Xylose, Lysine, Deoxycholate Agar

M031B

Xylose-Lysine Deoxycholate Agar is a selective medium recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of BP.

Composition**

Ingredients	Gms / Litre
Xylose	3.500
L-Lysine	5.000
Lacose monohydrate	7.500
Sucrose	7.500
Yeast extract	3.000
Phenol red	0.080
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Sodium chloride	5.000
Agar	13.500
pH after heating (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.8 grams of dehydrated medium in 1000 ml purified/ 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, which will require prolonged heating and may produce precipitate.

Principle And Interpretation

Enterobacteriaceae is a family of gram-negative, non-spore-forming bacilli that contains more than 100 species of bacteria that normally inhabit the intestines of humans and animals. Members forming part of the normal intestinal microflora are referred to as coliforms. The clinically significant genera of *Enterobacteriaceae* include *Cedecea, Citrobacter, Edwardsiella, Enterobacter, Escherichia, Ewingella, Hafnia, Klebsiella, Kluyvera, Proteus, Salmonella, Shigella* and *Yersinia* (1).

The Salmonellae are the most complex of all the *Enterobacteriaceae* . Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk, contaminated by human or animal excreta (2). A large number of media have been developed for the selective isolation and identification of enteric bacilli including *Salmonella* .

Xylose Lysine Deoxycholate Agar is a selective as well as differential medium formulated by Taylor (3-7) for the isolation and identification of enteric pathogens especially Shigellae from stool samples. It is also used for pharmaceutical testing and non-sterile product testing for the detection (or absence) of *Salmonella* after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth (M1491B). This medium is prepared according to BP(9) and is in accordance with the harmonized method of USP/EP/BP/JP (8-11).

Deoxycholate, ferric ammonium citrate and sodium thiosulphate are selective agents that inhibit gram-positive microorganisms. Essential nutrients, growth factors for growth of microorganism are provided by yeast extract. Xylose, sucrose and lactose are the fermentable sugars in this medium. Xylose is fermented by almost all the enteric bacteria except Shigellae, which enable the differentiation of Shigellae from Salmonellae. Salmonellae metabolize the xylose and decarboxylate lysine and thus change the pH to alkaline and mimic Shigellae reaction. However to prevent this reaction by lysine positive coliforms, lactose and sucrose are added in excess to produce acid and hence nonpathogenic H2S producers do not decarboxylate lysine. Sodium thiosulphate helps in reactivation of sulphur containing compounds and prevents the desiccation of these compounds during

storage. It also forms the substrate for enzyme thiosulphate reductase, which breaks it to form H2S. Thiosulphate and ferric ammonium citrate are the H2S indicators in the medium. Sodium thiosulphate is also inactivator of halogens, mercurial and aldehyde and can minimize its toxicity in the testing sample, if any during microbial limit tests. Sodium chloride maintains the osmotic equilibrium in this medium. Phenol red is the pH indicator.

Degradation of fermentable sugars proceed concurrently and generates acids, which cause pH indicator to give various shades of colour, causing a color change in the colonies and in the medium from red to yellow on prolonged incubation. Hydrogen sulfide production results in colonies with black centers under alkaline conditions, which can be inhibited by acid production by carbohydrate fermentation. Alkaline condition causes the color of the medium to change back to red. This medium is an ideal medium for screening samples containing mixed flora of enteric pathogens as recovery of Salmonellae and Shigellae is not conspicuous by even profuse growth of other species (12,13).

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.35% Agar gel

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

pH
7.20-7.60

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of BP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for 18 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 18-72 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100cfu (at 30-35°C for >=72 hours).

Cultural Response
M031B: Cultural characteristics observed after incubation at 30-35 °C for 18-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature	Incubation period
Growth Promoting + Indicative						
Salmonella Typhimurium ATCC 14028	50 -100	25 -100	>=50 %	red with black centres	30 -35 °C	18 -72 hrs
Salmonella Abony NCTC 6017	50 -100	25 -100	>=50 %	red with black centres	30 -35 °C	18 -72 hrs
Additional Microbiological testing						
Escherichia coli ATCC 8739	50 -100	10 -30	20 -30 %	yellow	30 -35 °C	18 -72 hrs
Escherichia coli ATCC 25922	50 -100	10 -30	20 -30 %	yellow	30 -35 °C	18 -72 hrs
Escherichia coli NCTC 9002	50 -100	10 -30	20 -30 %	yellow	30 -35 °C	18 -72 hrs
Proteus vulgaris ATCC 13315	50 -100	25 -100	>=50 %	grey with black centres	30 -35 °C	18 -72 hrs

Salmonella Paratyphi A ATCC 9150	50 -100	25 -100	>=50 %	red	30 -35 °C	18 -72 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	25 -100	>=50 %	red with black centres	30 -35 °C	18 -72 hrs
Salmonella Enteritidis ATCC 13076	50 -100	25 -100	>=50 %	red with black centres	30 -35 °C	18 -72 hrs
Salmonella Typhi ATCC 6539	50 -100	25 -100	>=50 %	red with black centres	30 -35 °C	18 -72 hrs
Shigella dysenteriae ATCC 13313	50 -100	25 -100	>=50 %	red	30 -35 °C	18 -72 hrs
Shigella flexneri ATCC 12002	50 -100	15 -40	30 -40 %	red	30 -35 °C	18 -72 hrs
Shigella sonnei ATCC 25931	50 -100	15 -40	30 -40 %	red	30 -35 °C	18 -72 hrs
Enterobacter aerogenes ATCC 13048	50 -100	10 -30	20 -30 %	yellow	30 -35 °C	18 -72 hrs
Enterobacter cloacae ATCC 13047	50 -100	10 -30	20 -30 %	yellow	30 -35 °C	18 -72 hrs
Staphylococcus aureus ATCC 25923	>=10^3	0	0%		30 -35 °C	>=72 hrs
Staphylococcus aureus ATCC 6538	>=10^3	0	0%		30 -35 °C	>=72 hrs
Enterococcus faecalis ATCC 29212	>=10^3	0	0%		30 -35 °C	>=72 hrs

Storage and Shelf Life

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

Reference

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