Sulfite Indole Motility Agar (SIM Agar)

M181I

Intended use

Recommended for determination of hydrogen sulphide production, indole formation and motility of *Clostridium* spp. The composition and performance criteria of this medium are as per the specifications laid down in ISO 15213:2-2023 and ISO 11133-2:2014 /Amd.2 :2020 (E)

Composition**

ISO 15213:2-2023		**SIM Agar**	**M181I**
Ingredients	**g / L**	**Ingredients**	**g / L**
Peptone	6.000	Peptone	6.000
Enzymatic digest of soy	20.000	Soya peptone #	20.000
Ferrous ammonium sulphate	0.200	Ferrous ammonium sulphate	0.200
Sodium thiosulphate	0.200	Sodium thiosulphate	0.200
Agar	2.50-4.50	Agar	3.000

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Soya peptone

Directions

Suspend 29.40 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Dispense 10ml in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in an upright position.

Principle And Interpretation

Clostridium is a Gram-positive, anaerobic, spore forming bacterium. It is a ubiquitous bacterium, predominantly found in soil, but also in the intestinal tract of humans and animals. The high numbers of *C. perfringens* in food can cause human illness, mainly diarrhoea. SIM Agar is recommended in ISO committee for the confirmation of *Clostridium* spp. from food, feed and environmental samples (1). Also the medium is used to differentiate enteric bacilli particularly *Salmonella* and *Shigella* on the basis of sulphide production, indole formation and motility (2).

SIM Agar enables determination of three characteristics by which the bacteria can be differentiated. Sodium thiosulphate is the indicator of H_2S production. This H_2S reacts with peptonized iron to form black precipitate of ferrous sulphide (3,4). Motile organisms intensify the H_2S reaction. Motile organisms grow away from line of inoculation showing diffused growth while non-motile organisms grow along the stab line. Motility detection is possible due to the semisolid nature of the medium. Growth radiating out from the central stab line indicates that the test organism is motile. Peptone and soya peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Tryptophan from peptone, is degraded by specific bacteria to produce indole. The indole is detected by the addition of chemical reagents following the incubation period. Inoculate fresh culture with a single stab using straight needle through the center of the medium. Following incubation, observe for motility (diffuse growth outward from the stab line or turbidity throughout the medium) and for H_2S production (blackening of the medium). To detect indole production, add three or four drops of Kovacs reagent and observe for development of red color (positive reaction). Determine motility and H_2S production prior to determination of indole production.

Type of specimen

Food and feed samples, Environmental samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (1).

After use, contaminated materials must be sterilized by autoclaving before discarding.

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. The test culture should be in pure form and other biochemical and serological tests are also to be carried out for complete identification.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

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

Cream to beige homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured slightly opalescent gel forms in tubes as butts

Cultural Response

Cultural characteristics observed after an incubation at 37±1°C for 22 ± 2 hours under anaerobic atmosphere.

Organism	Growth	Motility	Indole production(on addition of Kovac's	H_2S
Escherichia coli ATCC 25922 (00013*)	luxuriant	positive, growth away from stabline causing turbidity	positive reaction, red ring at the interface of the medium	negative reaction
Escherichia coli ATCC 8739(00012*)	luxuriant	positive, growth away from stabline causing turbidity	positive reaction, red ring at the interface of the medium	negative reaction
Clostridium perfringens ATCC 13124 (00007*)	luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	positive reaction, blackening of medium
Clostridium perfringens ATCC 12916 (00080*)	luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	positive reaction, blackening of medium
Clostridium perfringens ATCC 10543 (00174*)	luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	positive reaction, blackening of medium

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10- 30°C in a tightly closed container and the prepared medium at 15-30°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 (6,7).

Reference

1. Microbiology of the food chain —Horizontal method for detection and enumeration of *Clostridium* spp. ; ISO 15213-2:2023.
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5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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