HIMEDIA

Technical Data

Bismuth Sulphite Agar Medium

MU027

Intended Use:

Recommended for selective isolation of *Salmonellae* from faeces, urine, sewage and other materials in accordance with USP.

Composition**

Ingredients	g/ L
Peptone	5.000
HM peptone B #	5.000
Tryptone	5.000
Dextrose (Glucose)	5.000
Sodium phosphate	4.000
Ferrous sulphate	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters
#Equivalent to Beef extract

Directions

Suspend 52.32 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT OVERHEAT OR STERILIZE IN AUTOCLAVE** or by fractional sterilization since overheating may destroy the selectivity of the medium. Transfer to a water bath maintained at about 50°C.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

Bismuth Sulphite Agar Medium is prepared in accordance with USP (1) and is employed for the isolation and preliminary identification of *Salmonella* Typhi and other *Salmonellae* from pathological materials, sewage, water, food and other products. Bismuth Sulphite Agar is recommended by various Associations (2,3,4,5,6) for the isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water, food, pharmaceutical and other products. It is a modification of Wilson and Blair medium.

Brilliant green and bismuth sulphite incorporated into the medium inhibit the intestinal gram-negative and gram-positive bacteria, peptone, tryptone and HM peptone B are rich source for supplying essential nutrients for growth of the organism. The fermentable source of carbohydrate in this medium is dextrose, which provides energy for enhanced microbial growth. Phosphates incorporated in the medium act as a good buffering agent. The bismuth ions are reduced to metallic bismuth, which impart the metallic sheen around the colonies. Sulphite is reduced to black ferric sulphide giving the black colour with release of H_2S.

Salmonella Enteritidis and *Salmonella* Typhimurium typically grow as black colonies (rabbit eye colonies) with a surrounding metallic sheen. *Salmonella* Paratyphi A grow as light green colonies. This medium also favors use of larger inoculum and heavily contaminated samples as compared to other selective media, as it has unique inhibitory action towards gram-positive and coliform organisms. The medium 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 and also some Salmonellae like *S.*Sendai, *S.*Berta, *S.* Gallinarum, *S.*Abortus-equally are inhibited. *Proteus* species are inhibited but few strains give dull green or brown colonies with metallic sheen.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility (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. **DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM,** as it destroys the selectivity of the medium.
2. *S.*Typhi and *S.*Arizonae 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* (7)
6. Some Salmonella like *S.* Sendai, *S.* Berta, *S.* Gallinarum, *S.* Abortus-equi are also inhibited (7).

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and clarity of prepared medium

Yellow to greenish yellow opalescent with flocculant precipitate

Reaction

Reaction of 5.23% w/v aqueous solution. pH : 7.6±0.2

pH

7.40-7.80

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of USP. Cultural response was observed after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Cultural Response

Cultural characteristics observed after incubation at 30-35 °C for 24-48 hours. Recovery rate is considered as 100%for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of Colony
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	black or greenish-grey may have sheen
Salmonella Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	>=50 %	black with metallic sheen
Additional Microbiological testing					
#*Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	none-poor	0 -10	0 -10 %	brown-green (depends on the inoculum density)
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0	0%	
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	black with metallic sheen
Salmonella Typhi ATCC 6539	50 -100	luxuriant	50 -100	>=50 %	black with metallic sheen

Shigella flexneri ATCC 12022 (00126*)	50 -100	none-poor	0 -10	<=10 %	brown
Escherichia coli ATCC 8739 (00012*)	50 -100	none-poor	0 -10	<=10 %	Brown to green, depends on inoculum density

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. 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 (8,9).

Reference

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Revision :04/2024