

# BRILLIANT GREEN AGAR w/ and w/o NOVIOBIOCIN

## INTENDED USE

Remel Brilliant Green Agar is a solid medium recommended for use in qualitative procedures for selective isolation of *Salmonella* species other than *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi from fecal specimens or other materials.

## SUMMARY AND EXPLANATION

In 1925, Kristensen et al. described brilliant green agar for isolation of *Salmonella* spp.<sup>1</sup> Kauffmann reported increased isolation rates for *Salmonella* spp. from fecal specimens using a modification of Kristensen's formula.<sup>2</sup> Devenish and Cooke, in separate studies, added novobiocin to brilliant green agar to further improve selectivity.<sup>3,4</sup>

## PRINCIPLE

Casein and meat peptones provide nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Brilliant green dye is a selective agent which inhibits gram-positive bacteria and many gram-negative bacilli. *Salmonella* colonies are reddish or pink to nearly white in color with a red zone.<sup>5</sup> Lactose or sucrose fermenters that may occasionally grow on this medium appear as yellow-green colonies surrounded by a yellow-green zone. *Shigella* spp. grow poorly or not at all on this medium. Novobiocin is a selective agent which inhibits the growth of coliforms and *Proteus* spp.

## REAGENTS (CLASSICAL FORMULA)\*

Lactose.....	10.0 g	Yeast Extract.....	3.0 g
Sucrose.....	10.0 g	Phenol Red.....	0.08 g
Casein Peptone.....	5.0 g	Brilliant Green.....	12.5 mg
Meat Peptone.....	5.0 g	Agar.....	20.0 g
Sodium Chloride.....	5.0 g	Demineralized Water.....	1000.0 ml

pH 6.9 ± 0.2 @ 25°C

The following optional ingredient is available per liter of medium:

Novobiocin ..... 0.02 g

\*Adjusted as required to meet performance standards.

## PROCEDURE

1. An enrichment broth, such as Tetrathionate Broth (REF R07162), may be used in conjunction with Brilliant Green Agar (w/ or w/o Novobiocin) and a nonselective medium to increase the potential for recovering enteric pathogens.
2. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
3. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface. Streak the plate for isolation using a sterile inoculating loop.
4. Incubate the plates aerobically at 33-37°C for 18-24 hours.
5. Examine plates for typical colony morphology. Colonies of *Salmonella* appear red, pink, or white surrounded by a red zone. Lactose and sucrose fermenters form colonies which are yellow to yellow-green. (**Note:** This medium is inhibitory to *Salmonella* serovars Typhi and Paratyphi.)

## QUALITY CONTROL

All lot numbers of Brilliant Green Agar w/ and w/o Novobiocin have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

### CONTROL

#### Brilliant Green Agar:

*Salmonella enterica* serovar Typhimurium ATCC® 14028  
*Escherichia coli* ATCC® 25922  
*Staphylococcus aureus* ATCC® 25923

### INCUBATION

Aerobic, 18-24 h @ 33-37°C  
Aerobic, 18-24 h @ 33-37°C  
Aerobic, 18-24 h @ 33-37°C

### RESULTS

Pink colonies w/ pink zone  
Inhibition (partial to complete)  
No growth

#### Brilliant Green Agar w/ Novobiocin:

*Salmonella enterica* serovar Typhimurium ATCC® 14028  
*Proteus mirabilis* ATCC® 12453

Aerobic, 18-24 h @ 33-37°C  
Aerobic, 18-24 h @ 33-37°C

Pink colonies w/ pink zone  
No growth

## LIMITATIONS

1. *Shigella* spp., *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi are inhibited on Brilliant Green Agar w/ and w/o Novobiocin.<sup>6</sup>
2. Some strains of *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar and mimic *Salmonella*.<sup>6</sup>

## BIBLIOGRAPHY

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4. Cooke, V.M., R.J. Miles, R.G. Price, and A.C. Richardson. 1999. *Appl. Environ. Microbiol.* 65:807-812.
5. Isenberg, H.D. 2004. *Clinical Microbiology Procedures Handbook*. 2<sup>nd</sup> ed. ASM Press, Washington, D.C.
6. MacFaddin, J.F. 1985. *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*. Vol. 1. Williams & Wilkins, Baltimore, MD.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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