XLD Agar (Xylose Lysine Desoxycholate Agar) ISO

Cat. 1274

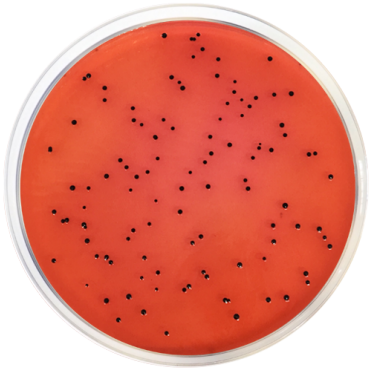
Selective medium for the isolation of Salmonella in food.

Practical information

Aplications	Categories
Selective isolation	Salmonella
Detection	Salmonella

Industry: Water / Food

Regulations: ISO 11133 / ISO 19250 / ISO 21567 / ISO 6579



Principles and uses

XLD Agar (Xylose Lysine Desoxycholate Agar) is prepared according to the formulation of the ISO 6579 norm. It is recommended for the identification of Salmonella in food products, after pre-enrichment in a non-selective fluid such as Buffered Peptone Water (Cat. 1402) and enrichment in a selective fluid medium such as Muller Kauffmann Broth Base with Brilliant Green & Novobiocin (MKTTN) (Cat. 1173), Rappaport Soy Broth (Vassiliadis) (Cat. 1174) or Modified Semisolid Rappaport Vassiliadis Medium (MSRV) (Cat. 1376).

The reactions are the degradation of the three fermentable carbohydrates: xylose, lactose, and sucrose, with the production of acid, manifested in the color change from red to yellow. Sodium thiosulfate serves as a reactive substance with Ferric ammonium citrate as an indicator of the formation of hydrogen sulfide under alkaline conditions. Lysine is included to enable the Salmonella group to be differentiated from the non-pathogens since, in its absence, salmonellae would quickly ferment the xylose, making it indistinguishable from non-pathogenic species. After the salmonellae terminate the xylose present, the lysine is attacked through the enzyme lysine decarboxylase with a change to an alkaline pH, similar to the Shigella reaction. The bacteria that decarboxylate the L-Lysine to cadaverine are identified by the presence of a purple-red color around the colonies due to the elevation of the pH. Phenol red is the pH indicator. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium desoxycholate is the selective agent and is thus inhibitory to Gram-positive microorganisms. Bacteriological Agar is the solidifying agent.

Typical colonies of Salmonella on XLD agar have a black center and lightly transparent zone of reddish color due to the color change of the indicator.

Salmonella H2S-negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive Salmonella grown on XLD agar are yellow with or without blackening.

Formula in g/L

Bacteriological agar	13,5	Ferric ammonium citrate	0,8
Lactose	7,5	L-Lysine hydrochloride	5
Phenol red	0,08	Sodium chloride	5
Sodium deoxycholate	1	Sodium thiosulfate	6,8
Sucrose	7,5	Xylose	3,75
Yeast extract	3		

Preparation

Suspend 54 grams of the medium in one liter of distilled water Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE.Cool the medium according to the applicable normative and pour into Petri dishes as soon as it has cooled.

Preparation of large volumes, overheating and prolonged storage in water bath is to be avoided. Precipitates may be formed but do not affect the performance of the culture media.

Instructions for use

* For detection of Salmonella spp. in food, animal feed, animal faeces, and environmental samples according to ISO 6579:

- Preenrichment in non-selective liquid medium:
Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 34-38 ºC for 18±2 h.
- Enrichment in/on selective media:
Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) or the Modified Semisolid Rappaport Vassiliadis medium (MSRV) (Cat. 1376), and the MKKTN Broth(Cat. 1173).
The Rappaport Soy Broth and the Modified Semisolid Rappaport medium are incubated at 41,5 ºC for 24±3 h, and the MKKTN Broth at 34-38 ºC for 24±3 h.
- Plating out on selective solid media:
From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (Salmonella Chromogenic Agar (Cat. 1122), Brilliant Green Agar (Cat. 1143), Bismuth Sulfite Agar (Cat. 1011), DCLS Agar(Cat. 1045), Desoxycholate Citrate Agar (Cat. 1067), Hektoen Enteric Agar (Cat. 1030), Salmonella Shigella Agar(Cat. 1064) and XLT4 Agar (Cat. 1159)).
Incubate the XLD plates inverted at 34-38 ºC for 24±3 h.
Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation:
Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Note: According to Annex D of ISO 6579-1: 2017, for the detection of enterica subspecies enterica serovars Typhi and Paratiphy, Selenite Cystine Broth (Cat. 1220) shlould be added as a selective enrichment medium and Bismuth Sulfite Agar (Wilson Blair) should be selected as a second selective medium (Cat. 1011).

* For detection of Salmonella spp. in water samples according to ISO 19250:

- Preenrichment in non-selective medium:
Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 34-38 ºC for 18±2 h.
- Enrichment in selective media:
Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) and the MKKTN Broth (Cat. 1173).
The Rappaport Soy Broth is incubated at 41,5±1 ºC and the MKKTN Broth at 34-38 ºC, both of them for 24±3 hours.
- Plating out on selective solid media:
From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (For instance, Brilliant Green Agar (Cat. 1143) or Bismuth Sulfite Agar (Cat. 1011))
Incubate the XLD plates inverted at 34-38 ºC for 24±3 hours.
Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation:
Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Pink	Red-orange	7,4±0,2

Microbiological test

According to ISO 11133:
Incubation conditions: Productivity, Selectivity ISO 6579 (34-38 °C / (24±3 h). Productivity, Selectivity ISO 19250 (36±2 °C) / (24±3 h).
Inoculation conditions: Productivity (10^3-10^4 CFU), Selectivity (10^4-10^6 CFU).

Microorganisms	Specification	Characteristic reaction
Salmonella enteritidis ATCC 13076	Good growth (2)	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
Salmonella typhimurium ATCC 14028	Good growth (2)	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
Escherichia coli ATCC 25922	Growth or partial inhibition (0-1)	Yellow colonies
Enterococcus faecalis ATCC 29212	Total inhibition (0)	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

International Standard UNE-EN-ISO 6579. Food Microbiology for human consumption and Animal Feed. Horizontal Method for the detection of Salmonella spp.
ISO 19250 water quality-detection of Salmonella spp
ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp. Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC.