PRODUCT SPECIFICATION SHEET

Brilliant Green Agar (Medium 16) (DM044I)

Intended Use

Brilliant Green Agar (Medium 16) (DM044I) is recommended for selective isolation of *Salmonellae* other than *Salmonella typhi* from faeces, foods, dairy products etc. in compliance with IP 2007.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *Salmonella typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and or neurological damage.[1] Infection can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*.

The composition of medium is is in accordance with Indian Pharmacopoeia.[2] Brilliant Green Agar Medium is used as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et al as medium for differentiation of paratyphoid B from other Gram negative enteric bacteria[3] It was further modified by Kauffmann for isolation of *Salmonella* from stool samples.[4] Brilliant green agar is also recommended by APHA[5,6] FDA.[7] The outstanding selectivity of this medium permits analysis of moderately heavy inocula and heavily contaminated samples, which should be evenly distributed over the surface. Brilliant Green Agar is used in the microbial limits test.[8] Brilliant Green Agar supplemented with novobiocin is used in food testing and pharmaceutical products.

Principles of the Procedure

Brilliant Green Agar medium contains peptone provide carbon, nitrogen and other essential growth nutrients. Yeast extract supplies essential amino acids and long chains of peptides for enhanced growth. Sodium chloride maintains the osmotic equilibrium of the medium. Lactose and sucrose are the fermentable carbohydrate sources. Phenol red serves as an acid base indicator giving yellow colour to lactose and or sucrose fermenting bacteria. Brilliant green inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella typhi*, *Shigella* species, *Escherichia coli*, *Proteus* species, *Pseudomonas* species, and *Staphylococcus aureus* are mostly inhibited.

Formula / Liter

Ingredients	Gms / Liter
Peptone	10.00
Yeast extract	3.00
Lactose	10.00
Sucrose	10.00
Sodium chloride	5.00
Phenol red	0.08
Brilliant green	0.0125
Agar	12.00
Final pH: 6.9 ± 0.2 at 25°C	
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.

Directions

1. Suspend 50.09 grams of medium in one liter of purified/distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. AVOID OVERHEATING.
5. Cool to 50°C. Mix well before pouring into sterile Petri plates.

PRODUCT SPECIFICATION SHEET

Quality Control Specifications

Dehydrated Appearance	Light yellow to light pink homogeneous free flowing powder
Prepared Medium	Greenish brown coloured clear to slightly opalescent gel forms in Petri plates
Reaction of % Solution	6.9 ± 0.2
Gel Strength	Firm, comparable with 1.2% agar gel.

Cultural Response:
Cultural response was carried out as per IP and was observed after an incubation at 30-35°C for 24-48 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				
		Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of Colony
1.	*Salmonella typhimurium ATCC 14028*	50-100	good-luxuriant	25-100	>=50 %	pinkish white
2.	*Salmonella abony NCTC 6017*	50-100	good-luxuriant	25-100	>=50 %	pinkish white
3.	*Salmonella enteritidis ATCC 13076*	50-100	good-luxuriant	25-100	>=50 %	pinkish white
4.	*Salmonella typhi ATCC 6539*	50-100	fair-good	15-40	30-40 %	reddish pink
5.	*Escherichia coli ATCC 8739*	50-100	none to poor	0-10	0-10 %	yellowish green
6.	*Escherichia coli ATCC 25922*	50-100	none to poor	0-10	0-10 %	yellowish green
7.	*Escherichia coli NCTC 9002*	50-100	none to poor	0-10	0-10 %	yellowish green
8.	*Staphylococcus aureus ATCC 6538*	>=10^3	inhibited	0	0%	--
9.	*Staphylococcus aureus ATCC 25923*	>=10^3	inhibited	0	0%	--

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

Test Procedure
1. Clinical specimens can be directly plated on this medium. Use standard procedures to obtain isolated colonies from specimens.
2. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery.
3. Cultures are enriched in Selenite F-Broth or Tetrathionate Bile Brilliant green broth and plated on atleast two of the following selective media Brilliant Green Agar, Bismuth Sulphite Agar, Xylose Lysine Deoxycholate Agar and Deoxycholate Citrate Agar.
4. Incubate plates, protected from light, at 30-35°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Results
1. Non-lactose fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation.
2. *Salmonella typhi* and *Shigella* species may not grow on this medium, moreover *Proteus, Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
3. Lactose and/or sucrose fermenters produce yellowish green colonies.

Storage
Store the sealed bottle containing the dehydrated medium at 2 - 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. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Brilliant Green Agar (Medium 16)
Product Code : DM044I
Available Pack sizes : 100gm/500gm

References

1. P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. The Indian Pharmacopoeia 1985, Goverment of India , Ministry of Health and Family Welfare, New Delhi.
3. Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
4. Kauffman F., 1935, Seit F. Hyg. 177:26.
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. Wehr H M and Frank J H., 2004, Standard Methods for the Examination of Dairy Products, 17th ed., APHA Inc., Washington, D.C.
7. FDA Bacteriological Analytical Manual, 2005, 18th ed., AOAC, Washington, DC.
8. United States Pharmacopeial Convention. 1995. The United States pharmacopeia, 23th ed. The United States Pharmacopeial Convention, Rockville. MD.

Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com

DM044IPSS,QAD/FR/024,Rev.00