XLD Agar, Modified

M031I

Intended use

Recommended for selective isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species. The composition and performance criteria of this medium are as per specifications laid down in ISO 6579-1:2017./ Amd: 2020, ISO 19250:2010(E) and APHA.

Composition**

ISO 6579-1 Specification - XLD Agar

Ingredients	g/ L
Yeast extract	3.000
L-Lysine hydrochloride	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.750
Sodium chloride (NaCl)	5.000
Sodium deoxycholate	1.000
Sodium thiosulphate	6.800
Iron (III) ammonium citrate	0.800
Phenol red	0.080
Agar	9.00-18.00
Final pH (at 25°C)	7.4±0.2

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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

- Equivalent to Iron (III) ammonium citrate

Directions

Suspend 55.43 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 which 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 (1-6) 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* species in accordance with ISO Committee, APHA (7-11). The incubation conditions has been revised as per the amendment 1, 2020 (8). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* . 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 H_2S 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_2S 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.

Type of specimen

Food and meat samples. milk and milk products, animal feed, animal faeces, environmental samples, Water samples

Specimen Collection and Handling:

Processesing : (7)

Pre-enrichment : Samples (25 grams in 225 ml) are pre-enriched in Buffered Peptone Water (M1494I)/(GM1494I) and incubated at 34°C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre-enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTTn broth (M1496I) and incubated at 36± 2°C for 24 ± 3 hours .

Isolation : The culture thus obtained is then plated on XLD Agar, Modified (M031I) and incubated at 36± 2°C for 24 ± 3 hours . Simultaneously plating on second isolation agar is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

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. XLD Agar is based on fermentation reaction and H_2S production hence second medium should be selected so as to detect lactose positive and H_2S negative strains.
3. *S.*Paratyphi A, *S.*choleraesuis, *S.*pullorum and *S.*gallinarum may form red colonies without H_2S, thus resembling *Shigella* species.
4. Atypical *Salmonella* species which are lactose positive and/or H_2S 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 5.54% 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 34°C to 38°C for for 24 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Productivity				
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	good	>=50 %	red with black centres
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good	>=50 %	red with black centres
Selectivity				
Escherichia coli ATCC 8739 (00012*)	>=10^4	growth or partial inhibition		yellow

Escherichia coli ATCC 25922 (00013*)	>=10^4	growth or partial inhibition		yellow
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0 %	-
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0 %	-

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 (12,13).

Reference

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