Bismuth Sulphite Agar Medium

MU027

Bismuth Sulphite Agar Medium is recommended for the selective isolation of Salmonellae from faeces, urine, sewage and other materials in accordance with United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	5.000
Beef extract	5.000
Peptic digest of animal tissue	5.000
Dextrose	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

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, Peptic digest of animal tissue, pancreatic digest of casein and beef extract 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 H2S.

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.

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

Cultural Response
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
MU027: 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
Cultural Response					
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	25 -100	>=50 %	black or greenish-grey may have sheen
Salmonella Abony NCTC 6017	50 -100	good-luxuriant	25 -100	>=50 %	black with metallic sheen
Additional Microbiological testing					
Enterobacter aerogenes ATCC 13048	50 -100	none-poor	0 -10	0 -10 %	brown-green (depends on the inoculum density)
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0	0%	
Salmonella Enteritidis ATCC 13076	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	50 -100	none-poor	0 -10	<=10 %	brown
Escherichia coli ATCC 8739	50 -100	none-poor	0 -10	<=10 %	Brown to green, depends on inoculum density

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°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.

Reference
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5. Murray PR, Baren EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors) 2003, Manual of clinical Microbiology, 8th ed., ASM, Washington, D.C..
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