



Ready Prepared Media

Technical Information

Xylose Lysine Deoxycholate Agar Plate

Product Code: PM 1031H

Application: Recommended as a selective medium 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

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* (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 (MH1491) in accordance with the harmonized method of USP/EP/BP/JP/IP (8,9,10,11,12). 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 (13,14).



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Type of specimen

Pharmaceutical samples

Specimen Collection and Handling:

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8,9,10,11,12). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Slight precipitation in the medium may occur, which is inheritant property of the medium, and does not affect the performance of the medium.
2. 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.
3. *S. Paratyphi A*, *S. Choleraesuis*, *S. Pullorum* and *S. Gallinarum* may form red colonies without H₂S, thus resembling *Shigella* species.
4. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
5. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
6. It is recommended to store the plates at 24-30°C to avoid minimum condensation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance

Sterile Xylose lysine deoxycholate Agar in 90 mm disposable plates with smooth surface and absence of black particles/bubbles/cracks

Colour of medium

Red coloured medium

Quantity of medium

25 ml of medium in 90 mm disposable plates.

pH

7.20-7.60

Sterility Check

Passes release criteria

Cultural Response

Cultural response was observed after an incubation at 35-37°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.



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Growth Promotion Test

Growth Promotion was carried out in accordance with the harmonized method of (USP/EP/BP/JP), after an incubation at 30-35 °C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar and fungus growth on Sabouraud Dextrose Agar.

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 ≤ 100 cfu (at 30-35°C for 24-48 hours).

Inhibitory properties

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

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation period
Growth promoting+Indicative						
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	≥ 50 %	red with black centres	18 -72 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	≥ 50 %	red with black centres	18 -72 hrs
Indicative						
Escherichia coli ATCC 8739 (00012*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Additional microbiological testing						
Escherichia coli ATCC 25922 (00013*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Proteus vulgaris ATCC 13315	50 -100	good-luxuriant	25-100	≥ 50 %	grey with black centres	18 -72 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	good-luxuriant	25-100	≥ 50 %	red	18 -72 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	good-luxuriant	25-100	≥ 50 %	red with black centres	18 -72 hrs
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	25-100	≥ 50 %	red with black centres	18 -72 hrs
Salmonella Typhi ATCC 6539	50 -100	good-luxuriant	25-100	≥ 50 %	red with black centres	18 -72 hrs
Shigella dysenteriae ATCC 13313	50 -100	good-luxuriant	25-100	≥ 50 %	red	18 -72 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair good	25-100	30-40 %	red	18 -72 hrs
Shigella sonnei ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
# Klebsiella aerogenes ATCC 13048	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Enterobacter cloacae ATCC 13047 (00083*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	$\geq 10^3$	inhibited	0	0%		≥ 72 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	$\geq 10^3$	inhibited	0	0%		≥ 72 hrs
Enterococcus faecalis	$\geq 10^3$	inhibited	0	0%		≥ 72 hrs



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ATCC 29212 (00087*)

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 20-30°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Further Reading

- 1.King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
- 2.The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
- 3.European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
- 4.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
- 5.The Japanese Pharmacopoeia, 17th edition, 2016, The Ministry of Health, Labour and welfare.
- 6.The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
- 7.Lowbury E J L., 1951, J.Clin.Path., 4:66.
- 8.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
- 9.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. , 11th Ed., 2015, Manual of Clinical Microbiology.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and developmentwork carried at **CDH** is true and accurate
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