Technical Information

Brilliant Green Agar Base, Modified

Product Code: DM 1016

Application: - Brilliant Green Agar (Modified) is used for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces and other materials.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	20.000
Final pH (25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Salmonella species cause different types of infections, ranging from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of Salmonella disease is self-limiting gastroenteritis with fever lasting for less than 2 days and diarrhoea less than 7 days [9]. Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of Salmonella species was first described by Kristensen et. al. [1] which was further modified by Kauffmann [2]. Brilliant Green Agar is also recommended by APHA [3, 4] FDA [5] and included in EP, BP and IP [6-8]. This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. Salmonella Typhi, Shigella species Escherichia coli, Pseudomonas species, Staphylococcus aureus are mostly inhibited. Clinical specimens can be directly plated on this medium. 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. Often cultures enriched in Selenite or Tetrathionate Broth is plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar. The medium contains proteose peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and/or sucrose in the medium results in the formation of acidic pH which is detected by phenol red indicator. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further be supplemented with sulphacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with Salmonella species [10]. Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation. 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.

Methodology

Suspend 29 grams of powder media in 500 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (MS2068). Mix well before pouring into sterile Petri plates.

Quality Control

Physical Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish brown clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH Range 6.70-7.10

Cultural Response/ characteristices

DM 1016: Cultural response was carried out 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.

Organism	Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of colony
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	25-100	>=50 %	pinkish white
Salmonella Abony NCTC 6017	50-100	good-luxuriant	25-100	>=50 %	pinkish white
Salmonella Enteritidis ATCC 13076	50-100	luxuriant	25-100	>=50 %	pinkish white
Salmonella Typhi ATCC 6539	50-100	fair-good	15-40	30-40%	reddish pink
Escherichia coli ATCC 25922	50-100	none-poor	0-10	0-10%	yellowish green
Escherichia coli ATCC 8739	50-100	none-poor	0-10	0-10%	yellowish green
Escherichia coli NCTC 9002	50-100	none-poor	0-10	0-10%	yellowish green
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	0%	
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0%	

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
2. Kauffman F., 1935, Seit F. Hyg. 177:26.
3. Downes F. P. and Ito K. (Ed), 2001, Compendium of Methods for Microbiological Examination of Foods, 4th Ed. APHA, Washington D.C.
4. Standard Methods for the Microbiological Examination of Dairy Products, 1995, 19th Ed, APHA, Washington, D.C.
5. Bacteriological Analytical Manual, 5th Ed, 1978, AOAC, Washington D.C.
6. The European Pharmacopoeia, 2008, Council or Europe, Strasbourg.
7. The British Pharmacopoeia, 2007 vol. II, London.
8. Indian Pharmacopoeia, 2007, Ministry of Health and Family Welfare, Govt., of India,

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