



## XLD Agar

M031F

### Intended Use:

Recommended for isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species in accordance with FDA BAM, APHA.

### Composition\*\*

	FDA BAM		M031F
Ingredients	g / L	Ingredients	g / L
Yeast extract	3.000	Yeast extract	3.000
L-Lysine	5.000	L-Lysine	5.000
Xylose	3.750	Xylose	3.750
Lactose	7.500	Lactose	7.500
Sucrose	7.500	Sucrose	7.500
Sodium desoxycholate	2.500	Sodium desoxycholate	2.500
Ferric ammonium citrate	0.800	Ferric ammonium citrate	0.800
Sodium thiosulphate	6.800	Sodium thiosulphate	6.800
Sodium Chloride	5.000	Sodium Chloride	5.000
Phenol red	0.080	Phenol red	0.080
Agar	15.000	Agar	15.000
Final pH	7.4±0.2	Final pH ( at 25°C)	7.4±0.2

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

### Directions

Suspend 56.93 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 was formulated by Taylor (1) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species. The media has been recommended for the identification of *Enterobacteriaceae* (2) water and dairy products (3,4). This has also been recommended by FDA BAM, APHA for the selective isolation and identification of *Salmonella* from food specimens (5,6). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media such as SS Agar, EMB Agar and Bismuth Sulphite Agar (7,1). The medium contains yeast extract, which provides nitrogen and vitamins required for growth. 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. 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 H<sub>2</sub>S 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 H<sub>2</sub>S producers do not decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies. 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 H<sub>2</sub>S, thus resembling *Shigella* species (8).

### Type of specimen

Food samples

## Specimen Collection and Handling:

According to the BAM procedure (5), 25g of the food sample is pretreated with suitable diluents such as Lactose Broth (M1003) or Buffered Peptone Broth (M614) or Universal Pre-enrichment Broth (M1372F); depending upon the type and nature of the sample. Typically, for specimens with low microbial load, sample to broth ratio has been recommended to be 1:9. Inoculated broth is further incubated at  $35 \pm 0.2^\circ\text{C}$  for  $24 \pm 2$  hrs. In case of food samples with high microbial load, 0.1 ml of sample mixture is mixed with 10 ml of Tetrathionate broth (M032F) and incubated at  $43 \pm 0.2^\circ\text{C}$  for  $24 \pm 2$  hrs. After incubation, 10  $\mu\text{l}$  of the corresponding broth is inoculated on Xylose Lysine Deoxycholate Agar (M031F). After incubation period of  $24 \pm 2$  hrs at  $35^\circ\text{C}$ , plates are checked for *Salmonella* colonies. Typical *Salmonella* colonies appear as pink to red colored with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies. Atypically a few *Salmonella* cultures produce yellow colonies with or without black centers. Cultures identified using XLD agar are further confirmed through biochemical tests.

## 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. 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  $\text{H}_2\text{S}$ , thus resembling *Shigella* species.
6. Atypical *Salmonella* species which are lactose positive and/or  $\text{H}_2\text{S}$  negative should be confirmed by biochemical and serological tests.

## 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 5.69% w/v aqueous solution at  $25^\circ\text{C}$ . pH :  $7.4 \pm 0.2$

### pH

7.20-7.60

### Cultural Response

Cultural characteristics observed after an incubation at  $35-37^\circ\text{C}$  for 22-26 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	$\geq 50\%$	pink-red with black centres
<i>Salmonella</i> Abony NCTC 6017	50 -100	luxuriant	$\geq 50\%$	pink-red with black centres
<i>Salmonella</i> Paratyphi A ATCC 9150	50 -100	good-luxuriant	$\geq 50\%$	pink
<i>Salmonella</i> Paratyphi B ATCC 8759	50 -100	good-luxuriant	$\geq 50\%$	pink-red with black centres
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	$\geq 50\%$	red with black centres
<i>Salmonella</i> Typhi ATCC 6539	50 -100	good-luxuriant	$\geq 50\%$	pink-red with black centres

Key : (\*)Corresponding WDCM numbers.

### 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

### Reference

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