

# TM 2008 – BISMUTH SULPHITE AGAR MEDIUM (as per USP)

### **INTENDED USE**

For the selective isolation of Salmonellae from faeces, urine, sewage and other materials.

### **PRODUCT SUMMARY AND EXPLANATION**

Bismuth Sulphite Agar Medium is prepared in accordance with USP 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 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.

#### COMPOSITION

Ingredients	Gms / Ltr		
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		

## PRINCIPLE

Brilliant green and bismuth sulphite incorporated into the medium inhibit the intestinal gram-negative and grampositive 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.

## **INSTRUCTION FOR USE**

- Dissolve 52.32 grams in 1000 ml 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.

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# QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Light yell
Appearance of prepared medium	: Yellow to
pH (at 25°C)	: 7.6±0.2

ight yellow to greenish yellow homogeneous free flowing powder fellow to greenish yellow opalescent with flocculant precipitate .6+0.2

# **INTERPRETATION**

Cultural characteristics observed after incubation.

Microorga nism	АТСС	Inoculu m (CFU)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
<i>Salmonella</i> Typhimuriu m	14028	50 -100	Luxuriant	>=70 %	Black or greenish-grey may have sheen	30-35 °C	24-48 Hours
Enterobact er aerogenes	13048	50 -100	None-Poor	0 -10 %	Brown-green (depends on the inoculum density)	30-35 °C	24-48 Hours
Enterococc us faecalis	29212	>=10 <sup>3</sup>	Inhibited	0%	-	30-35 °C	24-48 Hours
<i>Salmonella</i> Enteritidis	13076	50 -100	Luxuriant	>=70 %	Black with metallic sheen	30-35 °C	24-48 Hours
Salmonella Typhi	6539	50 -100	Luxuriant	>=70 %	Black with metallic sheen	30-35 °C	24-48 Hours
Shigella flexneri	12022	50 -100	None-Poor	0-10 %	Brown	30-35 °C	24-48 Hours
Escherichia coli	8739	50 -100	None-Poor	0-10 %	Brown to green, depends on inoculum density	30-35 °C	24-48 Hours

## PACKAGING:

In pack size of 100 and 500 gm bottles.

# STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.







**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

## DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

#### REFERENCES

- 1. Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
- 2. Murray PR, Baren EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors) 2003, Manual of clinical Microbiology, 8th ed., ASM, Washington, D.C..
- 3. Downes F P and Ito K. (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
- 4. United States Pharmacopoeia, 2009, U. S. Pharmacopoeial Convention, Inc., Rockville, MD.
- 5. Washington J.A., 1981, Laboratory Procedures in Clinical Microbiology, Springer-Verlag, New York.

6. 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.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. \*For Lab Use Only Revision: 08 Nov., 2019

