BIOSECTOR
CHROMOGENIC SALMONELLA AGAR II
XLD AGAR

Ready-to-use bi-plates

1-INTENDED USE

Selective and differential media for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella*.

2 - COMPOSITIONS -TYPICAL FORMULAS

CHROMOGENIC SALMONELLA AGAR II*

Peptones	10.0 g
Selective compounds, organic and inorganic salt	12.0 g
Chromogenic mix	0.9 g
Emulsifying agents	10.0 g
Opacifier	10.0 g
Antibiotic mix (cefsulodin, novobiocin, linezolid)	17.0 mg
Agar	15.0 g
Purified water	1000 mL

XLD AGAR*

Xylose	3.50 g
L-lysine	5.00 g
Lactose	7.50 g
Sucrose	7.50 g
Sodium chloride	5.00 g
Yeast extract	3.00 g
Sodium desoxycholate	2.50 g
Sodium thiosulphate	6.80 g
Ferric ammonium citrate	0.80 g
Phenol red	0.08 g
Agar	13.50 g
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

The bi-plate with Chromogenic Salmonella Agar II and XLD Agar culture media is indicated in industrial microbiology when determination in *Salmonella* spp. (including S.Typhi and *Salmonella* lac + strains) and *Shigella* spp is required.

CHROMOGENIC SALMONELLA AGAR II

is an evolution of Chromogenic Salmonella Agar, designed to improve its selective properties.

Chromogenic Salmonella Agar II is a selective and differential medium, suitable for the isolation of *Salmonella* spp. and for the presumptive identification of colonies. Chromogenic media are included as a second culture medium in ISO standards for the 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 as follows: cefsulodin, a third-generation cephalosporin antibiotic with highly specific activity against *P. aeruginosa* and *S. aureus;* novobiocin, linezolid and sodium deoxycholate, which inhibit the growth of Gram-positive and some Gram-negative bacteria. The contents of vial A are used to emulsify the ingredients of the culture medium.

Differentiation of *Salmonella* from other growing organisms is achieved by means of:

- a chromogenic substrate for the C8 esterase enzyme, which 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, form blue-green or dark blue colonies, even though they are esterase-positive, making them easy to differentiate from the magenta-red Salmonella colonies. The chromogenic and selective compounds in the medium also allow the detection of rare lactose-positive *Salmonella* strains, which are not detected on traditional media based on lactose fermentation. Chromogenic Salmonella Agar II is also useful for the detection of S. Typhi and S. Paratyphi. Ready-to-use plates appear uniformly opaque, which improves the visual differentiation of colony colours.

XLD AGAR

XLD Agar is a selective and differential medium intended for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella* from clinical specimens.[4-6] It is recommended for the detection of *Salmonella* in non sterile pharmaceutical products according to harmonized EP, USP, JP method[7] and by FDA-BAM for detection of *Salmonella* in food[8].

Yeast extract provides carbon, nitrogen, vitamins and trace elements for bacterial growth; sodium chloride maintains the osmotic balance in the medium; sodium desoxycholate is a selective agent for suppressing the growth of Gram positive bacteria. XLD Agar contains three indicator systems: xylose, lactose, and sucrose combined with phenol red, lysine hydrochloride and again phenol red, sodium thiosulfate and ferric ammonium citrate. Target bacteria are tentatively grouped by reading the effect of carbohydrate fermentation, lysine decarboxylation and formation of hydrogen sulphide.

4 - PHYSICAL CHARACTERISTICS

CHROMOGENIC SALMONELLA AGAR II

Medium appearance	yellowish opaque
Final pH at 20-25 °C	7.2 ± 0.2

XLD AGAR

Medium appearance	red, limpid
Final pH at 20-25 °C	7.4 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Biosector Chromogenic Salmonella Agar II / XLD Agar	Ready-to-use bi-plates	495351N	2 x 10 plates ø 90 mm with 2 sectors 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

Biosector Chromogenic Salmonella Agar II / XLD Agar is intended for the bacteriological processing of non clinical specimens such as non-sterile pharmaceutical products[7] and food[2,3,8]. Good laboratory practices for collection, transport and storage of specimens should be applied.[9] Consult appropriate standard methods for details of collection and preparation of specimens.[2,3,7,8]

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 media surfaces 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 inoculated bi-plates in aerobic conditions at 35-37°C for 18-24 hours.
Consult appropriate references for the detection of *Shigella* and *Salmonella* in non clinical specimens. [2,3,7,8]

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
The different microorganisms grow on Chromogenic Salmonella Agar II with the following characteristics:

Microorganism	Growth characteristics
Salmonella spp.	good growth, magenta-red colonies
Salmonella spp. lac+	good growth, magenta-red / violet colonies
Salmonella Typhi	good growth, magenta-red colonies
E.coli	poor growth with colourless colonies
Enterobacter spp.	growth with blue-green colonies or inhibited
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

Interpretation of colonies' colours on XLD Agar[10]:
Red colonies: alkaline reaction, non-fermentation of xylose/sucrose/lactose, or fermentation of xylose followed by decarboxylation of lysine: possible *Shigella* or *Providencia* or *Pseudomonas* spp. or *Salmonella* sp. H_2S negative
Red colonies with black centre: xylose fermentation only, lysine positive, H_2S positive, rapid depletion of xylose and resultant alkalinity due to lysine decarboxylation, black centre due to H_2S production possible only in alkaline pH environment: suspect *Salmonella* H_2S positive.
Opaque yellow colonies: xylose fermentation, lysine negative and non fermentation of lactose and sucrose, acid pH: possible *E.coli*, *Klebsiella/Enterobacter, Citrobacter*, *Serratia, Proteus* spp.
Yellow colonies: lactose or sucrose fermentation, lysine negative, acid pH: possible coliforms or sucrose-positive *P.vulgaris*

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 his own 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
CHROMOGENIC SALMONELLA AGAR II		
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
K. pneumoniae ATCC 700603	35-37°C / 18-24h / A	growth, blue-green colonies
P. aeruginosa ATCC 27853	35-37°C / 18-24h / A	inhibited
XLD AGAR		
S.Typhimurium ATCC 14028	30-35 or 35-37°C / 18-24h / A	growth, red colonies with black centre
S.flexneri ATCC 12022	30-35 or 35-37°C / 18-24h / A	growth, red colonies
E.faecalis ATCC 29212	30-35 or 35-37°C / 18-24h / A	inhibited
E.coli ATCC 25922	30-35 or 35-37°C / 18-24h / A	partially inhibited, yellow colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection
Incubation temperature depends of the followed Standard (CLSI[13] or EuPh[7])

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of ready to use bi-plates of Chromogenic Salmonella Agar II / XLD Agar and of the raw materials used for the production of bi-plates (dehydrated Chromogenic Salmonella Agar II REF 405350N and XLD Agar, REF 402206) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

CHROMOGENIC SALMONELLA AGAR II

Productivity is tested by a quantitative test with 2 target strains: S. Enteritidis ATCC 13076, S. Typhimurium ATCC 14028; CSA II plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 35-37°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is ≥ 0.7 and if the colonies morphology and colour are typical (magenta-red colonies) the results are considered acceptable and conform to the specifications. Furthermore, the productivity characteristics are tested by semi-quantitative ecometric technique with the target strain *S. diarizonae* ATCC 19934 *(*lactose positive strain). After incubation, colonies' colour (light purple colonies) and the amount of growth is evaluated and recorded.

Specificity is tested by semi-quantitative ecometric technique with the non-target strain *K. pneumoniae* ATCC 700603 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 25922, *P. vulgaris* ATCC 13315, *A. calcoaceticus* ATCC 19606*, P. aeruginosa* ATCC 27853, *A. hydrophila* ATCC 7966, Mucor sp environmental isolate. The growth of non-target strains *E. faecalis, P. aeruginosa, A. calcoaceticus, A. hydrophila* and *Mucor* is inhibited at the dilution 10^{-1}, the growth of *E. coli* and *P. vulgaris* is partially inhibited.

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

XLD AGAR

Productivity is tested by a quantitative test with 2 target strains: S. Enteritidis ATCC 13076, S.Typhimurium ATCC 14028; XLD Agar plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 30-35°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is ≥ 0,7 and if the colonies morphology and colour are typical (red colonies with black centre) the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the target strain *S.flexneri* ATCC 12022. After incubation, 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^{-3} of a 0.5 McFarland suspension of the non-target strains *E.faecalis* ATCC 19433 and *E.coli* ATCC 25922. The growth of non-target strain *E.faecalis* is inhibited at the dilution 10^{-1}, the growth of Gram negative non-target strain is partially inhibited and the colonies show typical yellow colour, according to the specifications.

12 - LIMITATIONS OF THE METHOD

- Some strains of *Pseudomonas, Acinetobacter* and *Aeromonas,* resistant to antimicrobial agents of Chromogenic Salmonella Agar, 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 Chromogenic Salmonella Agar or grow colourless (e.g., *Salmonella enterica* serovar Dublin grows with white colonies).
- On XLD Agar non-enteric organisms such as *Pseudomonas* may grow; *Pseudomonas* and *Providencia rettgeri* may both exhibit red colonies. Some *Proteus* spp. may develop black centres. [10]
- On XLD Agar S.Parathyphi A, S.Cholerae-suis, S.Pullorum and S.Gallinarum may form red colonies without black centre, thus resembling *Shigella* spp. [10]
- Incubation exceeding 48 hours may lead to false positive results.[10]
- 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.

13 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved 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 bi-plate is for single use only.
- Ready-to-use bi-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. Dispose the unused medium and the 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at +2°C/ +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°C/ +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. 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

2. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella.
3. ISO19250:2010 Water quality — Determination of Salmonella species
4. Vandepitte J Verhaegen J Engbaek K Rohner P Piot P Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve:World Health Organization
5. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
6. 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.
7. European Pharmacopoeia, current edition.
8. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019
9. 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.
10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	Manufacturer	This side up	Keep away from direct light	Fragile, handle with care
Temperature limitation	Contents sufficient for <n>	Consult Instructions for Use	Use by	For single use only	

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First edition	2025/12
Revision 1	Update of quality control method	2026/02

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