Technical Information

Inositol Brilliant Green Bile Agar (Plesiomonas Differential Agar)

Product Code: DM 1574

Application: - Inositol Brilliant Green Bile Agar (Plesiomonas Differential Agar) is recommended for selective isolation of *Plesiomonas shigelloides* and *Aeromonas* species from faeces and foodstuffs.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Meat extract	5.000
Meso-Inositol	10.000
Bile salts mixture	8.500
Sodium chloride	5.000
Brilliant green	0.00033
Neutral red	0.025
Agar	13.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Plesiomonas shigelloides is an opportunistic pathogen. This organism was first isolated on MacConkey Agar from faecal specimen by Ferguson and Henderson (1947) *P.shigelloides* has also been isolated from fresh water, freshwater fish, shell fish and from many types of animals. The organism may be present in unsanitary water, which has been used as drinking water, recreational water, or used to rinse foods that are consumed without cooking or heating. Human infections from *P.shigelloides* are mostly waterborne and have been implicated in gastroenteritis. Like other bacteria it is also identified by common bacteriological analysis, serotyping, and antibiotic sensitivity testing [1]. Other organism's responsible for human waterborne diarrhoea includes *Aeromonas* species.

Several media and methods have been designed to selectively isolate *P.shigelloides.* Strains of *P.shigelloides* grow in the presence of brilliant green and are also resistant to bile salts. There compounds are usually added in media to inhibit the growth gram-positive bacteria. Most bacterial species fail to ferment meso- inositol, where as almost all strains of *P.shigelloides* ferment this to naturally occurring cyclic polyhydroxyl alcohol. Schubert [2] studied advantage of the three properties as discussed above and prepared Inositol Brilliant Green Bile Salts Agar for differential diagnosis of P. Shigelloides from faces & food stuff [5].

It is a differential medium for inositol utilizers and non-utilizers. Proteose peptone and meat extract supply nitrogenous nutrients required for the growth of organisms. Bile salts and brilliant green inhibit all gram-positive bacteria and most of the gram-negative bacilli, other than coliforms respectively. Meso-inositol is a fermentable carbohydrate source in the medium while neutral red is the pH indicator. Oxidase test must be performed during the identification procedure to differentiat *Plesiomonas from other* members of the *Enterobacteriaceae* [4, 5].

Samples, depending upon consistency and expected numbers of organism are diluted and directly streaked on PL Agar (DM2173) and Inositol Brilliant Green Bile Agar (DM1574) (4). Another 10 grams of the sample is inoculated into 90 ml of Tetrathionate Broth Base (DM1032). Plates are incubated at 35-37°C and broth at 40°C. Following an incubation of 24 hours, presumptive *P. shigelloides*

Colonies are inoculated into TSI slants (DM1021) and Inositol Gelatin Medium Butts (DM2161). Growth from DM1032 is streaked onto PL Agar (DM2173) and BGBA (DM1574)

Methodology

Suspend 52.05 grams of powder media in 1000 ml distilled water. Shake well & heat to boiling to dissolve to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

Reddish orange coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.2% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range 7.00-7.40

Cultural Response/Characteristics

DM 1574: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Aeromonas hydrophila *ATCC 7966*	50-100	*luxuriant*	>=50%	colourless
Klebsiella pneumoniae *ATCC 13883*	50-100	*good*	40-50%	pink
Klebsiella pneumoniae *ATCC 13883*	50-100	*luxuriant*	>=50%	pink
Staphylococcus aureus *ATCC 25923*	>=10^3	inhibited	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: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1 .Foodborne Pathogenic Microorganisms and Natural Toxins Handbook Centre for Food Safety and Applied Nutrition, US Food and Drug Administration.

2.Cooper R. G., and Brown G. W., 1968, Plesiomonas shigelloides Schubert R. H. W., 1977, Ueber den Nachweis von Plesiomonas shigelloides Habs and Schubert, 1962, und ein Elektivmedium, den Inositol-Brilliantgrun-Gallesalz-Agar. Ernst Rodenwaldt Arch. 4:97-103.

3.Appelbaum D. C., Bowen A. J., Adhikari M., et al, 1978, J. Pediatr., 92:676.

4.Bhat P., Shanthakumari S. and Rajan D., 1974, Ind. J. Med. Res. ,62:1051.

5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimor

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