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 Report[4] 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 ß-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

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SALMONELLA SELECTIVE SUPPLEMENT VIAL B

Appearance of lyophilised pellet	short dense pastille
Appearance of solution	colourless limpid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Salmonella Agar Base	Dehydrated medium	4053502	500 g (13,2 L) CND: W0104010101; EDMA:14.01.01.01: RDM:1858078/R
Salmonella Selective Supplement	Selective supplement	4240013	5 + 5 vials, each for 500 mL of medium CND :W01040101045; EDMA: 14.01.01.04 ; RDM:1892762/R

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
Salmonella spp.	good growth, magenta-red colonies
Salmonella spp. lac+	good growth, magenta-red colonies
Salmonella Typhi	good growth, magenta-red colonies
E.coli	poor growth with colourless colonies
Enterobacter spp.	growth with blue-green colonies
Klebsiella spp.	poor growth with blue-green colonies
Pseudomonas spp.	inhibited
Proteus 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 it is responsibility of the end-user to perform Quality Control testing 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
E.aerogenes ATCC 13048	35-37°C / 18-24h / A	growth, blue-green colonies
P.aeruginosa 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 study[3], 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.
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.aerogenes* ATCC 13048, *E.coli* ATCC 8739, *P.vulgaris* ATCC 13315, *A.calcoaceticus* ATCC 19606, *P.aeruginosa* ATCC 27853, *A.hydrophila* ATCC 7965. The growth of non-target strains *E.faecalis, P.vulgaris, P.aeruginosa, A.calcoaceticus, A.hydrophila* is inhibited at the dilution 10^{-1} , the growth of *E.aerogenes* is very slightly inhibited, the growth of *E.coli* is partially inhibited. According to the specifications, the non-target strains colonies show typical blue-green colour or are colourless.

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.
- 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.
- Apply Good Manufacturing Practice in the preparation process of plated or bottled media.
- The selective supplement is sterilized by membrane filtration.
- 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.
- 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. 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/tubes/bottles), and the storage method applied (temperature and packaging).
Selective 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. 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 the period of validity of the finished products, according to the type (plates/tubes/bottles), and the storage method applied (temperature and packaging).

16 - REFERENCES

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6. ISO19250:2010 Water quality — Determination of Salmonella species
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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* 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

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

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