

Xylose Lysine Deoxycholate HiCynth™ Agar (XLD HiCynth™ Agar)

MCD031

Xylose-Lysine Deoxycholate HiCynth™ Agar (XLD HiCynth™ Agar) is a selective medium recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.5*	4.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Synthetic detergent No. III	1.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptones

Directions

Suspend 56.68 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.

Principle And Interpretation

XLD Agar was formulated by Taylor (1-5). XLD HiCynth™ Agar is chemically defined media free from animal and vegetable peptones for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species. XLD Agar has been recommended for the identification of *Enterobacteriaceae* (7) and for the microbiological testing of foods, water and dairy products (8-12). It exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar, EMB Agar and Bismuth Sulphite Agar (2, 4, 6, and 13-16). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (17). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (18).

The medium contains HiCynth™ Peptone No.5, which provides nitrogen and carbon source, long chain amino acids and vitamins required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by *Shigellae* but practically by all enterics. This helps in the differentiation of *Shigella* species. 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 that mimics the *Shigella* reaction. 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 decarboxylase lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies (1).

XLD HiCynth™ 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 (19).

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 5.67% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

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.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response				
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	≥50 %	red with black centres
<i>Salmonella Abony</i> NCTC 6017	50 -100	good-luxuriant	≥50 %	red with black centres
<i>Escherichia coli</i> ATCC 8739	50 -100	fair	20 -30 %	yellow
<i>Escherichia coli</i> ATCC 25922	50 -100	fair	20 -30 %	yellow
<i>Escherichia coli</i> NCTC 9002	50 -100	fair	20 -30 %	yellow
<i>Proteus vulgaris</i> ATCC 13315	50 -100	good-luxuriant	≥50 %	grey with black centres
<i>Salmonella Paratyphi A</i> ATCC 9150	50 -100	good-luxuriant	≥50 %	red
<i>Salmonella Paratyphi B</i> ATCC 8759	50 -100	good-luxuriant	≥50 %	red with black centres
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	good-luxuriant	≥50 %	red with black centres
<i>Salmonella Typhi</i> ATCC 6539	50 -100	good-luxuriant	≥50 %	red with black centres
<i>Shigella dysenteriae</i> ATCC 13313	50 -100	good-luxuriant	≥50 %	red
<i>Shigella flexneri</i> ATCC 12022	50 -100	fair-good	30 -40 %	red
<i>Shigella sonnei</i> ATCC 25931	50 -100	fair-good	30 -40 %	red
<i>Enterobacter aerogenes</i> ATCC 13048	50 -100	fair	20 -30 %	yellow
<i>Enterobacter cloacae</i> ATCC 13047	50 -100	fair	20 -30 %	yellow

<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0%
<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	inhibited	0%
<i>Enterococcus faecalis</i> ATCC 29212	$\geq 10^3$	inhibited	0%

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

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