# B.G. Sulpha Agar (Brilliant Green Sulpha Agar) M492

## Intended Use:

A highly selective medium for isolation and detection of *Salmonella* species in food, especially eggs and egg products.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Yeast extract | 3.000 |
| Proteose peptone | 10.000 |
| Lactose | 10.000 |
| Sucrose | 10.000 |
| Sodium sulphapyridine | 1.000 |
| Sodium chloride | 5.000 |
| Brilliant green | 0.0125 |
| Phenol red | 0.080 |
| Agar | 20.000 |
| Final pH ( at 25°C) | 6.9±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 59.09 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. To maintain selectivity of the medium. DO NOT OVER STERILIZE OR OVERHEAT the medium. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

## Principle And Interpretation

*Salmonella* species are ubiquitous in the environment. These enter the gastrointestinal tract of animals due to consumption of contaminated feed. Stringent animal husbandry practices are used in the meat (food) industry and inedible raw materials are recycled and discarded. Thus the organisms are further returned to the environment and stay in the global food chain (1,2). Eggshell and its contents are usually sterile at the time of oviposition. Subsequently it gets contaminated on contact with the nest, the floor and litter of other birds (3,4,5).

*Salmonella* species are usually the causative agents of a self-limiting gastroenteritis. In some cases they may also cause typhoid fever. *Salmonella* contamination is most frequently encountered in the poultry industry. Brilliant Green Sulpha Agar is used for the selective isolation and detection of *Salmonella* species in foods especially from eggs and egg products. Brilliant Green Agar was first formulated by Kristensen, Lester and Jargens (6). This was further modified by Osborne and Stokes (7) by the addition of 0.1% sodium sulphapyridine to the original formulation. This addition helped to increase the selective properties of the medium. Colonies of *Salmonella* may sometimes vary from red to pink to white depending upon the time and length of incubation and the strain of *Salmonella*.

Yeast extract and proteose peptone provide essential growth nutrients, amino acids and vitamins. Brilliant green used in the medium is inhibitory to gram-positive and most gram-negative lactose/sucrose fermenting bacilli. Sulphapyridine enhances the selectivity of the medium. The medium does not support luxuriant growth of *Salmonella* Typhi. *Shigella* species also fail to grow on Brilliant Green Sulpha Agar (8). Since Brilliant Green Sulpha Agar is highly selective, a less inhibitory medium should be simultaneously used to recover organisms from the pre-enriched culture (Selenite Cystine Medium).

## Type of specimen

Food samples - Eggs and Egg products

## Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations :

1. Do not autoclave the medium for more than 15 minutes as it decreases the selectivity of the medium.
2. Further biochemical tests are needed for a final identification of the isolated organisms.
3. 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 light pink homogeneous free flowing powder

**Gelling**

Firm, comparable with 2.0% Agar gel.

**Colour and Clarity of prepared medium**

Greenish brown coloured, clear to slightly opalescent gel forms in Petri plates.

**Reaction**

Reaction of 5.9% w/v aqueous solution at 25°C. pH : 6.9±0.2

**pH**

6.70-7.10

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|---|---|---|---|---|
| *Enterococcus faecalis ATCC 29212* (00087*) | >=$10^4$ | inhibited | 0% | |
| *Escherichia coli ATCC 25922* (00013*) | 50-100 | none-poor | <=10% | yellow green surrounded by intense yellow-green zone |
| *Proteus vulgaris ATCC 13315* | >=$10^4$ | inhibited | 0% | |
| *Salmonella* Enteritidis *ATCC 13076* (00030*) | 50-100 | good | >=50% | pink-white, surrounded by a brilliant red-zone |
| *Salmonella* Typhimurium *ATCC 14028* (00031*) | 50-100 | good | >=50% | pink - white |
| *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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

## Reference

1. Doyle M. P., (Ed.), 1989, Foodborne Bacterial Pathogens, Marcel Dekker, Inc., New York. 327-445
2. DAoust J. Y., 1994, Int. J. Food Microbiol. 24: 11-31.
3. Brooks and Taylor, 1955, Rep. Rd. Invest. Bd. 60, H. M. S. O., London, England.

4. Forsythe, Ayres and Radlo, 1953, Food Technol., 7:49.

5. Stadelman, Ikeme, Roop and Simmons, 1982, Poultry Sci., 61:388.

6. Kristensen M., Lester V., and Jargens A., 1925, Brit. J. Exp. Pathol. , 6:291.

7. Osborne W. W. and Stokes J. L., 1955, Ottawa; Food and Drug Laboratories, 1962.

8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams & Wilkins, Baltimore, MD.

9. 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.

10. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

11. 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.

Revision : 03/2024