PRODUCT SPECIFICATION SHEET

Bismuth Sulphite Agar Medium (Twin Pack) (DM039I)

Intended Use

Bismuth Sulphite Agar Medium (Twin Pack) (DM039I) is recommended for selective isolation and identification of *Salmonellae*, in compliance with IP.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. *Salmonellae* constitute the major part of taxonomically complex group of bacteria among *Enterobacteriaceae*. Ingestion of food, water or milk contaminated by human or animal excreta are the most common causes of human *Salmonella* infections. Humans are the only reservoirs of *S.typhi*.[1] Four clinical types of *Salmonella* infections may be distinguished[2] namely gastroenteritis, bacteremia or septicemia, enteric fever and a carrier state. Bismuth Sulfite Agar is a modification of the Wilson and Blair[3-5] formula. Wilson[6,7] and Wilson and Blair[3-5] clearly showed the superiority of Bismuth Sulfite medium for isolation of *S. typhi*. Cope and Kasper[8] increased their positive findings of typhoid from 1.2 to 16.8% among food handlers and from 8.4 to 17.5% among contacts with Bismuth Sulfite Agar. Employing this medium in the routine laboratory examination of fecal and urine specimens, these same authors[9] obtained 40% more positive isolations of *S.typhi*than were obtained on Endo medium. Gunther and Tuft,[10] employing various media in a comparative way for the isolation of typhoid from stool and urine specimens, found Bismuth Sulfite Agar most productive. Bismuth Sulfite Agar was stable, sensitive and easier to prepare. Green and Beard,[11] using Bismuth Sulfite Agar, claimed that this medium successfully inhibited sewage organisms. The value of Bismuth Sulfite Agar as a plating medium after enrichment has been demonstrated by Hajna and Perry.[12] Since these earlier references to the use of Bismuth Sulfite Agar, this medium has been generally accepted as routine for the detection of most *Salmonella*. For food testing, the use of Bismuth Sulfite Agar is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milks and butter.[13-16] The use of Bismuth Sulfite Agar is also recommended for use in testing clinical specimens.[17,18] In addition, Bismuth Sulfite Agar is valuable when investigating outbreaks of *Salmonella* spp., especially *S. typhi*.[19-21]
Bismuth Sulphite Agar is recommended by various Associations[22-27] 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.

Principles of the Procedure

Bismuth Sulphite Agar Medium contains peptone and beef extract which 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 H_2S.

Formula / Liter

Ingredients	Gms / Liter
Part A (Solution 1)	--
Beef extract	6.00
Peptone	10.00
Brilliant green	0.01
Ferric citrate	0.40
Agar	24.00
Part B (Solution 2)	--
Ammonium bismuth citrate	3.00
Sodium sulphite	10.00
Anhydrous disodium hydrogen phosphate	5.00
Dextrose monohydrate	5.00
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. The medium should be stored at 2-8°C for 5 days before use.

Directions

4. Suspend 40.4 grams of Part A (Solution 1) in one liter of purified/distilled water.
5. Heat to boiling to dissolve the medium completely. Sterilize by maintaining at 115°C, 10 lbs pressure for 30 minutes.
6. Suspend 22.54 grams of dehydrated medium of Part B (Solution 2) in 100 ml distilled water.
7. Heat if necessary, to dissolve the medium completely.
8. DO NOT STERILIZE IN AUTOCLAVE or by fractional sterilization since overheating may destroy the selectivity of the medium. Add one volume of Solution II to ten volumes of Solution I previously melted and cooled to a temperature of 55°C. Mix well before pouring into sterile Petri plates.
9. 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.

Quality Control Specifications

Dehydrated Appearance	Part A: Light yellow to greenish yellow homogeneous free flowing powder Part B: White to cream homogeneous free flowing powder
Prepared Medium	Greenish yellow coloured, opalescent gel with flocculent precipitate forms in Petri plates
Reaction of % solution	Not Applicable
Gel Strength	Firm, comparable with 2.4% Agar gel

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.

Expected Cultural Response:

Sr. No	Organisms	Results to be achieved					Incubation period
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	
	Test for specified microorganism						
1.	*Salmonella typhimurium ATCC 14028*	50-100	good-luxuriant	25 -100	>=50 %	black or green colony	18 -24 hrs
2.	*Salmonella abony NCTC 6017*	50-100	good-luxuriant	25 -100	>=50 %	black or green colony	18 -24 hrs
	Additional Microbiological testing						
3.	*Salmonella Enteritidis ATCC 13076*	50-100	good-luxuriant	25 -100	>=50 %	black or green colony	18 -24 hrs
4.	*Salmonella Typhi ATCC 6539*	50-100	good-luxuriant	25 -100	>=50 %	black or green colony	18 -24 hrs
5.	*Enterobacter aerogenes ATCC 13048*	50-100	none-poor	0 -10	0-10 %	brown-green (depends on the inoculums density)	18 -24 hrs
6.	*Escherichia coli ATCC 8739*	50 -100	none-poor	0 -10	0-10 %	brown to green, depends on inoculums density	18 -24 hrs
7.	*Escherichia coli ATCC 9002*	50 -100	none-poor	0 -10	0-10 %	brown to green, depends on inoculums density	18 -24 hrs
8.	*Shigella flexneri ATCC 12022*	50 -100	none-poor	0 -10	0-10 %	brown	18 -24 hrs
9.	*Enterococcus faecalis ATCC 29212*	>=10^3	inhibited	0	0%	--	18 -24 hrs

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. For isolation of *Salmonella* spp. from food, samples are enriched and selectively enriched. Streak 10 µL of selective enrichment broth onto Bismuth Sulfite Agar.
2. Incubate plates for 24-48 hours at 35°C. Examine plates for the presence of *Salmonella* spp.
3. Refer to appropriate references for the complete procedure when testing food samples.[13-16]
4. For isolation of *Salmonella* spp. from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the Bismuth Sulfite Agar plate and streak for isolation. This will permit the development of discrete colonies.
5. Incubate plates at 35°C. Examine at 24 hours and again at 48 hours for colonies resembling *Salmonella* spp.
6. For additional information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[17-21]

Results

1. The typical discrete *S.typhi* surface colony is black and surrounded by a black or brownish-black zone which may be several times the size of the colony. By reflected light, preferably daylight, this zone exhibits a distinctly characteristic metallic sheen.
2. *S.typhi* in a heavily inoculated plate may not show this reaction except near the margin of the mass inoculation, intead they appear as small light green colonies which emphasize the importance of inoculating plates so that some areas are sparsely populated with discrete *S.typhi* colonies.
3. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.
4. Generally, *Shigella* spp. other than *S. flexneri* and *S. sonnei* are inhibited. *S. flexneri* and *S. sonnei* strains that do grow on this medium produce brown to green, raised colonies with depressed centers and exhibit a crater-like appearance.
5. *Escherichia coli* is partially inhibited. Occasionally a strain will be encountered that will grow as small brown or greenish glistening colonies. This color is confined entirely to the colony itself and shows no metallic sheen.
6. A few strains of *Enterobacter aerogenes* may develop on this medium, forming raised, mucoid colonies. *Enterobacter* colonies may exhibit a silvery sheen, appreciably lighter in color than that produced by *S. typhi*.
7. Some members of the coliform group that produce hydrogen sulfide may grow on the medium, giving colonies similar in appearance to *S. typhi*. These coliforms may be readily differentiated because they produce gas from lactose in differential media, for example, Kligler Iron Agar or Triple Sugar Iron Agar.
8. The hydrolysis of urea, demonstrated in Urea Broth or on Urea Agar Base, may be used to identify *Proteus* sp. To isolate *S.typhi* for agglutination or fermentation studies, pick characteristic black colonies from Bismuth Sulfite Agar and subculture them on MacConkey Agar.
9. The purified colonies from MacConkey Agar may then be picked to differential tube media such as Kligler Iron Agar, Triple Sugar Iron Agar or other satisfactory differential media for partial identification.
10. All cultures that give reactions consistent with *Salmonella* spp. on these media should be confirmed biochemically as *Salmonella* spp. before any serological testing is performed.
11. Agglutination tests may be performed from the fresh growth on the differential tube media or from the growth on nutrient agar slants inoculated from the differential media. The growth on the differential tube media may also be used for inoculating carbohydrate media for fermentation studies.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. It is important to streak for well-isolated colonies. In heavy growth areas, *S.typhi*appears light green and may be misinterpreted as negative growth for *S.* Typhi.

2. *S.typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. Brown zones are not produced by other members of the *Enterobacteriaceae*. However, *S. arizonae* is usually inhibited.
3. Colonies on Bismuth Sulfite Agar may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium (e.g., MacConkey Agar).
4. Typical *S.typhi* colonies usually develop within 24 hours; however, all plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.
5. DO NOT AUTOCLAVE. Heating this medium for a period longer than necessary to just dissolve the ingredients destroys its selectivity.
6. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Bismuth Sulphite Agar Medium (Twin Pack)
Product Code : DM039I
Available Pack sizes : 100gm / 500gm

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Further Information

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