XYLOSE LYSINE DESOXYCHOLATE (XLD) AGAR ISO FORMULATION

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Selective and differential medium for the detection of *Salmonella* and *Shigella* from foodstuffs and waters according to ISO Standards.

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

DEHYDRATED AND READY-TO-USE MEDIUM

Xylose	3.75 g
L-lysine hydrochloride	5.00 g
Lactose	7.50 g
Sucrose	7.50 g
Sodium chloride	5.00 g
Yeast extract	3.00 g
Sodium deoxycholate	1.00 g
Sodium thiosulphate	6.80 g
Ferric ammonium citrate	0.80 g
Phenol red	0.08 g
Agar	14.50 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

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, especially of *Shigella.*[1] In 1965, xylose lysine deoxycholate (XLD) agar was introduced by Welton I. Taylor for the enhanced recovery of *Shigella*.[2] Several evaluations demonstrated the relatively high efficiency of XLD Agar in the primary isolation of *Shigella* and *Salmonella*.[3-5]

XLD Agar ISO Formulation is a selective and differential medium intended for the detection of *Salmonella* in food chain samples according to ISO 6579,[6] in water samples according to ISO 19250[7] and for the detection of *Shigella* in foods according to ISO 21567[8].

XLD ISO Formulation contains a lower concentration of sodium deoxycholate and a slightly higher concentration of xylose than the standard XLD Agar formulation (REF 402206).

Yeast extract provides carbon, nitrogen, vitamins and trace elements for bacterial growth; sodium chloride maintains the osmotic balance in the medium; sodium deoxycholate 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.

Sugars' fermentation lowers the pH and the phenol red indicator registers this by changing from red to yellow. Most enteric bacteria, including *Salmonella*, can ferment the xylose to produce acid; *Shigella* does not ferment the xylose, does not cause acidification of the medium, and therefore, grows with red colonies. After exhausting the xylose supply, *Salmonella* colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. 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.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 54.9 g in 1000 mL of cold purified water. 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.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
XLD Agar ISO Formulation	Dehydrated medium	4022082 4022084	500 g (9.1 L) 5 kg (91 L)
XLD Agar ISO Formulation	Ready-to-use plates	542208	2 x 10 plates ø 90 mm

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.

8 - SPECIMENS

Food chain and water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[6-8]

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

9 - TEST PROCEDURE

Detection of *Salmonella* in foods[6]

The detection of *Salmonella* in foods and other samples of sanitary interest, necessitates 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 Rappaport Vassiliadis Soy (RVS) Broth (REF 4019819) or MSRV Medium (ref 401982) incubated at 41.5 °C ± 1 for 24 h ± 3 h and from MKTT Broth (REF 401745) incubated between 34 °C and 38 °C for 24 h ± 3 h, transfer a loopful of growth on a plate of XLD Agar ISO Formulation and on another selective medium for *Salmonella,* based on different diagnostic characteristics to those of XLD agar (e.g., Chromogenic Salmonella Agar REF 405350).
Incubate the XLD plates inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use

Detection of *Shigella* in foods[8]

From Shigella broth containing 0,5 µg/ml of novobiocin (Shigella Broth Base REF 4020402 supplemented with Novobiocin Antimicrobic Supplement REF 4240047), incubated anaerobically at 41.5 ±1°C for 16 h to 20 h, transfer a loopful of growth on plates of MacConkey Agar REF 401670 (low selectivity), XLD Agar ISO Formulation (moderate selectivity), and Hektoen Enteric Agar REF 401541 (greatest selectivity).
Incubate the plating-out media at 37 °C for 20 h to 24 h. If no typical colonies are seen and the growth of other microorganisms is weak (particularly on the more selective agar), re-incubate the plates for a further 24 h. Examine them again for typical *Shigella* colonies.

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.
Typical colonies of *Salmonella* on XLD Agar ISO Formulation have a black centre and a lightly transparent zone of reddish colour due to the colour change of the indicator. *Salmonella* H_2S-negative variants grown with pink colonies with a darker pink centre. Lactose positive *Salmonella* grown with yellow colonies with or without blackening.
Typical colonies of *Shigella* on XLD Agar ISO Formulation are translucent with red/cerise centre, same colour as the agar.
The recognition of colonies of *Salmonella* is to a large extent a matter of experience, and their appearance can vary somewhat. Here below a summary of the interpretation criteria for colonies grown on XLD Agar[9]:
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*.
For presumptive *Salmonella* spp. identification, it is advised to screen the colonies by testing with one drop of MUCAP Reagent (REF 191500) and observing 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.[10]

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, red colonies with black centre
S. flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, red colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, yellow colonies

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready to use XLD Agar ISO Formulation are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by a quantitative test with the target strains S. Enteritidis ATCC 13076 and S. Typhimurium ATCC 14028; the 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 (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 suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *E. faecalis* ATCC 19433 and *E. coli* ATCC 8739. The growth of *E. faecalis* is inhibited while the growth of *E. coli* is partially inhibited and the colonies exhibit typical yellow colour.

13 - LIMITATIONS OF THE METHOD

- A single medium is only rarely useful to recover all pathogens contained in a specimen.
- Non-enteric organisms such as *Pseudomonas* may grow; *Pseudomonas* and *Providencia rettgeri* may both exhibit red colonies. Some *Proteus* spp. may develop black centres.[9]
- S. Parathyphi A, S. Cholerae-suis, S. Pullorum and S. Gallinarum may form red colonies without black centre, thus resembling *Shigella* spp.[9]
- Incubation exceeding 48 hours may lead to false positive results.[9]
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 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 production process of prepared media.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheets 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

Ready to use plates

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).

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).

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). According to ISO 6579 the self-prepared plates can be stored at +2°C +8°C, protected from drying, for up to four weeks.[6]

16 - REFERENCES

1. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010
2. Taylor WI. Isolation of shigellae I. Xylose lysine agars; new media for isolation of enteric pathogens. Am J Clin Pathol 1965; *44*:471-475
3. Taylor WI, Schelhart D. Isolation of shigellae VI. Performance of media with stool specimens. Appl Microbiol 1968;*16*:1387-1393
4. Taylor WI, Schelhart D. Isolation of shigellae VIII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar and Eosin Methylene Blue Agar with stool specimens Appl Microbiol 1971; 21:32-7
5. Zajc-Satler J, Gragas AZ. Xylose Lysine Deoxycholate Agar for the isolation of Salmonella and Shigella from clinical specimens. Zentralbl Bakteriol Orig 1977; A237:196-200
6. ISO 6579-1:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella —Part 1: Detection of Salmonella spp.
7. ISO 19250:2010 Water quality — Determination of Salmonella species
8. ISO 21567:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
10. 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.

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	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/08

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it