XLT4 AGAR BASE
XLT4 SUPPLEMENT

Dehydrated culture medium and selective supplement

1 - INTENDED USE

XLT4 Agar Base is used with XLT4 Supplement for the preparation of XLT4 Agar, a highly selective medium for the detection of *Salmonella* spp. other than S.Typhi.

2- COMPOSITION

XLT4 AGAR BASE

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptone	1.60 g
Yeast extract	3.00 g
Xylose	3.75 g
Sucrose	7.50 g
Lactose	7.50 g
L-lysine	5.00 g
Sodium thiosulfate	6.80 g
Ferric ammonium citrate	0.80 g
Sodium chloride	5.00 g
Phenol red	0.08 g
Agar	17.00 g

XLT4 SUPPLEMENT (BOTTLE CONTENT)

Tergitol 4 (Niaproof 4/Sodium tetradecilsulfate)	100 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

In the first half of the twentieth century, several culture media were developed and proposed for the isolation of enteric pathogens from faeces and other materials. Some of them were moderately selective and allowed the growth of faecal contaminants, others showed excessive toxicity for the growth of pathogens.[1] In 1990, xylose-lysine-tergitol 4 (XLT4) agar was developed by Miller and Tate[2] as a modification of xylose lysine deoxycholate agar, for the enhanced recovery of non-Typhi *Salmonella* spp. Several evaluations demonstrated that XLT4 medium significantly improved *Salmonella* isolation from chicken farm environmental drag-swab samples over the other selective plating media.[3-5] XLT4 agar was found to strongly inhibit *Proteus, Pseudomonas, Providencia*, and many other non-salmonellae and to provide good differentiation between *Salmonella* and *Citrobacter*.[3]
XLT4 Agar is included in the FDA-BAM list of rapid methods and specialty substrate media for detection of foodborne bacteria.[6]
Yeast extract provides carbon, nitrogen, vitamins and trace elements for bacterial growth. XLT4 Agar contains three indicator systems: xylose, lactose, sucrose and lysine hydrochloride combined with 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.
Sugars' fermentation lowers the pH and the phenol red indicator registers this by changing from red to yellow. After exhausting the xylose supply, *Salmonell*a colonies will decarboxylate lysine, increasing the pH once again to alkaline. To prevent similar pH reversion by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Moreover *Salmonella* spp. produce thiosulphate reductase that cause the release of a sulphide molecule from the sodium thiosulfate; this sulphide 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. The addition of low concentrations of peptone produces blacker *Salmonella* colonies in shorter incubation times (increased hydrogen sulphide production), while still maintaining strong inhibition of competing bacteria.[7] The selectivity relies on the use of the anionic surfactant Niaproof 4 (formerly Tergitol 4) that largely inhibits the unwanted accompanying flora. On XLT4 agar, *Salmonella* spp. produces black or black-centred colonies with a yellow or pink periphery. Other Gram-negative bacteria are markedly inhibited or produce yellow or pink colonies without black coloration.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 58 g in 1000 mL of cold purified water and add 4.6 mL of XLT4 Supplement (REF 4240097). Heat to boiling with frequent agitation, to dissolve completely. Do not overheating, do not autoclave. Cool to 47-50°C, mix well and distribute into sterile Petri dishes. Do not leave the medium more than 45 minutes into the water batch.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	red-pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red, limpid
Final pH at 20-25 °C	7.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
XLT4 Agar Base	Dehydrated medium	4022072	500 g (8.6 L)
XLT4 Supplement	Liquid supplement	4240097	100 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

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8 - SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

9 - TEST PROCEDURE

The detection of *Salmonella* in foods and other samples of sanitary interest, may require four successive stages: pre-enrichment in non-selective liquid medium, enrichment in one or two selective liquid media, plating out and recognition, confirmation.
Allow plates to come to room temperature and to dry the surface of the medium.
From a suitable *Salmonella* enrichment broth (e.g., Mueller Kauffman Tetrathionate Broth or Tetrathionate Broth or Selenite Cystine Broth) transfer a loopful of growth on a plate of XLT4 Agar.
Streak the inoculum over the four quadrants of the plate to obtain well isolated colonies.
Incubate between 34 °C and 38 °C and examined after 24 and 48 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Do not examine areas of confluent growth as false negative fermentation reactions may occur.
Salmonella spp. ferment the xylose with acidification of the medium, and decarboxylate the lysine with consequent inversion of the pH of the medium to alkaline values. Typical colonies of *Salmonella* on XLT4 Agar after 18-24 hours incubation have a black centre and a lightly transparent zone of yellow colour. After 48 hours incubation *Salmonella* colonies became entirely black or pink to red with black centres. H_2S-negative variants grown with pinkish-yellow colonies. Lactose-positive *Salmonella* strains grown with yellow colonies with or without blackening.
Shigella is partially inhibited and grows with red colonies.
Coliforms bacteria such as *E. coli, Enterobacter, Citrobacter* are markedly inhibited and colonies appear yellow without blackening. Growth of other Gram-negative bacteria is markedly to completely inhibited. Gram positive bacteria are totally 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, colonies with black centre
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, yellow colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated XLT4 Agar Base supplemented with XLT4 Supplement (REF 4240097) is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by a semi-quantitative ecometric tecnique with the following target strains: *S. arizonae* ATCC 13314, S. Enteritidis ATCC 13076, S. Typhimurium ATCC 14028, S. Gallinarum CB 506, S. Dublin CB 092, S. Choleraesuis CB X4. After incubation at 37°C for 24 hours, colonies' colour and the amount of growth is evaluated: target strains exhibit good growth with black centred colonies.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *E. faecalis* ATCC 19433, *E. coli* ATCC 25922, *C. freundii* ATCC 8090, E. *aerogenes* ATCC 13048, *P.mirabilis* ATCC 10005. The growth of *E. faecalis, E. aerogenes, P. mirabilis* is totally inhibited while the growth of *E. coli* and *C. freundii* is partially or not inhibited and the colonies exhibit typical yellow colour without blackening.
CB: Biolife microbial strains collection.

13 - LIMITATIONS OF THE METHOD

- A single medium is only rarely useful to recover all pathogens contained in a specimen.
- XLT4 agar is not intended for the detection of S.Typhi.
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.
- Non-*Salmonella* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Salmonella.*

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are 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.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. XLT4 Supplement is classified as hazardous. Before use, consult the Material Safety Data Sheets.
- This 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 the 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.
- Be careful when opening the metal ring of the supplement vial to avoid injury.
- The supplement is not supplied sterile; it must be autoclaved with the medium base.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.

- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium 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 materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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 +10°C /+30°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).

Supplement

Upon receipt, store the product in the original package at +10°C /+30°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. The supplement bottle can be opened several times until the contents are exhausted. Before use, examine the product and discard if there are obvious signs of deterioration (e.g., turbidity, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010
2. Miller RG, Tate CR. XLT4: A highly selective plating medium for the isolation of Salmonella. The Maryland Poultryman, 1990 April: 2-7.
3. Tate CR, Mallinson ET, Scherrer JA. Xylose-lysine-tergitol 4: an improved selective agar medium for the isolation of Salmonella. Poult Sci. 1991;70:2429.
4. Miller RG, Tate CR, Mallinson ET, Scherr JA. Xylose-lysine-tergitol 4: an improved selective agar medium for the isolation of Salmonella. Poult Sci. 1992; 71:398.
5. Miller RG, Tate CR, Mallinson ET. Evaluation of two Isolation and two non isolation methods for detecting naturally occurring Salmonellae from broiler flock environmental drag-swab samples. J Food Prot. 1992; 55:964-967.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual. Appendix 1, Rapid Methods for Detecting Foodborne Pathogens. Version: January 2001. Content current as of June 18, 2009.
7. Miller RG, Tate CR, Mallinson ET Improved XLT4 Agar: Small addition of peptone to promote stronger production of hydrogen-sulfide by Salmonellae. J Food Prot 1994; 57:854-858.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/12

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