

## TM 039 – BISMUTH SULPHITE AGAR

### INTENDED USE

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

### PRODUCT SUMMARY AND EXPLANATION

The Salmonellae constitute the most taxonomically complex group of bacteria among *Enterobacteriaceae*. 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*. Four clinical types of *Salmonella* infections may be distinguished 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. Bismuth Sulphite Agar is a modification of original Wilson and Blair Medium. It is also recommended by various Associations for the isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water, food and other products. *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. Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action towards gram-positive organisms and coliforms.

### COMPOSITION

Ingredients	Gms / Ltr
Peptone	10.000
Beef extract	5.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate, anhydrous	4.000
Ferrous sulphate, anhydrous	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000

### PRINCIPLE

Peptone and beef extract serve as sources as carbon, nitrogen, long chain amino acids, vitamins and essential growth factors. Dextrose is the carbon source. Disodium 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.

### INSTRUCTION FOR USE

- Dissolve 52.33 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.
- 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 sterile Petri plates.

### QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Light yellow to greenish yellow homogeneous free flowing powder.  
**Appearance of prepared medium** : Greenish yellow coloured, opalescent with flocculent precipitate forms in Petri plates.  
**pH (at 25°C)** : 7.7±0.2

### INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
<i>Klebsiella aerogenes</i>	13048	50-100	None-poor	0-10%	Brown-green (depends on the inoculum density)	37 ± 1°C	24 ± 3 hour
<i>Enterococcus faecalis</i>	29212	50-100	Inhibited	0%	-	37 ± 1°C	24 ± 3 hour
<i>Escherichia coli</i>	25922	50-100	None-poor	0-10%	Brown-green (depends on the inoculum density)	37 ± 1°C	24 ± 3 hour
<i>Salmonella Enteritidis</i>	13076	50-100	Good-luxuriant	≥50%	Black with metallic sheen	37 ± 1°C	24 ± 3 hour
<i>Salmonella</i> Typhi	6539	50-100	Good-luxuriant	≥50%	Black with metallic sheen	37 ± 1°C	24 ± 3 hour
<i>Salmonella</i> Typhimurium	14028	50-100	Good-luxuriant	≥50%	Black with metallic sheen	37 ± 1°C	24 ± 3 hour
<i>Shigella flexneri</i>	12022	50-100	None-poor	0-10%	Brown	37 ± 1°C	24 ± 3 hour
<i>Escherichia coli</i>	8739	50-100	None-poor	0-10%	Brown-green (depends on the inoculum density)	37 ± 1°C	24 ± 3 hour

### PACKAGING:

In pack size of 100 gm and 500 gm bottles.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°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.

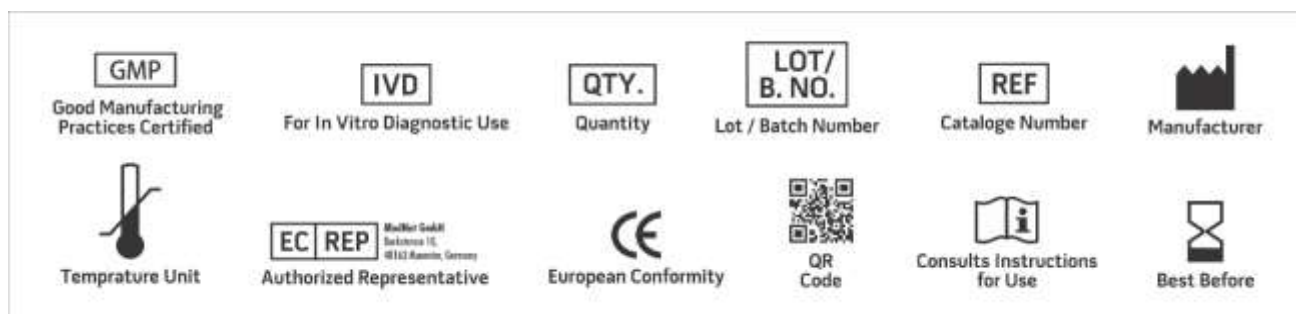


## DISPOSAL

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

## REFERENCES

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
3. Gunter and Tuft, 1939, J. Lab. Clin. Med., 24:461.
4. Indian Pharmacopoeia, 1996, Ministry of Health and Family Welfare, Govt. of India, Volume 2.
5. MacFaddin J. F., 2000, (Ed.), Biochemical Tests for Identification of Medical Bacteria, 3rd Edition, Lippincott, Williams & Wilkins, New York.
6. Mandell G. L., Douglas R. G. Jr., Bennet J. E., (Eds.), 1985, Principles and Practice of Infectious Diseases, 2nd Ed., 660-669, John Wiley & Sons New York.
7. Murray P. R., Baron J. H., Pfaller M. A., Tenover F. C. and Tenover F. C., (Eds.), 1999, Manual of Clinical Microbiology, 7th Ed., American Society for Microbiology, Washington, D.C.
8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
9. Tindall B. J., Crimont P. A. D., Gorritty G. M., EUZESY B. P., 2005, Int. J. Sys. Evol. Microbiol., 55:521
10. Wilson and Blair, 1926, J. Pathol. Bacteriol., 29:310.
11. Wilson and Blair, 1927, J. Hyg., 26:374
12. Wilson and Blair, 1931, J. Hyg., 31:138



**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For Lab Use Only**  
Revision: 08 Nov., 2020