Ready Prepared Media

Technical Information

Brilliant Green Agar Modified Plate

Product Code: PM 1016

Application: - Recommended for selective isolation of *Salmonellae* other than *Salmonella* Typhi from faeces, food, dairy products.

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
Sodium sulphaacetamide	1.000
Sodium mandelate	0.250
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Salmonella species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever.The most common form of Salmonella disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days. Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of Salmonellaspecies was first described by Kristensen et. al. (1) and further modified by Kauffmann (2). Brilliant Green Agar is also recommended by APHA (3,4) FDA (5) and described in EP, BP and IP (6,7,8).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 inhibitorymedium to increase the chances of recovery. Often cultures enriched in Selenite or Tetrathionate Broth is plated on Brilliant Green Agar along with Bismuth SulphiteAgar, SS Agar, MacConkey Agar.

The medium contains proteose peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential lnutrients. 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. Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation.

Type of specimen

Clinical : faeces; Food and dairy samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,9,10). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (11).For clinical samples follow appropriate techniques for handling specimens as per established guidelines (12,13).

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the pack. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and cultures. 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. Though this medium is selective for Salmonella other species of Enterobacteriaceae may grow.
2. Salmonella Typhi and Shigella species may not grow on this medium.
3. Moreover Proteus, Pseudomonas and Citrobacter species may mimic enteric pathogens by producing small red colonies.
4. Further confirmation has to be carried out on presumptive Salmonella isolates.

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.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate .

Quality Control

Appearance
Sterile Brilliant Green Agar in 90 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles.
pH
6.70-7.10
Quantity of medium
25 ml of medium in 90 mm disposable plates .
Colour of medium
Greenish brown coloured medium .
Sterility Test
Passes release criteria
Cultural Response
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.

Oragnism	**Inoculum (CFU)**	**Growth**	**Recovery**	**Colour of Colony**
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	>=50%	pinkish white
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50%	pinkish white
Salmonella Abony NCTC 6017 (00029*)	50-100	good-luxuriant	>=50%	pinkish white
Salmonella Typhi ATCC 6539	50-100	fair-good	30-40%	reddish pink
Escherichia coli ATCC 25922 (00013*)	50-100	non-poor	0-10%	yellowish green
Escherichia coli ATCC 8739 (00012*)	50-100	non-poor	0-10%	yellowish green
Escherichia coli NCTC 9002	50-100	non-poor	0-10%	yellowish green
*Staphylococcus aureus*subsp. *aureus* ATCC 25923 (00034*)	>=10^3	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538(00032*)	>=10^3	inhibited	0%	

Key : *Corresponding WDCM numbers.

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Storage and Shelf Life

On receipt store between 2-8°C. Use before expiry date on the label. Product performance is best if used with instated 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

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. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed.,American Public Health Association, 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. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
7. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
8. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
9. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
11. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed.Washington DC:APHA Press; 2023.
12. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
13. 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.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
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