Xylose-Lysine Deoxycholate Agar (XLD Agar)

M031F

Xylose-Lysine Deoxycholate Agar (XLD Agar) is a selective medium recommended for the isolation, identification and enumeration of *Salmonella* Typhi and other *Salmonella* species in accordance with FDA BAM 1998.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
L-Lysine	5.000
Xylose	3.750
Lactose	7.500
Sucrose	7.500
Sodium desoxycholate	2.500
Ferric ammonium citrate	0.800
Sodium thiosulphate	6.800
Sodium Chloride	5.000
Phenol red	0.080
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.93 grams in 1000 ml distilled water. Heat with frequent agitation until the medium boils. DO NOT AUTOCLAVE OR OVERHEAT. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates . It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate.

Note: Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.

Principle And Interpretation

XLD Agar was formulated by Taylor (1) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species. The media has been recommended for the identification of *Enterobacteriaceae* (2) water and dairy products (3 , 4). This has also been recommended by FDA BAM 1998, for the selective isolation and identification of *Salmonella* from food specimens(5). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media such as SS Agar, EMB Agar and Bismuth Sulphite Agar (1,6) .

The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. Salmonellae rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylated by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. To add to the differentiating ability of the formulation, an H2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H2S producers do not decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies. XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms. Some Proteus strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies. *S* . Paratyphi A, *S* . Choleraesuis, *S* . Pullorum and *S* . Gallinarum may form red colonies without H2S, thus resembling *Shigella* species (7).

According to the BAM procedure (5), 25g of the food sample is pretreated with suitable diluents such as Lactose Broth (M1003) or Buffered Peptone Broth (M614) or Universal Pre-enrichment Broth (M1372F); depending upon the type and nature of the sample. Typically, for specimens with low microbial load, sample to broth ratio has been recommended to be 1:9. Inoculated broth is further incubated at 35 ± 0.2°C for 24 ± 2 hrs. In case of food samples with high microbial load, 0.1 ml of sample mixture is mixed with 10 ml of Tetrathionate broth (M032F) and incubated at 43 ± 0.2°C for 24 ± 2hrs. After incubation, 10 µl of the corresponding broth is inoculated on Xylose Lysine Deoxycholate Agar (M031F). After incubation period of 24 ± 2 hrs at 35°C, plates are checked for *Salmonella* colonies. Typical *Salmonella* colonies appear as pink to red colored with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies. Atypically a few *Salmonella* cultures produce yellow colonies with or without black centers. Cultures identified using XLD agar are further confirmed through biochemical tests.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.69% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 22-26 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response				
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	>=50 %	pink-red with black centres
Salmonella Abony NCTC 6017	50 -100	luxuriant	>=50 %	pink-red with black centres
Salmonella Paratyphi A ATCC 9150	50 -100	good-luxuriant	>=50 %	pink
Salmonella Paratyphi B ATCC 8759	50 -100	good-luxuriant	>=50 %	pink-red with black centres
Salmonella Enteritidis ATCC 13076	50 -100	good-luxuriant	>=50 %	red with black centres
Salmonella Typhi ATCC 6539	50 -100	good-luxuriant	>=50 %	pink-red with black centres

Storage and Shelf Life

Store below 30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference

1.Taylor, W. L. and Schelhart, B. 1969. Appl. Microbiol., 18: 393-395.
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3.Wehr, H.M. and Frank, J.H. 2004. Standard Methods for the Examination of Dairy Products. 17 ed.
4.Andrew, E. D., Rice, E. W., Greenberg, A. E. and S, Clesceri L. 2005. APHA Washington, D.C.
5.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
6.Dunn, C. and Martin, W. J. 1971. Appl. Microbiol., 22: 17-22.
7.MacFaddin, J. F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. vol. 1. Baltimore: Williams and Wilkins.

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