Xylose Lysine Deoxycholate Agar

Intended Use
Xylose Lysine Deoxycholate Agar is moderately selective medium used for isolation and differentiation of *Salmonella* and *Shigella* species.

Summary
XLD Agar is a differential medium used for the isolation of *Salmonella* and *Shigella* from clinical and non-clinical specimens like faeces and foods. It was developed by Taylor in order to increase the efficiency of isolation of the enteric pathogens, particularly *Shigella* from faecal specimens. The pathogens are differentiated not only from the non-pathogenic lactose fermenters but also from many non -pathogens, which do not ferment lactose or sucrose. Also, the medium was formulated to increase the frequency of growth of more fastidious pathogens, which in other formulations have often failed to grow due to the inclusion of excessively toxic inhibitors. This medium is used in the microbial limit test for screening specimens for the detection of *Salmonella* and is recommended by APHA for the examination of foods, dairy products and water.

Principle
XLD Agar is both, a selective and differential medium. Yeast extract provides nutrients while sodium deoxycholate inhibits Gram-positive organisms. Xylose is fermented practically by all enterics except *Shigella*, which enables the differentiation of *Shigella* species. Incorporation of lysine enables the *Salmonella* group to be differentiated from the non-pathogens since, without lysine, *Salmonella* would rapidly ferment xylose and be indistinguishable from non-pathogenic species. After *Salmonella* exhausts the supply of xylose, lysine is attacked, with reversion to an alkaline pH, which mimics the *Shigella* reaction. However, to prevent this reaction by lysine positive coliforms, lactose and sucrose are added in excess to produce acid and hence non-pathogenic H_2S producers do not decarboxylate lysine. The acid reaction produced by them prevents the blackening of the colonies. Sodium thiosulphate and ferric ammonium citrate are included for the visualization of hydrogen sulphide production, resulting in the formation of colonies with black centers. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients	g/L
Sucrose	7.5
Lactose	7.5
Sodium Thiosulphate	6.8
L-Lysine	5.0
Sodium Chloride	5.0
Xylose	3.5
Yeast Extract	3.0
Sodium Deoxycholate	2.5
Ferric Ammonium Citrate	0.8
Phenol Red	0.08
Agar	15.0
Final pH (at 25°C)	7.4 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples - Faeces; Food samples.

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 56.68 g of the powder in 1000 mL purified / distilled water and mix thoroughly.
2. Heat with frequent agitation until the medium just boils to dissolve the powder completely.
3. DO NOT AUTOCLAVE OR OVERHEAT. Overheating causes precipitation.
4. Cool immediately in a water bath at 45°C-50°C and pour into sterile petridishes.

Note: It is advisable not to prepare large volumes that will require prolonged heating.

Quality Control

Dehydrated Appearance: Light yellow to pink coloured, homogeneous free flowing powder.
Prepared Appearance: Light red to red coloured, clear to slightly opalescent gel forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP and growth is observed after an incubation at 30°C-35°C for 18 to 48 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.
Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating > 100 cfu of the appropriate microorganism at at 30°C-35°C for > 48 hours.

Organism (ATCC)	**Growth**	**Colour of Colony**
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Red with black centres
Salmonella enterica subsp. *enterica* serovar *Abony* (NCTC 6017)	Good	Red with black centres
Inhibitory		
Staphylococcus aureus subsp. *aureus* (6538)	Inhibited	-

Note:
1. For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.
2. For inhibition no growth of test microorganism should occur.
3. Inoculum for good growth is 10 - 100 cfu and that for inhibition is greater than 100 cfu.

Interpretation of Results

1. Degradation of xylose, lactose and sucrose leads to formation of acid products, causing a colour change in the medium from red to yellow.
2. H_2S production under alkaline conditions causes colonies to form black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.
3. Lysine decarboxylation in the absence of lactose and sucrose fermentation causes reversion to alkaline condition and the colour of the medium changes back to red.

Typical colonial morphology and reactions on XLD Agar:
E. coli.....Large, flat, yellow; some strains may be inhibited
Proteus.....Red to yellow, most strains have black centers.
Salmonella.....Red- yellow with black centers.
H_2S negative *Shigella, Salmonella* .....Red.
Gram-positive bacteria.....No growth or slight growth.

Precautions/Limitations

1. It is advisable not to prepare large volumes, which will require prolonged heating.
2. Longer incubation may result in false positive results.
3. Some species of *Salmonella* like *S. paratyphi A, S. choleraesuis, S. gallinarum* and *S. pullorum* form red colonies without black centers, which resemble *Shigella* colonies.
4. Also, a few species of *Shigella* that ferment lactose, and *Salmonella* that fail to decarboxylate lysine would not be detected on this medium.
5. Red, false positive colonies may occur with some *Proteus* and *Pseudomonas* species. Some *Proteus* strains will give black centered colonies on XLD Agar.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Taylor W I; 1965, Am. J. Clin. Path; 44: 471.
2. Downes and Ito (ed.) 2001, Compendium of Methods for The Microbiological Examination of Foods,4th edition, APHA Washington DC.
3. H. Wehr and J. Frank, 2004, Std. Methods for The Examination of Dairy Products, 17th Edition; APHA, Washington, DC.
4. Greenberg AE; Clesceri LS and Eaton AD (Eds), 1998, Std thMethods for The Examination of Water and Wastewater, 20th edition, APHA, Washington, DC.
5. US Pharmacopeial Convention, Inc. 2001. The United States Pharmacopoeia 25/NF 20-2002. The US Pharmacopeial Convention, Inc; Rockville, Md.
6. IP, 1996, Ministry of Health and Family Welfare, Govt. of India, Vol. 2.
7. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
8. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
201240020100	Dehydrated Culture Media	100 g
201240020500	Dehydrated Culture Media	500 g