Inositol Brilliant Green Bile Agar (Plesiomonas Differential Agar) M574

Intended Use:

Recommended for selective isolation of *Plesiomonas shigelloides* and *Aeromonas* species from faeces and food stuffs.

Composition**

Ingredients	g / L
Proteose peptone	10.000
HM 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
Equivalent to Meat extract

Directions

Suspend 52.03 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. 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 diarrhoea. It is identified by common bacteriological analysis, serotyping, and antibiotic sensitivity testing (5).Other organisms implicated in human waterborne diarrhoea include *Aeromonas* species.

Inositol Brilliant Green Bile Agar is a medium described by Schubert (1) and is recommended for selective isolation of *P.shigelloides* (an opportunistic pathogen) and *Aeromonas* species from faeces and other foodstuffs (2). 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 (3) 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 HM 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)

Type of specimen

Clinical samples - faeces; Food samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (8). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(9) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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 (00063*)	50-100	luxuriant	>=50%	colourless
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good	40-50%	pink
Plesiomonas shigelloides ATCC 14029	50-100	luxuriant	>=50%	pink
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

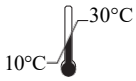
Reference

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.
6.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
9.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 04/2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.