Bismuth Sulphite Agar Modified

M1004

Intended use

Recommended for the selective isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water supplies, food etc.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM Peptone B#	5.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate	4.000
Ferrous sulphate	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.016
Agar	12.700
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 40 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. DO NOT STERILIZE IN AUTOCLAVE or by fractional sterilization since overheating may destroy the selectivity of the medium. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, which should be dispersed before pouring into the sterile Petri plates.

Principle And Interpretation

The Salmonellae constitute the most taxonomically complex group of bacteria among *Enterobacteriaceae* (12). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Humans are the only reservoirs of *S.* Typhi (8). Four clinical types of *Salmonella* infections may be distinguished (10) namely gastroenteritis, bacteremia or septicemia, enteric fever and a carrier state. Of the various media employed for the isolation and preliminary identification of Salmonellae, particularly *Salmonella* Typhi; Bismuth Sulphite Agar is the most productive (4).

Bismuth Sulphite Agar, Modified is a modification of the original formulation of Wilson and Blair Medium (14). It is also recommended for the isolation of *Salmonella* Typhi and other Salmonella (2, 5).

*S.*Typhi, *S.*Enteritidis and *S.*Typhimurium typically grow as black colonies with a surrounding metallic sheen resulting from hydrogen sulphide production and reduction of sulphite to black ferric sulphide. *Salmonella* Paratyphi A grows as light green colonies. Bismuth Sulphite Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms. *Shigella* species are mostly inhibited on this medium; exceptions being *S. flexneri* and *S.*sonnei (9) and also some *Salmonella* like *S.*Sendai, *S.*Berta, *S.*Gallinarum, *S.*Abortus-equi are inhibited (9). Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action toward gram-positive organisms and coliforms.

Peptone and HM peptone B serve as sources of carbon, nitrogen, vitamins and essential growth factors. Dextrose (Glucose) is the carbon source. Disodium hydrogen phosphate maintains the osmotic equilibrium. Bismuth sulphite indicator along with brilliant green inhibits the intestinal gram-positive and gram-negative bacteria. Ferrous sulphate aids in detection of hydrogen sulphide production. Clinical samples can be directly used to inoculate Bismuth Sulphite Agar. In case of food samples, pre enrichment of the sample is done prior to inoculation.

Type of specimen

Clinical samples - Blood, faeces and other pathological specimen ; Food and dairy samples ; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,11,13).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic 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. DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM, as it destroys the selectivity of the medium.
2. *S.*Typhi exhibit typical brown colonies, with or without metallic sheen.
3. This medium is highly selective and must be used in parallel with less selective media for isolation.
4. With certain *Salmonella* species, typical black colonies with metallic sheen is observed near heavy inoculation and isolated colonies may show green colonies.
5. *Shigella* species are mostly inhibited on this medium; exceptions being *S. flexneri* and *S. sonnei* .
6. Some *Salmonella* like *S.* Sendai, *S.* Berta, *S.* Gallinarum, *S.* Abortus-equi are also inhibited .

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 greenish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.27% Agar gel.

Colour and Clarity of prepared medium
Greenish yellow coloured opalescent with flocculent precipitate forms in Petri plates.

Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH
7.40-7.80

Cultural Response
M1004: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Preparation of test strain Cultural Response				
# Klesiella aerogenes ATCC 13048 (00175)*	50-100	none-poor	<=10%	brown-green(depends on inoculum density)
Enterococcus faecalis ATCC 29212 (00087)*	>=10^3	inhibited	0%	
Escherichia coli ATCC 25922 (00013)*	50-100	none-poor	<=10%	brown-green(depends on inoculum density)
Salmonella Typhi ATCC 19430	50-100	good-luxuriant	>=50%	black with metallic sheen

Salmonella Paratyphi B ATCC 8759	50-100	good-luxuriant	>=50%	black with metallic sheen
Salmonella Enteritidis ATCC 13076 (00030)*	50-100	good-luxuriant	>=50%	black with metallic sheen
Shigella flexneri ATCC 12022 (00126)*	50-100	none-poor	<=10%	brown
Salmonella Typhimurium ATCC 14028 (00031)*	50-100	good-luxuriant	>=50%	black with metallic sheen

Key : (*) - Corresponding WDCM numbers.

(#)- Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C, but not for more than 2 days as after which dye oxidizes to give green medium that could be inhibitory to some Salmonellae. 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. Use before expiry date on the label.
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 (6,7).

Reference

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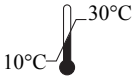
IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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