



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Brilliant Green Agar is a highly selective medium for the isolation of *Salmonella* other than typhoid bacilli from feces and other materials.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - Streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of **Trypticase™** Soy Broth diluted to yield 10^6 – 10^7 CFU/mL.
 - Incubate at $36 \pm 1^\circ\text{C}$ under appropriate atmospheric conditions.
 - Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls for inhibited strains.
- Examine plates after 18 – 48 h for growth, colony color and selectivity.
- Expected Results

Organisms	ATCC™	Recovery	Colony Color
* <i>Enterococcus faecalis</i>	29212	Inhibition (partial to complete)	N/A
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)	Yellow-green
* <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	Moderate to heavy growth	Pink to red with red zone
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Enteritidis	13076	Moderate to heavy growth	Pink to red with red zone

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of 6.9 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at $33 - 37^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Brilliant Green Agar is a highly selective medium used for the isolation of *Salmonella* species other than typhoid bacilli from feces and other materials.

V SUMMARY AND EXPLANATION

Brilliant Green Agar was first described by Kristensen et al. and then modified slightly by Kauffman.^{1,2} Brilliant green dye is used in this medium to inhibit gram-positive bacteria and most gram-negative bacilli for the selective isolation of *Salmonella* species, except for *Salmonella* Typhi and *Salmonella* Paratyphi, from feces and other clinical specimens.^{3,4}

The highly selective nature of this medium enables the use of a large inoculum, which facilitates the detection of *Salmonella* present in small numbers in a specimen.³ To further increase the chance of recovering pathogens, a less inhibitory medium with a broader microbial coverage should also be inoculated.

VI PRINCIPLES OF THE PROCEDURE

Enzymatic digests of animal tissue and casein provide the amino acids and other complex nitrogenous substances necessary to support bacterial growth. Yeast extract primarily supplies the B-complex vitamins. Sodium chloride maintains osmotic equilibrium.

Lactose and sucrose are energy sources. Fermentation of these carbohydrates is detected by a visible color change of the medium. If the inoculated organism ferments lactose or sucrose, acids are produced that lower the pH of the surrounding medium, causing the color of the pH indicator, phenol red, to change from red to yellow. Generally, lactose- or sucrose-fermenting organisms produce yellow or greenish colonies surrounded by a bright yellow-green halo. In contrast, typical *Salmonella* colonies appear reddish to pink or nearly white, and are surrounded by bright pink to red medium. Colony morphology may vary depending on the cultures and the length of incubation.

Brilliant green dye is a selective agent that inhibits gram-positive bacteria and most gram-negative bacilli for the selective isolation of *Salmonella* species, with the exception of *Salmonella* Typhi and *Salmonella* Paratyphi.

VII REAGENTS

Brilliant Green Agar

Approximate Formula* Per Liter Purified Water

Yeast Extract.....	3.0 g	Sucrose.....	10.0 g
Peptic Digest of Animal Tissue.....	5.0 g	Phenol Red	0.08 g
Pancreatic Digest of Casein.....	5.0 g	Agar	20.0 g
Lactose.....	10.0 g	Brilliant Green.....	12.5 mg
Sodium Chloride	5.0 g		

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁵⁻⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.^{4,9-11}

Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Brilliant Green Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs should be streaked onto the medium with a sterile inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora. Consult appropriate texts for information about the processing and inoculation of feces and other materials.⁹⁻¹¹

Incubate the plates in an inverted position (agar side up) at 35°C for up to 48 h.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After 48 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Salmonella typically produces white to pink or red colonies surrounded by pink to red medium. *Salmonella* Typhi and *Salmonella* Paratyphi are generally inhibited on this medium.

Typical colonies of lactose or sucrose fermenters are yellow or greenish with a bright yellow-green halo in the surrounding medium.

XI LIMITATIONS OF THE PROCEDURE

Brilliant Green Agar should not be used for examination of specimens suspected of containing dysentery organisms because they generally grow poorly or not at all on this medium.

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit the growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

Some diagnostic tests may be performed with the primary plate, but a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate texts for further information.^{4,11,12}

XII AVAILABILITY

Cat. No.	Description
295963	BBL™ Brilliant Green Agar, Pkg. of 20 plates

XIII REFERENCES

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12. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual® of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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