SALMONELLA SHIGELLA AGAR/XLD AGAR

INSTRUCTIONS FOR USE THE READY-TO-USE BI-PLATED MEDIUM

Cat. no:	Medium type:	Packaging:
2025PD90 **202025**	Solid medium on a bi-plate	1x10 pcs (90 mm)

1. Intended use

Detection and isolation of Gram-negative enteric pathogens, especially *Shigella* and *Salmonella* species in human clinical specimens and other specimens.

Salmonella Shigella Agar/XLD Agar of Gram-negative enteric pathogens, especially *Shigella* and *Salmonella* species.
The function of Salmonella Shigella Agar/XLD Agar is to support the diagnosis of patients with symptoms indicating potential infection with Gram-negative enteric pathogens, particularly those of the genus *Shigella* and *Salmonella.*
Salmonella are some of the most common etiologic agents of food poisoning. The pathogenicity of these microorganisms varies from one serovar to another and can vary within the same subspecies. Some serovars are responsible for invasive diseases, but there are also those that cause self-limiting food poisoning. The most isolated serovars of *Salmonella enterica* subsp. *enterica* species are S. Enteritidis, S. Typhimurium, S. Virchow, S. Hadar or S. Infantis.
The genus *Shigella* includes four species: *S. dysenteriae*, S. *flexneri*, *S. boydii* and *S. sonnei.* All species are obligate pathogens and cause bacterial dysentery.

2. Principle of the procedure

Salmonella Shigella Agar
The presence of bile salts, malachite green and sodium citrate inhibits the growth of Gram-positive microorganisms and *Enterobacterales* other than *Salmonella* and *Shigella.* Differentiation of *Enterobacteriales* is possible due to the addition of lactose. Lactose fermenting bacteria produce acid and form red coloured colonies, due to the pH indicator in the medium being neutral red. In contrast, lactose non-fermenting microorganisms form colorless colonies. Ferric citrate is an indicator of hydrogen sulfide production. *Salmonella* produce thiosulfate reductase, which releases sulfide molecules present in sodium thiosulfate. These molecules combine with hydrogen ions to form H_2S, which reacts with ferric ammonium citrate. This reaction results in formation of precipitates, visible as black spots in the center of bacterial colonies.

XLD Agar
Yeast extract is a source of nutrients in the medium. The presence of sodium deoxycholate inhibits the growth of Gram-positive bacteria. Differentiation of bacteria is possible due to three indicating systems:
- lactose, xylose and sucrose in combination with phenol red, which is a pH indicator,
- L-lysine hydrochloride and phenol red,
- sodium thiosulfate and ferric ammonium citrate.

Fermentation of xylose lower the pH of the medium and causes it to change colour from red to yellow. Most enteric pathogens including *Salmonella* are able to ferment xylose, causing acidification of the medium. Since bacteria of the *Shigella* genus are lactose non-fermenting, they do not produce acids and thus form red colonies.
Lysine allows to differentiate *Salmonella* bacteria from other, non-pathogenic bacteria. Once xylose is depleted, *Salmonella* bacteria utilize L-lysine in the process of decarboxylation, which changes the pH level of the medium to alkaline. To prevent similar reversion of the pH level, by lysine-positive coliforms, lactose and sucrose are added to produce acids in excess.
Ferric ammonium citrate is an indicator of hydrogen sulfide production. *Salmonella* produce thiosulfate reductase, which releases sulfide molecules present in sodium thiosulfate. These molecules combine with hydrogen ions to form H_2S, which reacts with ferric ammonium citrate to form precipitates, visible as black centers in bacterial colonies. Non-pathogenic bacteria that produce H_2S do not decarboxylate L-lysine. Therefore, the acid reaction produced by them prevents the blackening of the colonies. Sodium chloride maintains the osmotic balance.

3. Medium composition

In g/l distilled water:

Salmonella Shigella Agar		XLD Agar	
Beef extract	5,0 g	Yeast extract	3,0 g
Enzymatic digest of animal tissue	2,5 g	Sucrose	7,5 g
Enzymatic digest of casein	2,5 g	Agar	13,5 g
Lactose	10,0 g	Sodium deoxycholate	2.5 g
Sodium citrate	8,5 g	Sodium thiosulfate	6,8 g
Bile salts	8,5 g	Xylose	3,5 g
Sodium thiosulfate	8,5 g	Lactose	7,5 g
Brilliant green	0,00033 g	L-lysine hydrochloride	5,0 g
Ferric citrate	1,0 g	Phenol red	0,08 g
Agar	13,5 g	Ferric ammonium citrate	0.8 g
Neutral red	0,025 g	Sodium chloride	5,0 g
pH 7,0 **±** 0.2 at 25° C.		**pH** 7.4 **±** 0.2 at 25° C.	
Appearance of the medium - Clear, salmon		**Appearance of the medium -** Clear, red.	

4. Medium preparation

The medium is ready to use. Bring the medium to room temperature immediately before use.

5. Equipment required, not provided

Standard microbiological laboratory equipment necessary for testing including an incubator.

6. Precautions

- The product is intended for professional use only.
- Non-automated product.
- The medium contains components of animal origin, which may be associated with the presence of biological pathogens, therefore must be handled in accordance with principles of handling potentially infectious biological material.
- Do not use plates if the medium shows signs of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
- Do not use damaged plates.
- Do not use plates after the expiration date.
- Re-incubation of previously inoculated plates is not allowed.
- To ensure correct test results, follow these instructions.
- If the handling of the medium differs from that described in this manual, the laboratory is obliged to validate the procedure adopted.

7. Storage

Store plates at 2-12ºC until the expiration date. Store plates in their original packaging, in an inverted position (agar side up), away from direct light sources. To avoid freezing of agar, do not store plates close to the refrigerator walls. To avoid the appearance of water condensation on the plate lid, do not open the refrigerator more often than necessary and do not store

plates in an overfilled refrigerator.

8. Expiration date

The medium stored at 2-12ºC retains its properties up to 3 months from the date of production.

9. Specimen types

Test specimens are fresh stool specimens containing blood or mucus strands, rectal swabs, as well as other clinical specimens. Place the stool specimens in an airtight, sterile container with a screw cap. Do not allow the specimens to dry out. If the patient is unable to pass stool, take a rectal swab sample.
Deliver specimens for testing to the laboratory within 2 hours of collection. If the sample is not delivered to the laboratory within this time, it should be placed in Cary-Blair or Amies transport medium and refrigerated. At refrigerator temperature, samples in transport medium are stable for up to 2 days.

10. Test procedure

1. Allow the medium to warm to room temperature before inoculation.
2. Inoculate the specimen by spreading it directly on the agar surface.
3. If the specimen is collected on a swab - gently rotate the tip of the swab on a small area of agar just around the edges of the plate, and then inoculate specimen using streak plate method with a sterile loop.
4. Incubate the inoculated plate at 35 ± 2°C
5. Examine for growth after 18-24 hours of incubation.

11. Reading and interpretation

After the incubation, observe:

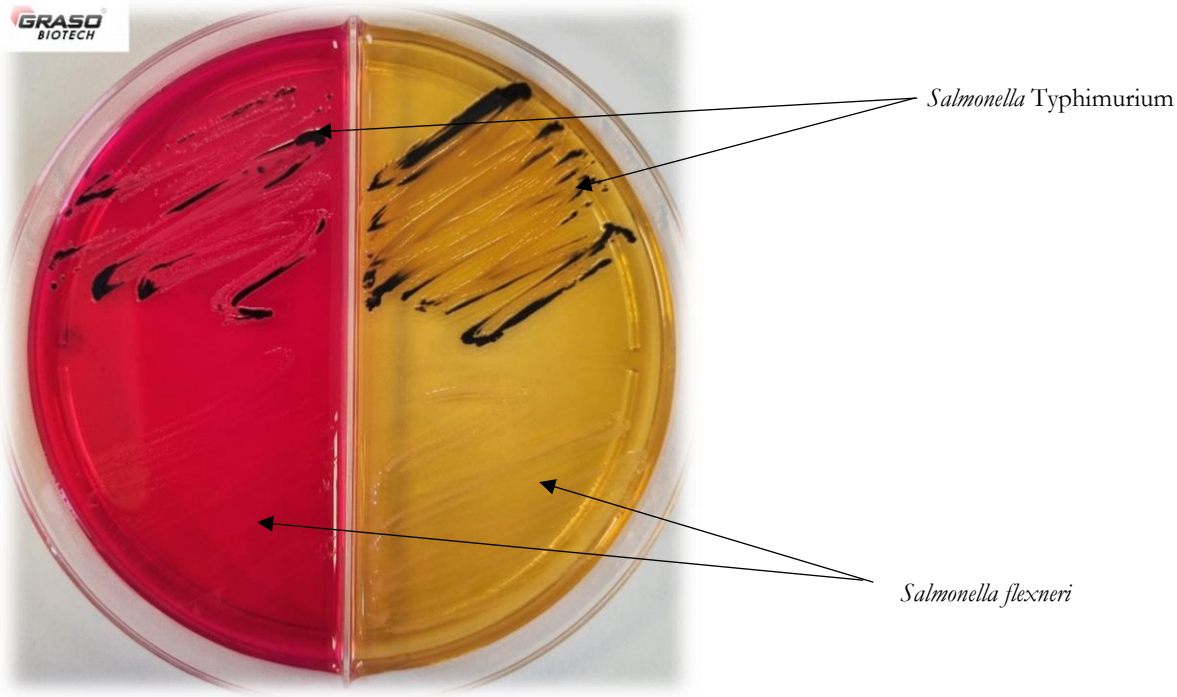
- the presence of bacterial colony growth,
- colony morphology,
- colony staining

Typical morphology of bacterial colonies grown on Salmonella Shigella Agar:

Microorganism	**Typical colony morphology/medium colour**
Escherichia coli	Poor growth pink to red colonies
Klebsiella, Enterobacter	Poor growth, pink colonies
Proteus	Colourless colonies with a black center
Salmonella	Colourless colonies, usually with a black center
Shigella	Colourless colonies
Pseudomonas	Poor growth, irregular colonies
Gram-positive bacteria	No growth

Typical morphology of bacterial colonies grown on XLD Agar:

Microorganism	**Typical colony morphology**
Escherichia coli	Colonies large, yellow, no growth in some strains
Klebsiella, Enterobacter	Mucous colonies, yellow
Proteus	Red to yellow colonies, most strains with black
Salmonella H_2S-positive	Red colonies, with black centers
Shigella, *Salmonella* H_2S-negative	Red colonies
Pseudomonas	Red colonies
Gram-positive bacteria	No growth or very weak growth



Colony morphology and growth pattern of *Salmonella* and *Shigella* on Salmonella Shigella Agar/XLD Agar

For the final identification of cultured microorganisms, additional tests and/or confirmatory tests should be carried out using other methods used in the laboratory.

12. Quality control

The nutritional properties and selectivity of the medium should be checked using reference strains giving the expected positive and negative reactions. The test should be performed using pure, 18 – 24 hour (or older) cultures of reference strains giving the desired reactions. Use the following reference strains to perform medium quality control:

Salmonella Shigella Agar

Reference strain:	**Growth intensity:**	**Colonies morphology:**
Salmonella typhimurium ATCC 14028	good growth	colourless colonies with a black center
Shigella flexneri ATCC 12022	good growth	colourless
Escherichia coli ATCC 25922	poor growth or no growth	pink to red colonies
Enterococcus faecalis ATCC 29212	no growth	-

XLD Agar

Reference strain:	**Growth intensity:**	**Colonies morphology:**
Salmonella typhimurium ATCC 14028	good growth	red colonies with a black center
Shigella flexneri ATCC 12022	good growth	red colonies
Escherichia coli ATCC 25922	poor growth or no growth	yellow colonies

Other reference strains may be used in accordance with the laboratory's procedures and instructions Quality control procedures should meet the requirements of applicable regulations and guidelines/recommendations.

13. Limitations of the method

- Due to variability in nutritional requirements, some strains may grow poorly, or not grow at all on Salmonella Shigella Agar/XLD Agar
- *Proteus* spp. colonies may resemble *Salmonella* in appearance, so additional confirmatory tests are recommended.

- Some strains of lactose-fermenting *Salmonella* and *Shigella* bacteria can form coliform-like colonies on Salmonaella Shigella Agar.
- Some strains of *Pseudomonas* may form red colonies on the medium, while *Proteus* bacteria may form red colonies with a black center.

14. Characteristics of the method

Salmonella Shigella Agar: Identifying the etiologic agent of infectious diarrhea is significant not only for the diagnostic process and determining the adequate treatment, but also for epidemiological reasons. In many cases, the treatment of this type of infection is conservative and consists of electrolyte supplementation and rehydration, but from the epidemiological standpoint it is greatly important to find out the etiological agent, as it helps to identify the carriers and prevent the infection from spreading. There are many commercially available media designed for isolation of Enterobacteriaceae including Salmonella and Shigella, of varying diagnostic value. One of such media is Salmonella Shigella Agar.
Ruiz J. et al. in published a study results in Eur. J. Clin. Microbiol. Infect. Dis., which assessed the ability of six different culture media to isolate Enterobacteriales from fecal samples (Rambach Agar, Salmonella Shigella Agar, Novobiocin-brilliant green-glycerol-lactose NBGL, modified semi-solid Rappaport-Vassiliadis MSRV medium, and Salmonella Detection and Identification-2 SM2). 500 human fecal samples were tested. 81 samples were positive for Salmonella on at least one culture medium. Based on the study results, the specificity of the Salmonella Shigella Agar was set at 99.3% and its sensitivity at 69.1%. Similar studies were conducted by the same authors, analyzing five media: Salmonella Shigella Agar, Hektoen Enteric Agar, Bismuth Sulfite Agar, Novobiocin-brilliant green-glycerol-lactose NBGL, and Salmonella
Detection and Identification-2 SM2. 1,000 fecal samples were analyzed. Each sample was inoculated twice. Firstly, by applying the material directly to solid medium and secondly, to Selenite F enrichment broth. On solid medium positive cultures were obtained for 74 samples. The sensitivity for Salmonella Shigella Agar was 64.9% and the positive predictive value was 18.7%. After enrichment in Selenite F broth, 88 positive samples were obtained. The sensitivity for Salmonella, Shigella Agar was 92% and the positive predictive value was 17%.

XLD Agar: *Enterobacterales* are a large, closely related group of bacteria comprising more than forty genera, more than half of which are related to humans. Many *Enterobacterales* inhabit the digestive tract of healthy humans and animals. Others, such as *Salmonella*, *Shigella* and *Yersinia* include obligate pathogens responsible for specific diseases.
There are many commercially available media designed for detection and isolation of these microorganisms. Which medium is used depends on the specifics of the laboratory and often the preferences of its employees. One of such media is XLD medium. It was formulated relatively early and has been commercially available for decades.
Diagnosing infections caused by *Enterobacterales* may present a challenge, due to the difficulties in isolation of *Shigella* and *Salmonella*.
In 1968, Taylor W.I. and Schelhart D. studied the ability of three nutrient broths and four solid media for the isolation of *Salmonella* and *Shigella*. They found that direct inoculation onto solid media only recovered 50% of *Salmonella* and 61% of *Shigella*. These values increase significantly after prior incubation in enrichment broth. Four solid media were tested: MacConkey (MAC), with deoxycholate citrate (DC), XLD and XLBG. The individual media yielded *Salmonella* growth rates of 94% for XLD, 71% for XLBG, 55% for MAC and 35% for DC. For *Shigella,* the values were as follows: XLD - 89%, MAC - 75%, XLBG - 63% and DC - 27%.
Over the years, new media were developed, but the diagnostic value of XLD Agar remains high, especially in combinations with other media. Maddocks S. et al. compared CHROMagar Salmonella medium with XLD Agar, Salmonella Shigella Agar and Hektoen Agar. They found that the specificity of *Salmonella* detection after direct inoculation on chromagar medium was 100%, similar to SS and XLD medium, however these media required prior incubation with enrichment broth.
Nye K.J. et al. conducted similar studies using XLD, DCA, MLCB and alpha-beta chromogenic (ABC) substrate. MLCB substrate yielded the highest isolation rate when used alone. The isolation rate from XLD and DCA medium correlated with prior incubation in enrichment broth. In contrast, the combination of XLD and MLCB substrate yielded the highest isolation rate.

15. Disposal of used material

Used and unused materials should be disposed of in accordance with current medical waste handling regulations and laboratory procedures for the disposal of infectious and potentially infectious materials.

16. Reporting of adverse events

According to current regulations, adverse events and incidents that can be directly linked to the described medium must be reported to the manufacturer and to the competent authorities.

17. References

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10. Nye K.J., Fallon D., Frodsham D., Gee B., Graham C., Howe S., Messer S., Turner T., Warren R.E., An evaluation of the performance of XLD, DCA, MLCB and ABC agars as direct plating media for the isolation of Salmonella enterica from faeces, J. Clin. Pathol. Apr 202, 286-8.
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History of document changes

Date of change	Section	Description of the change
2023/06/01	Entire document	Adaptation to the requirements of EU Regulation 2017/746

NOTE
The revision history of the document does not include editorial changes.

SYMBOL	NAME OF SYMBOL	DESCRIPTION	REF.
	Manufacturer	Indicates the medical device manufacturer.	5.1.1
	Date of manufacture	Indicates the date after which the medical device is not to be useed.	5.1.3
	Catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be used..	5.1.6
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.5

	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an invitro diagnostic medical device.	5.5.1
	Do not re-use	Indicates a medical device that is intended for one single use only.	5.4.2
	Contains sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.	5.5.5
	Use –by date	Indicates the date after which the medical device is not to be used	5.1.4
	Temperature limit	Indicates the temperature limits of temperature shall be indicates adjacent to the upper and lower horizontal lines.	5.3.7
	Safety symbol (Compliance with EU requirements)	The CE marking on a product is a manufacturer's declaration that the product complies with the essential requirements of the relevant European Union health, safety and environmental regulations.	nd.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	5.4.3
	Sterilized using aseptic processing techniques	Indicates a medical device that has been manufactured using accepted aseptic techniques.	5.2.2
	Do not use if package is damaged and consult instructions for use	Indicates that a medical device that should not be used if the package has been damaged or opened and that the user should consult the instructions for use for addictional information.	5.2.8
	Contains biological material of animal origin	Indicates a medical device that contains biological tissue, cells, or their derivatives, of animal origin	5.4.8

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