



## Brilliant Green Agar Medium

MU016

### Intended Use:

Recommended for selective isolation of *Salmonellae* other than *Salmonella typhi* from faeces, food, dairy products etc. in accordance with USP.

### Composition\*\*

Ingredients	g / L
Peptone	5.000
Tryptone	5.000
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
pH after sterilization (at 25°C)	6.9±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 58.09 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. AVOID OVERHEATING. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Brilliant Green Agar medium is recommended as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et al as medium for differentiation of paratyphoid B from other Gram negative enteric bacteria (1). Kauffmann further modified it for isolation of *Salmonella* from stool samples (2). Brilliant green agar is also recommended by APHA (3,4) FDA (5) and is in accordance with United States Pharmacopoeia (6). This medium is employed in testing clinical specimens. Heavy inocula and heavily contaminated samples can be analyzed due to the outstanding selectivity of this medium. Brilliant Green Agar is used in the microbial limits test and with novobiocin for testing food and pharmaceutical products. Combination of peptone, tryptone and yeast extract makes the medium highly nutritious and supplies amino acids and long chains of peptides. Sodium chloride maintains the osmotic equilibrium. Lactose and sucrose are the fermentable carbohydrate sources. Phenol red serves as an acid base indicator giving yellow colour to lactose and or sucrose fermenting bacteria. This medium also contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive, bacteria. *Salmonella Typhi*, *Shigella* species, *Escherichia coli*, *Proteus* species, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited.

However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite or Tetrathionate Broth are plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar. Non-lactose fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation. *Salmonella typhi* and *Shigella* species may not grow on this medium, moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.

### Type of specimen

Foodstuffs & dairy samples; Pharmaceutical samples.

### Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,7,8). 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. 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.

## 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 clear to slightly opalescent gel forms in Petri plates

### pH

6.70-7.10

### Growth Promotion Test

Growth Promotion was observed in accordance with USP.

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature
<b>Growth Promotion Test</b>						
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	good-luxuriant	25 -100	≥50 %	pinkish white	24 -48 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	≥50 %	pinkish white	24 -48 hrs
<b>Additional Microbiological testing</b>						
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	Good-luxuriant	25 -100	≥50 %	pinkish white	24 -48 hrs
<i>Salmonella</i> Typhi ATCC 6539	50 -100	poor-good	15 -40	30 -40 %	reddish pink	24 -48 hrs
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	none-poor	0 -10	0 -10 %	yellowish green	24 -48 hrs
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	none-poor	0 -10	0 -10 %	yellowish green	24-48 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923(00034*)	≥10 <sup>3</sup>	inhibited	0	0%		24 -48 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	≥10 <sup>3</sup>	inhibited	0	0%		24 -48 hrs

Key : \*Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°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 (9,10).

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## Reference

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6. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
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