

## Technical Information

### XLD Agar (Xylose-Lysine-Deoxycholate Agar)

#### Product Code: DM 1031H

**Application:** 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 USP/EP/BP/JP/IP (Medium 11).

#### Composition\*\*

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

\*\*Formula adjusted, standardized to suit performance parameters

#### Principle & 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* <sup>(1)</sup>.

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

Xylose Lysine Deoxycholate Agar a selective as well as differential medium formulated by Taylor <sup>(3-7)</sup> in being used 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 (DM1491) in accordance with the harmonized method of USP/EP/BP/JP/IP <sup>(8-12)</sup>.

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 H<sub>2</sub>S 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 H<sub>2</sub>S. Thiosulphate and ferric ammonium citrate are the H<sub>2</sub>S 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 <sup>(13, 14)</sup>.

## Methodology

Suspend 54.8 grams of dehydrated medium in 1000 ml purified/ distilled water. Shake well & heat with frequent agitation until the medium boils. DO NOT HEAT IN AN AUTOCLAVE. 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.

## Quality Control

### Physical 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 range** 7.20-7.60

### Cultural Response

Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP. 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  $\leq 100$  cfu (at 30-35°C for  $\leq 18$  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  $\leq 100$  cfu (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  $\geq 100$  cfu (at 30-35°C for  $\geq 72$  hours).

### Cultural Response

DM 1031H: 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)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	Luxuriant	25-100	$\geq 50\%$	Red with black centres	18-72 hrs
<i>Salmonella Abony</i> NCTC 6017	50-100	Good-Luxuriant	25-100	$\geq 50\%$	Red with black centres	18-72 hrs
<b>Growth Promoting + Indicative</b>						
<i>Escherichia coli</i> ATCC 8739	50-100	Fair	10-30	20-30%	Yellow	18-72 hrs
<i>Escherichia coli</i> ATCC 25922	50-100	Fair	10-30	20-30%	Yellow	18-72 hrs
<i>Escherichia coli</i> NCTC 9002	50-100	Fair	10-30	20-30%	Yellow	18-72 hrs
<i>Proteus vulgaris</i> ATCC 13315	50-100	Good-Luxuriant	25-100	$\geq 50\%$	Grey with black centres	18-72 hrs
<i>Salmonella Paratyphi A</i> ATCC 9150	50-100	Good-Luxuriant	25-100	$\geq 50\%$	Red	18-72 hrs



Dehydrated Culture Media  
Bases / Media Supplements

<i>Salmonella Paratyphi B</i> ATCC 8759	50-100	Good-Luxuriant	25-100	>=50%	Red with black centers	18-72 hrs
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	Good-Luxuriant	25-100	>=50%	Red with black centers	18-72 hrs
<i>Salmonella Typhi</i> ATCC 6539	50-100	Good-Luxuriant	25-100	>=50%	Red with black centers	18-72 hrs
<i>Shigella dysenteriae</i> ATCC 13313	50-100	Good-Luxuriant	25-100	>=50%	Red	18-72 hrs
<i>Shigella flexneri</i> ATCC 12002	50-100	Fair-good	15-40	30-40%	Red	18-72 hrs
<i>Shigella sonnei</i> ATCC 25931	50-100	Fair-good	15-40	30-40%	Red	18-72 hrs
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	Fair	10-30	20-30%	Yellow	18-72 hrs
<i>Enterobacter cloacae</i> ATCC 13047	50-100	Fair	10-30	20-30%		18-72 hrs
<i>Staphylococcus aureus</i> ATCC 25923	>=10 <sup>3</sup>	inhibited	0	0%		>=72 hrs
<i>Staphylococcus aureus</i> ATCC 6538	>=10 <sup>3</sup>	inhibited	0	0%		>=72 hrs
<i>Enterococcus faecalis</i> ATCC 29212	>=10 <sup>3</sup>	inhibited	0	0%		>=72 hrs

## Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

**Prepared Media:** 2-8<sup>0</sup> in sealable plastic bags for 2-5 days.

## Further Reading

1. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed., Tata McGraw-Hill Publishing Company Ltd, New Delhi.
2. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippincott Company.
3. Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
4. Taylor W. L. and Harris B., 1965, Am. J. Clin. Pathol., 44:476.
5. Taylor W. L. and Harris B., 1967, Am. J. Clin. Pathol., 48:350.
6. Taylor W. L. and Schelhart B., 1967, Am. J. Clin. Pathol., 48:356.
7. Taylor W. L. and Schelhart B., 1968, Am. J. Clin. Pathol., 16:1387.2. ,,
8. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
9. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia.
10. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
11. Japanese Pharmacopoeia, 2008.
12. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India.
13. McCarthy M.D., 1966, N.Z. J. Med. Lab. Technol., 20: 127.
14. Isenberg H.D., Kominos S. and Siegal M., 1969, Appl. Microbiol., 18:656.

## Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specifications for identity and performance parameters.

