



BIOSECTOR

CHROMOGENIC SALMONELLA AGAR II

HEKTOEN ENTERIC 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

HEKTOEN ENTERIC AGAR*

Tryptose	12.000 g
Yeast extract	3.000 g
Bile salts n° 3	9.000 g
Lactose	12.000 g
Sucrose	12.000 g
Salicin	2.000 g
Sodium chloride	5.000 g
Sodium thiosulphate	5.000 g
Fe-ammonium citrate	1.500 g
Bromothymol blue	0.065 g
Acid fuchsin	0.100 g
Agar	15.000 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 II Agar and Hektoen Enteric 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.

HEKTOEN ENTERIC AGAR

Hektoen Enteric Agar is a selective and differential medium intended for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella*.⁴ Hektoen Enteric Agar is recommended by ISO 21567⁵ as plating medium for the detection of *Shigella* and by FDA-BAM⁶ for detection of *Salmonella*, in food.

Animal peptone and yeast extract provide carbon, nitrogen, vitamins and trace elements for bacterial growth; the high concentration of bile salts n°3 and dyes inhibits Gram-positive organisms and most of the non-pathogenic coliform flora of the intestinal tract. Since the enteric pathogens *Salmonella* and *Shigella* can tolerate these inhibitory substances, they generally grow faster and larger than the coliforms.⁷ Lactose, sucrose and salicin are fermented by coliforms, that are able to grow in the presence of the bile salts, and by some *Proteus* species with production of acids. The acid condition causes the bromothymol blue indicator to change from its neutral green colour to an orange-yellow colour and to bile salts to precipitate appearing as a hazy zone around the colonies. Ferric ammonium citrate is an indicator of the formation of hydrogen sulphide. *Salmonella* spp. produce thiosulphate reductase that cause 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****CHROMOGENIC SALMONELLA AGAR II**

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

HEKTOEN ENTERIC AGAR

Medium appearance dark green, limpid or lightly opalescent
 Final pH at 20-25 °C 7.5 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Biosector Chromogenic Salmonella Agar II / Hektoen Enteric Agar	Ready-to-use bi-plates	495350N	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

Bisector Chromogenic Salmonella Agar II / Hektoen Enteric Agar is intended for the bacteriological processing of non clinical specimens such as food and animal feeding stuffs^{6,7}. Good laboratory practices for collection, transport and storage of specimens should be applied. Consult appropriate standard methods for details on food sample collection and preparation.^{2,3,5}

8 - TEST PROCEDURE

MEDIA RECOGNITION: Chromogenic Salmonella Agar II: opaque yellowish medium; Hektoen Enteric Agar: deep green medium. Maximum recovery of Salmonella is achieved using enrichment in a selective liquid medium followed by plating. For the isolation of Shigella, enrichment in GN Broth is recommended.

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.^{2,3,5}

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
<i>Salmonella</i> spp.	good growth, magenta-red colonies
<i>Salmonella</i> spp. lac+	good growth, magenta-red/purple 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 colonies
Gram-positive bacteria	inhibited

The different colour characteristics of isolates on Hektoen Enteric Agar may be interpreted as follows:⁷

Greenish-blue, light green, or transparent colonies with black centres: no fermentation present, H₂S production present: suspect *Salmonella*.

Greenish-blue, light green, or transparent colonies: no fermentation present, H₂S production absent: suspect *Shigella* or H₂S negative *Salmonella*.

Yellow colonies with an orange-yellow precipitate: fermentation of lactose, sucrose, or salicin: not likely to be *Salmonella* or *Shigella*.

Salmon to orange colonies: fermentation of salicin, H₂S production absent: not likely to be *Salmonella* or *Shigella*.

Yellow, salmon to orange colonies with black centre: fermentation of lactose or sucrose, or salicin, H₂S production present: not likely to be *Shigella* or *Salmonella* (other than rare lactose positive *Salmonella*).

Since some *Proteus* spp. may grow with greenish blue colonies with 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.

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



**HEKTOEN ENTERIC AGAR**

<i>S. Typhimurium</i>	ATCC 14028	35-37°C / 18-24h / A	growth, green colonies with black centre
<i>S. flexneri</i>	ATCC 12022	35-37°C / 18-24h / A	growth, light green 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, yellow to salmon colonies

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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of ready to use bi-plates of Chromogenic Salmonella Agar / Hektoen Enteric Agar and of the raw materials used for the production of bi-plates (dehydrated Chromogenic Salmonella Agar II REF 405350N and Hektoen Enteric Agar, REF 401541) 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.

HEKTOEN ENTERIC AGAR

Productivity is tested by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with 6 target strains: *S. Enteritidis* NCTC 5188, *S. Typhimurium* ATCC 14028, *S. Gallinarum* clinical isolate, *S. arizonae* clinical isolate, *S. flexneri* ATCC 12022 and *S. sonnei* ATCC 9290. *Salmonella* colonies are green with black centre, *Shigella* colonies are light green; 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 of a 0.5 McFarland suspension of the non-target Gram positive strain *E. faecalis* ATCC 29212 and 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 and *E. aerogenes* ATCC 13048. The growth of non-target strain *E. faecalis* is totally inhibited, the growth of Gram negative non target strains are partially inhibited and the colonies show typical chromatic characteristics, 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).
- Do not examine areas of confluent growth on Hektoen Enteric Agar as false negative fermentation reactions may occur.
- Be aware that carbohydrates non fermenting strains of *Proteus* spp. may or may not be inhibited on Hektoen Enteric Agar and colonies may resemble *Salmonella*.⁴ Rapid differentiation between very similar colonies may be performed on Chromogenic Salmonella Agar II.
- Some lactose fermenting *Shigella* and *Salmonella* strains may resemble coliforms and not recognized on Hektoen Enteric Agar.
- Do not incubate longer than 24 hours since a loss of yellow/salmon colour may occur on Hektoen Enteric Agar due to the utilisation of peptones for growth with the productions of alkaline end products.⁷
- Over time and during the shelf-life, bile salts in Hektoen Enteric Agar sector 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.

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. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. ISO 21567 :2004. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019.
7. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010.

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.

