Bismuth Sulphite Agar Medium (Twin Pack)

MM027

Bismuth Sulphite Agar Medium is recommended for the selective isolation and identification of Salmonellae in accordance with Indian Pharmacopoeia , 1996.

Composition**

Ingredients	Gms / Litre
Part A (Solution 1)	-
Beef extract	6.000
Peptone	10.000
Brilliant green	0.010
Ferric citrate	0.400
Agar	24.000
Part B (Solution 2)	-
Ammonium bismuth citrate	3.000
Sodium sulphite	10.000
Anhydrous disodium hydrogen phosphate	5.000
Dextrose monohydrate	5.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.4 grams of Part A (Solution 1) in 1000 ml ditilled water. Heat to boiling to dissolve the medium completely. Sterilize by maintaining at 115ºC for 30 minutes.

Suspend 22.54 grams the equivalent weight of dehydrated medium per litre)of Part B (Solution 1) in 100 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE
Add one volume of Part B solution to ten volumes of Part I solution previously melted and cooled at a temperature of 55°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.

Note : The medium should be stored at 2-8°C for 5 days before use .

Principle And Interpretation

Bismuth Sulphite Agar is recommended by various Associations (1, 2, 3, 4, 5, 6) for the isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water, food 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 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 of enhanced microbial growth. Phosphates incorporated in the medium acts as a good buffering agent. The bismuth ions are reduced to metallic bismuth, which impart 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

Part A

Light yellow to greenish yellow homogeneous free flowing powder

Part B

White to cream homogeneous free flowing powder

Gelling

Firm, comparable with 2.4% agar gel.

Colour and Clarity of prepared medium

Greenish yellow coloured, opalescent gel with flocculent precipitate forms in Petri plates.

Cultural Response

Growth Promotion is carried out in accordance with IP. Cultural response was observed after an incubation at 36-38°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature
Test for specified microorganism						
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	25 -100	>=50 %	Black or green colony	18 -24 hrs
Salmonella Abony NCTC 6017	50 -100	luxuriant	25 -100	>=50 %	Black or green colony	18 -24 hrs
Cultural Response						
Additional microbiological testing						
Salmonella Enteritidis ATCC 13076	50 -100	luxuriant	25 -100	>=50 %	Black or green colony	18 -24 hrs
Salmonella Typhi ATCC 6539	50 -100	luxuriant	25 -100	>=50 %	Black or green colony	18 -24 hrs
Enterobacter aerogenes ATCC 13048	50 -100	none-poor	0 -10	0 -10 %	brown-green (depends on the inoculum density)	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	none-poor	0 -10	0 -10 %	brown to green (depends on inoculum density)	18 -24 hrs
Escherichia coli ATCC 8739	50 -100	none-poor	0 -10	0 -10 %	Brown to green, depends on inoculum density	18 -24 hrs
Shigella flexneri ATCC 12022	50 -100	none-poor	0 -10	0 -10 %	brown	18 -24 hrs
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0	0%		18 -24 hrs

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C for 5 days before use. Use before expiry date on the label.

Reference

1. Washington J. A., 1981, Laboratory Procedures in Clinical Microbiology, Springer - verlag, New York.
2. Eaton A. D., Clesceri L. S. and Greenberg A W.,(Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
3. Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
4. Murray PR, Baren EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors) 2003, Manual of clinical Microbiology, 8th ed., ASM, Washington, D.C.
5. Downes F P and Ito K. (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
6. Indian Pharmacopoeia, 1996, Ministry of Health and Family Welfare, Govt. of India.

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