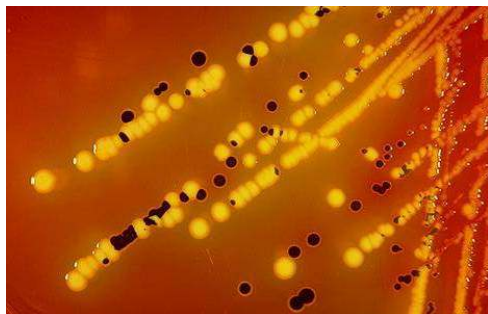


**INSTRUCTIONS FOR USE**

## XLD AGAR

### Dehydrated culture medium



XLD Agar: *Salmonella* colonies with large black centre and *E.aerogenes* with yellow colonies

**1 - INTENDED USE**

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

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

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		

\*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*.<sup>1</sup> In 1965, Xylose Lysine Desoxycholate (XLD) agar was introduced by Welton I. Taylor for the enhanced recovery of *Shigella*.<sup>2</sup> Several clinical evaluations demonstrated the relatively high efficiency of XLD Agar in the primary isolation of *Shigella* and *Salmonella*.<sup>3-5</sup>

XLD Agar is a selective and differential medium intended for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella* from clinical specimens.<sup>6-8</sup> It is recommended for the detection of *Salmonella* in non sterile pharmaceutical products according to harmonized EP, USP, JP method<sup>9</sup> and by FDA-BAM for detection of *Salmonella* in food<sup>10</sup>. The XLD formula recommended by ISO norms for the detection of *Salmonella* and *Shigella* in food and water contains a lower concentration of sodium desoxycholate and corresponds to Biolife medium XLD Agar ISO Formulation (REF 402208).

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.

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 present in the medium; this sulphide molecule couples with a hydrogen ion to form H<sub>2</sub>S 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 55 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, to dissolve completely. 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	Dehydrated medium	4022062	500 g (9.1 L)
		4022064	5 kg (91 L)

**7 - MATERIALS REQUIRED BUT NOT PROVIDED**

Water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

**8 - SPECIMENS**

XLD Agar is intended for the bacteriological processing of clinical specimens such as faeces, rectal swab, urine, bile,<sup>6-8</sup> non-sterile pharmaceutical products<sup>9</sup> and food<sup>10</sup>. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.<sup>11</sup> Consult appropriate standard methods for details of collection and preparation of non-clinical specimens.<sup>9,10</sup>





## 9 - 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 four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. 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 the enrichment step in Selenite Broth followed by subculture to XLD Agar and to a second plating medium.<sup>8</sup>

For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised, followed by subculture on two different selective media: XLD Agar and a second less selective medium (Mac Conkey Agar).<sup>8</sup>

Incubate inoculated XLD Agar 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.

For the detection of *Salmonella* in non-sterile pharmaceuticals products the technique recommended by European Pharmacopoeia<sup>9</sup>, and summarized below, should be followed:

- Prepare a sample using a 1:10 dilution of not less than 1 g of the product to be examined and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate the suitable amount of Tryptic Soy Broth. Mix and incubate at 30-35°C for 18-24 h.
- Shake the container, transfer 0,1 mL of Tryptic Soy Broth to 10 mL of Rappaport Vassiliadis Enrichment Salmonella Broth EP (REF 401979) and incubate at 30-35°C for 18-24 h.
- Subculture on a plate of XLD Agar and incubate at 30-35 °C for 18-48 h.

Consult appropriate references for the detection of *Salmonella* in food.<sup>10</sup>

## 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.

Interpretation of colonies' colours<sup>12</sup>:

**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<sub>2</sub>S negative

**Red colonies with black centre:** xylose fermentation only, lysine positive, H<sub>2</sub>S positive, rapid depletion of xylose and resultant alkalinity due to lysine decarboxylation, black centre due to H<sub>2</sub>S production possible only in alkaline pH environment: suspect *Salmonella* H<sub>2</sub>S 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 the colonies 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<sub>8</sub> esterase enzyme, typical of *Salmonella* spp.<sup>14</sup>

## 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.<sup>13</sup>

CONTROL STRAINS		INCUBATION T° / T / ATM	EXPECTED RESULTS
S.Typhimurium	ATCC 14028	35-37°C / 18-24h / A	growth, red colonies with black centre
<i>S.flexneri</i>	ATCC 12022	35-37°C / 18-24h / A	growth, red 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 colonies

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 XLD Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

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 35-37°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is  $\geq 0.7$  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<sup>-1</sup> to 10<sup>-4</sup> 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 at the dilution 10<sup>-1</sup>, the growth of *E.coli* is partially inhibited and the colonies show typical yellow colour, according to the specifications.

## 13 - LIMITATIONS OF THE METHOD

- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella* and/or *Shigella*, with lower selectivity such as Mac Conkey Agar and with higher selectivity such as SS Agar, should be used; it is suggested to inoculate additional media for the isolation of other enteric pathogens with the specimen.<sup>8</sup>
- Non-enteric organisms such as *Pseudomonas* may grow; *Pseudomonas* and *Providencia rettgeri* may both exhibit red colonies. Some *Proteus* spp. may develop black centres.<sup>12</sup>
- *S.Paratyphi* A, *S.Cholerae-suis*, *S.Pullorum* and *S.Gallinarum* may form red colonies without black centre, thus resembling *Shigella* spp.
- Incubation exceeding 48 hours may lead to false positive results.<sup>12</sup>





- 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 diseases; 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.

**14 - 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.
- 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](http://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 preparation process of plated or bottled media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates 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 Sheet of the product are available on the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it).
- Notify Biolife Italiana Srl ([complaint@biolifeitaliana.it](mailto:complaint@biolifeitaliana.it)) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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**

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 for the validation of the period of validity of the finished products, according to the type (plates/bottles), the added supplements and the storage method applied (temperature and packaging).

**16 - REFERENCES**

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**TABLE OF APPLICABLE SYMBOLS**

REF Catalogue number	Batch code	<i>In vitro</i> Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

**REVISION HISTORY**

Version	Description of changes	Date
Revision 2	Updated layout and content	2020/05
Revision 3	Update of "precautions and warnings" and "storage conditions and shelf life"	2022/01
Revision 4	Removal of obsolete classification	2023/04

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

