

TM 414 – BISMUTH SULPHITE AGAR (as per IP) (DOUBLE PACK)

INTENDED USE

For selective isolation and identification of Salmonellae.

PRODUCT SUMMARY AND EXPLANATION

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 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.

COMPOSITION

Ingredients	Gms / Ltr
Part I	
Beef extract	6.000
Peptone	10.000
Brilliant green	0.010
Ferric citrate	0.400
Agar	24.000
Part II	
Ammonium bismuth citrate	3.000
Sodium sulphite	10.000
Anhydrous disodium hydrogen phosphate	5.000
Dextrose monohydrate	5.000

PRINCIPLE

Brilliant green 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. Phosphates incorporated in the medium act as a good buffering agent.

INSTRUCTION FOR USE

- Dissolve 40.4 grams of Part I (Solution 1) in 1000 ml distilled water.



- Heat to boiling to dissolve the medium completely. Sterilize by maintaining at 115°C for 30 minutes.
- Suspend 22.54 grams the equivalent weight of dehydrated medium per litre) of Part II (Solution 1) in 100 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- DO NOT AUTOCLAVE Add one volume of Part II 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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Part I- Light yellow to greenish yellow homogeneous free flowing powder. Part II- White to cream homogeneous free flowing powder.
Appearance of prepared medium	: Greenish yellow coloured, opalescent gel with flocculent precipitate forms in Petri plates.
pH (at 25°C)	: 7.6 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
<i>Salmonella Typhimurium</i>	14028	50-100	Luxuriant	≥70%	Black or greenish-grey may have sheen	36-38°C	18-24 Hours
<i>Enterobacter aerogenes</i>	13048	50-100	None-poor	0-10%	Brown-green (depends on the inoculum density)	36-38°C	18-24 Hours
<i>Enterococcus faecalis</i>	29212	50-100	Inhibited	0%	-	36-38°C	18-24 Hours
<i>Salmonella Enteritidis</i>	13076	50-100	Luxuriant	≥70%	Black with metallic sheen	36-38°C	18-24 Hours
<i>Salmonella Typhi</i>	6539	50-100	Luxuriant	≥70%	Black with metallic sheen	36-38°C	18-24 Hours
<i>Shigella flexneri</i>	12022	50-100	None-poor	0-10%	Brown	36-38°C	18-24 Hours
<i>Escherichia coli</i>	8739	50-100	None-poor	0-10%	Brown to green, depends on inoculum density	36-38°C	18-24 Hours

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 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. 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, Tenover FC, Tenover 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.

 Good Manufacturing Practices Certified	 For In Vitro Diagnostic Use	 Quantity	 Lot / Batch Number	 Catalogue Number	 Manufacturer
 Temperature Unit	 Authorized Representative <small>MedNet GmbH Borkstrasse 10, 48163 Münster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

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

***For Lab Use Only**
Revision: 08 Nov., 2019