------------|
| Appearance of solution | pale yellow slightly opalescent |
|------------------------|---------------------------------|



**SALMONELLA SELECTIVE SUPPLEMENT VIAL B**

Appearance of lyophilised pellet

short dense pastille

Appearance of solution

colourless limp

6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|----------------------------------|----------------------|---------|---|
| Chromogenic Salmonella Agar Base | Dehydrated medium | 4053502 | 500 g (13,2 L) |
| Chromogenic Salmonella Agar Base | Dehydrated medium | 4053504 | 5 kg (132 L) |
| Salmonella Selective Supplement | Selective supplement | 4240013 | 5 vials A+ 5 vials B, each for 500 mL of medium |

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, 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

Chromogenic Salmonella Agar Base added with Salmonella Selective Supplements is intended for the bacteriological processing of clinical specimens such as faeces and rectal swabs. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.⁷ Consult appropriate standard methods for details of collection and preparation of non-clinical specimens.^{5,6}

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.

Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment step in Selenite Broth followed by sub-culture to Chromogenic Salmonella Agar and to a second plating medium.

Incubate inoculated plates with the specimen or with specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.

Consult appropriate references for the detection of *Salmonella* in food and water.^{5,6}

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Interpretation of colonies' colours:

| Microorganism | Growth characteristics |
|-----------------------------|---|
| <i>Salmonella</i> spp. | good growth, magenta-red colonies |
| <i>Salmonella</i> spp. lac+ | good growth, magenta-red colonies |
| <i>Salmonella</i> Typhi | good growth, magenta-red colonies |
| <i>E.coli</i> | poor growth with colourless colonies |
| <i>Enterobacter</i> spp. | growth with blue-green colonies |
| <i>Klebsiella</i> spp. | poor growth with blue-green colonies |
| <i>Pseudomonas</i> spp. | inhibited |
| <i>Proteus</i> spp. | poor growth with pale brown or green colonies |
| Gram-positive bacteria | inhibited |

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 / 18-24h / A | growth, magenta-red colonies |
| S.Enteritidis ATCC 13076 | 35-37°C / 18-24h / A | growth, magenta-red colonies |
| <i>E.aerogenes</i> ATCC 13048 | 35-37°C / 18-24h / A | growth, blue-green colonies |
| <i>P.aeruginosa</i> ATCC 27853 | 35-37°C / 18-24h / A | inhibited |

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

12 - PERFORMANCES CHARACTERISTICS

Chromogenic Salmonella Agar was evaluated by Babic-Ergeg et al.² on 3,000 stool specimens, 45 of which positive for *Salmonella*, including SS Agar as the reference medium. The authors reported a sensitivity of 100% and a specificity of 99% in the isolation and preliminary identification of *Salmonella* colonies.

In another independent study³, 50 pure cultures of *Salmonella* of clinical origin, gave all the specific chromatic reactions; among the other 80 strains of Gram-negative bacteria tested, not belonging to the *Salmonella* genus, 3 out of 3 strains of *P.aeruginosa* and 1 out of 3 strain of *A.baumannii* provided chromatic results similar to *Salmonella* spp. (red-pink colonies), the remaining 76 strains of *Enterobacteriaceae* gave non-typical chromatic reactions; 20 out of 20 strains of Gram-positive bacteria were totally inhibited.

Chromogenic Salmonella Agar performance was evaluated with an in-house study, compared to Hektoen Enteric Agar (HEA). Productivity, selectivity and specificity have been evaluated by semi-quantitative ecometric technique, incubating at 35-37°C for 18-24 hours, using 43 bacterial strains: 8 target strains and 35 non target strains. 8 *Salmonella* strains, including 2 *S.Typhi*, showed a good





growth with magenta-red colonies; 3 *Shigella* strains showed a poorer growth than on HEA with colourless colonies; 22 *Enterobacteriaceae* strains belonging to 9 genera showed a poorer growth than on HEA with colourless or blue-green colonies; 4 *P.aeruginosa* strains were totally inhibited; 2 non-fermenters strains were totally inhibited and *A.hydrophila* grew with magenta red colonies; 1 Gram-positive strain was totally inhibited and 1 yeast strain was partially inhibited showing colourless colonies.

Prior to release for sale a representative sample of all lots of dehydrated Chromogenic Salmonella Agar supplemented with Salmonella Selective Supplement 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.Enteritidis* ATCC 13076 and *S.Typhimurium* ATCC 14028. After incubation at 35-37°C for 18-24 hours, colonies' colour and the amount of growth is evaluated and recorded.

Specificity is tested by semi-quantitative ecometric technique with the non-target strain *E. aeruginosa* ATCC 13048 which, after incubation at 35-37°C for 18-24 hours, grows with green -blue colonies.

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10⁻¹ to 10⁻⁶ of a 0.5 McFarland suspension of the non-target strains *E.faecalis* ATCC 19433, *E.coli* ATCC 8739, *P.vulgaris* ATCC 13315, *A.calcoaceticus* ATCC 19606, *P.aeruginosa* ATCC 27853, *A.hydrophila* ATCC 7966. The growth of non-target strains *E.faecalis*, *P.vulgaris*, *P.aeruginosa*, *A.calcoaceticus*, is inhibited at the dilution 10⁻¹, the growth of *E.coli* and *A.hydrophila* is partially inhibited.

According to the specifications, the non-target strains colonies show typical blue-green colour or are colourless.

Only *A.hydrophila* grows very slightly with magenta-red colonies.

13 - LIMITATIONS OF THE METHOD

- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella*, with lower selectivity such as Mac Conkey Agar should be used; other media for the isolation of other enteric pathogens should be inoculated with the specimen.
- Some strains of *Pseudomonas*, *Acinetobacter* and *Aeromonas*, resistant to antimicrobial agents of the medium, may grow with red-pink colonies, differentiable from *Salmonella* with oxidase test.
- The growth rate on the plates also depends on the nutritional requirements of *Salmonella*. It is possible that some strains with particular metabolic characteristics may not grow on the medium or grow colourless (e.g., *Salmonella enterica* serovar Dublin grows with white colonies).
- 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

- The medium base and the supplement are qualitative *in vitro* diagnostics, 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 must be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Material Safety Data Sheets.
- The 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.
- The freeze-dried supplement undergoes membrane sterilisation, the liquid supplement undergoes autoclave sterilisation.
- Be careful when opening the metal ring of the vials to avoid injury.
- 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, supplements and the sterilized plates 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 material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the products 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

Dehydrated medium

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).

Salmonella Selective Supplement Vial A

Upon receipt, store the product in the original package at +2°C /+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. Once the vial has been opened the product should be used immediately. During storage some needles or crystals can be formed. They dissolve with slight agitation after returning the reagent to room temperature. This characteristic does not affect the performance of the product.



**Salmonella Selective Supplement Vial B**

Upon receipt, store the product in the original package at +2°C /+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. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. 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 for the validation of their shelf life, according to the type (plates/bottles), and the storage method applied (temperature and packaging).

16 - REFERENCES

1. Pontello M, Russolo S, Carozzi F, Bottiroli U. Evaluation of a new rapid method (MUCAP Test) for the presumptive identification of Salmonella on primary isolation media. 5th Int. Simp. on Rapid Method and Aut. in Microb. and Immunol. Florence 4-6 nov. 1987
2. Babic-Erceg A et al. 12th European Congress of Clinical Microbiology and Infectious Diseases. Milan, April 24-27, 2002
3. Andreoni S. et al. Microbiologia Medica, 2002.
4. Istituto Superiore di Sanità Le infezioni da Salmonella: diagnostica, epidemiologia e sorveglianza. Caterina Graziani, Pasquale Galetta, Luca Busani, Anna Maria Dionisi, Emma Filetici, Antonia Ricci, Alfredo Caprioli, Ida Luzzi 2005, 49 p. Rapporti ISTISAN 05/27
5. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella
6. ISO19250:2010 Water quality — Determination of Salmonella species
7. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carroll KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.

4240013A SALMONELLA SELECTIVE SUPPLEMENT VIAL A

SDS rev 2

Regulation (EU) 2020/878

Contains: Sodium tetradecyl sulfate**Classification**

Skin corrosion, category 1B H314 Causes severe skin burns and eye damage.
Serious eye damage, category 1 H318 Causes serious eye damage.

Labelling

Hazard pictograms:

**Signal words Danger**

Hazard statements:

H314 Causes severe skin burns and eye damage.

Precautionary statements:

P260 Do not breathe dust / fume / gas / mist / vapours / spray.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].

P280 Wear protective gloves/ protective clothing / eye protection / face protection.

P310 Immediately call a POISON CENTER / doctor / . . .

P264 Wash . . . thoroughly after handling.

4240013B SALMONELLA SELECTIVE SUPPLEMENT VIAL B

SDS rev 2

Regulation (EU) 2020/878

Contains : cefsulodin**Classification**

Respiratory sensitization, category 1 H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Skin sensitization, category 1 H317 May cause an allergic skin reaction.

Labelling

Hazard pictograms:

**Signal words Danger**

Hazard statements:

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.





H317 May cause an allergic skin reaction.
 Precautionary statements:
 P261 Avoid breathing dust / fume / gas / mist / vapours / spray.
 P280 Wear protective gloves.
 P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER / doctor / . . .
 P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
 P333+P313 If skin irritation or rash occurs: Get medical advice / attention.
 P362+P364 Take off contaminated clothing and wash it before reuse.

TABLE OF APPLICABLE SYMBOLS

| | | | | | |
|----------------------------|----------------------------------|---|--------------|--------------|-----------------------------|
| or REF Catalogue number | Batch code | <i>In vitro</i> Diagnostic Medical Device | Manufacturer | This side up | Store in a dry place |
| Temperature limitation | Content sufficient for <n> tests | Consult Instructions for Use | Use by | Fragile | Keep away from direct light |

REVISION HISTORY

| Version | Description of changes | Date |
|------------|---|---------|
| Revision 1 | Updated layout and content | 2020/05 |
| Revision 2 | Modifications of "precautions and warnings, "storage conditions and shelf life"., | 2022/01 |
| Revision 3 | Inclusion of SDS summary. | 2022/03 |
| Revision 4 | Removal of obsolete classification | 2023/04 |

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

