# XLD Agar, Modified

**M031I**

XLD Agar, Modified is recommended for the isolation and enumeration of *Salmonella Typhi* and other *Salmonella* species.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Yeast extract | 3.000 |
| L-Lysine hydrochloride | 5.000 |
| Lactose | 7.500 |
| Sucrose | 7.500 |
| Xylose | 3.750 |
| Sodium chloride | 5.000 |
| Sodium deoxycholate | 1.000 |
| 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 55.43 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.

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 was formulated by Taylor (1-5) for the isolation and differentiation of enteric pathogens including *Salmonella Typhi* from other *Salmonella* species. XLD Agar, Modified (M031I) is recommended for selective isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species in accordance with ISO Committee (20).

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (7) and for the microbiological testing of foods, water and dairy products (8-12). 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) (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 (M1482) (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 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 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 5.54% 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 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

**Cultural Response**

| Organism | Inoculum (CFU) | Growth | Observed Lot value (CFU) | Recovery | Colour of Colony | Incubation temperature |
|---|---|---|---|---|---|---|
| **Cultural Response** | | | | | | |
| *Salmonella Typhimurium ATCC 14028* | 50 -100 | luxuriant | 25 -100 | >=50 % | red with black centres | 18 -72 hrs |
| *Salmonella Abony NCTC 6017* | 50 -100 | good-luxuriant | 25 -100 | >=50 % | red with black centres | 18 -72 hrs |
| *Escherichia coli ATCC 8739* | 50 -100 | fair | 10 -30 | 20 -30 % | yellow | 18 -72 hrs |
| *Escherichia coli ATCC 25922* | 50 -100 | fair | 10 -30 | 20 -30 % | yellow | 18 -72 hrs |
| *Escherichia coli NCTC 9002* | 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 |
| *Proteus mirabilis ATCC 25933* | 50 -100 | good-luxuriant | | >=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* | 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 12002* | 50 -100 | fair-good | 15 -40 | 30 -40 % | red | 18 -72 hrs |
| *Shigella sonnei ATCC 25931* | 50 -100 | fair-good | 15 -40 | 30 -40 % | red | 18 -72 hrs |
| *Enterobacter aerogenes ATCC 13048* | 50 -100 | fair | 10 -30 | 20 -30 % | yellow | 18 -72 hrs |
| *Enterobacter cloacae ATCC 13047* | 50 -100 | fair | 10 -30 | 20 -30 % | yellow | 18 -72 hrs |
| *Staphylococcus aureus ATCC 25923* | >=$10^3$ | inhibited | 0 | 0% | | >=72 hrs |
| *Staphylococcus aureus ATCC 6538* | >=$10^3$ | inhibited | 0 | 0% | | >=72 hrs |

| | | | | |
|---|---|---|---|---|
| *Enterococcus faecalis ATCC 29212* | >=$10^3$ | inhibited | 0 | 0% | >=72 hrs |

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 8°C. Use before expiry date on the label.

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