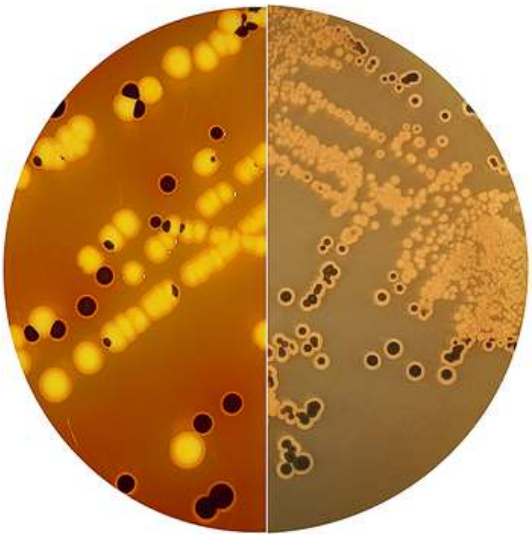
INSTRUCTIONS FOR USE

BIOSECTOR
XLD AGAR / SS AGAR

Ready-to-use bi-plates



Black *Salmonella* sp.colonies
on XLD Agar (left) and SS Agar (right)

1-INTENDED USE

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

2 - COMPOSITIONS -TYPICAL FORMULAS

XLD AGAR*

Xylose	3.50 g	Sodium desoxycholate	2.50 g
L-lysine	5.00 g	Sodium thiosulphate	6.80 g
Lactose	7.50 g	Ferric ammonium citrate	0.80 g
Sucrose	7.50 g	Phenol red	0.08 g
Sodium chloride	5.00 g	Agar	13.50 g
Yeast extract	3.00 g	Purified water	1000 mL

SS AGAR*

Beef extract	5.000 g	Ferric citrate	1.000 g
Peptocomplex	5.000 g	Neutral red	0.025 g
Lactose	10.000 g	Agar	13.500 g
Bile salts n°3	8.500 g	Brilliant green	0.330 mg
Sodium thiosulphate	8.500 g	Purified water	1000 mL
Sodium citrate	8.500 g		

*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 XLD Agar and SS Agar culture media is indicated in clinical and industrial microbiology when determination in *Salmonella* spp. and *Shigella* spp. is required.

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.[6-8] It is recommended for the detection of *Salmonella* in non sterile pharmaceutical products according to harmonized EP, USP, JP method[9] and by FDA-BAM for detection of *Salmonella* in food[10].

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.

SS AGAR

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 H_2S 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

XLD AGAR

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

SS AGAR

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
Biosector 32 XLD Agar/SS Agar	Ready-to-use bi-plates	491032	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.

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7 - SPECIMENS

Biosector XLD Agar/SS Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swab[3,6] and non clinical specimens such as food and animal feeding stuffs [6,7]. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[2] Consult appropriate standard methods for details on food sample collection and preparation.[4,5]

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.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment step in Selenite Broth.[3]
For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised.[3]
Incubate inoculated bi-plates with the specimen or with a specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours. Colonies on XLD agar may require 48 hours incubation for full colour and black precipitate development.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Interpretation of colonies' colours on XLD Agar: [6]
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*
Interpretation of colonies' colours on SS Agar: [6]
Smooth, opaque colourless colonies with black centres: no fermentation present, H_2S production present: suspect *Salmonella*.
Smooth, opaque colourless colonies without black centre: no fermentation present, H_2S production absent: suspect H_2S 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.

On both media, 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 C_8 esterase enzyme, typical of *Salmonella* spp.[7]

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, 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.[8]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
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
SS AGAR				
S.Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, colourless colonies with black centre
S.flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, colourless colonies
E.faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E.coli	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, representative samples of all lots of ready to use bi-plates of XLD Agar / SS Agar and of the raw materials used for the production of bi-plates (dehydrated XLD Agar REF 402206 and SS Agar, REF 40275) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
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.
SS AGAR

Productivity is tested by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with 7 target strains: *S*.Enteritidis ATCC 13076, *S*.Typhimurum ATCC 14028, *S*.Gallinarum, clinical isolate, *S.arizonae*, clinical isolate, *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.

12 - LIMITATIONS OF THE METHOD

- 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. [6]
- 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. [6]
- Incubation exceeding 48 hours may lead to false positive results. [6]
- Be aware that *Proteus* spp. may or may not be inhibited on SS Agar and colonies may resemble *Salmonella*.[6] Rapid differentiation between very similar colonies may be performed with the MUCAP Test.[7]
- Some lactose fermenting *Shigella* and *Salmonella* strains may resemble coliforms and are not recognized on SS Agar.
- 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.
- 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 disease; 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is 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 media contain 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 these products do not contain any transmissible pathogen. 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 by Biolife Italiana S.r.l. 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.
- 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.

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. 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
2. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
3. 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.
4. European Pharmacopoeia, current edition.
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. 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.
8. 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

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 0	First edition, in compliance with IVDR 2017/746	2021/09
Instructions for Use (IFU) - Revision 1	Removal of obsolete classifications	2023/03

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

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