Inositol Brilliant Green Bile Agar (Plesiomonas Differential Agar)

M574

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

Directions

Suspend 52.03 grams in 1000 ml distilled water. 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.

Principle And Interpretation

Plesiomonas shigelloides is an opportunistic pathogen. Ferguson and Henderson (1947) first isolated this organism on MacConkey Agar from faecal specimen. *P.shigelloides* has been isolated from fresh water, freshwater fish, shell fish and from many types of animals. Human infections from *P.shigelloides* are mostly waterborne. The organism may be present in unsanitary water, which has been used as drinking water, recreational water, or water used to rinse foods that are consumed without cooking or heating. *P.shigelloides* has been implicated in gastroenteritis. Its significance as an enteric (intestinal) pathogen is presumed because of its predominant isolation from stools of patients with diarrhea. It is identified by common bacteriological analysis, serotyping, and antibiotic sensitivity testing (1). Other organisms implicated in human waterborne diarrhoea include *Aeromonas* species.

Inositol Brilliant Green Bile Agar is a medium described by Schubert (2) and is recommended for selective isolation of *P.shigelloides* (an opportunistic pathogen) and *Aeromonas* species from faeces and other foodstuffs (3).

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 that are usually incorporated in media to inhibit gram-positive bacteria. Most bacterial species do not ferment meso- inositol, but almost all strains of *P.shigelloides* ferment this to naturally occurring cyclic polyhydroxyl alcohol. Schubert (2) took advantage of the three properties as discussed above and designed Inositol Brilliant Green Bile Salts Agar.

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. *Plesiomonas* may be misidentified as a member of the *Enterobacteriaceae* , if oxidase test is not performed during the identification procedure (4, 5).

Samples, depending upon consistency and expected numbers are diluted and directly streaked on PL Agar (M1173) and Inositol Brilliant Green Bile Agar (M574) (4). Another 10 grams of the sample is inoculated into 90 ml of Tetrathionate Broth Base (M032). 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 (M021) and Inositol Gelatin Medium Butts (M1161). Growth from M032 is streaked onto PL Agar (M1173) and BGBA (M574)

Quality Control

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
7.00-7.40

Cultural Response
M574: 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
Plesiomonas shigelloides ATCC 14029	50-100	luxuriant	>=50%	pink
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

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, Baltimore.

Revision : 1 / 2011

CE