



Xylose-Lysine Deoxycholate Agar (XLD Agar)

M031

Intended use

Recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
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

Directions

Suspend 56.68 grams 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 that will require prolonged heating, thereby producing precipitate. **Note :** Slight precipitation in the medium may occur, which is inheritant property of the medium, and does not affect the performance of the medium.

Principle And Interpretation

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (1) and for the microbiological testing. XLD Agar was formulated by Taylor (2-6) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species of foods, water and dairy products (7-11). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar (M108), EMB Agar (M022) and Bismuth Sulphite Agar (M027) (3,5,7,12-15). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (16). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (M1482) (17). It is also recommended by FDA (18).

The medium contains yeast extract, which provides nitrogen 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 decarboxylate 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 (2).

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

Type of specimen

Clinical samples - Faeces; Food and dairy samples; Water samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (19,20). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. 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 clinical 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. This medium is general purpose medium and may not support the growth of fastidious organisms.
3. Some *Proteus* strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions.
4. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies.
5. *S. Paratyphi A*, *S. Choleraesuis*, *S. Pullorum* and *S. Gallinarum* may form red colonies without H₂S, thus resembling *Shigella* species.

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.

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.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation period
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs

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<i>\$ Proteus hauseri</i> ATCC 13315	50 -100	good-luxuriant	25 -100	≥ 50 %	grey with black centres	18 -72 hrs
<i>Salmonella</i> Paratyphi A ATCC 9150	50 -100	good-luxuriant	25 -100	≥ 50 %	red	18 -72 hrs
<i>Salmonella</i> Paratyphi B ATCC 8759	50 -100	good-luxuriant	25 -100	≥ 50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	25 -100	≥ 50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Typhi ATCC 6539	50 -100	good-luxuriant	25 -100	≥ 50 %	red with black centres	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 12022 (00126*)	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>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Enterobacter cloacae</i> ATCC 13047 (00083*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	$\geq 10^4$	inhibited	0	0%		≥ 72 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	$\geq 10^4$	inhibited	0	0%		≥ 72 hrs
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	$\geq 10^4$	inhibited	0	0%		≥ 72 hrs

Key : *Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes* \$ Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (19,20).

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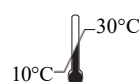
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