

# BRILLIANT GREEN WITH NOVOBIOCIN / XLT4 AGAR (BI-PLATE)

**PRODUCT:**

**Plated Media:**

Brilliant Green + Novobiocin/XLT4                      P4058

**PURPOSE:**

Brilliant Green with Novobiocin/XLT4 agar is a highly selective, differential media designed for the isolation of *Salmonella* species in fecal specimens. The two media are designed for use in food and dairy processing facilities.

**PRINCIPLE:**

Kristensen et al.<sup>2</sup> first described the use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella*. Later, the formula was modified by Kauffmann<sup>1</sup> who used the medium in conjunction with an enrichment, tetrathionate broth.

Brilliant Green agar supplemented with Novobiocin has been found to reduce or completely inhibit nuisance organisms commonly seen on agar media when isolating *Salmonella* as described by Miller and Tate<sup>3</sup>.

Miller and Tate<sup>3</sup> have also demonstrated that XLT4 (Xylose-Lysine-Tergitol™ 4) agar helps differentiate between *Salmonella* and non-*Salmonella* species. The selective agent, XLT4, used in this medium causes near-complete inhibition of gram-positive and competing gram-negative organisms.

**FORMULA:**

Approximate, per liter deionized filtered water.

**Brilliant Green agar medium, with Novobiocin:**

Peptic Digest of Animal Tissue .....	5.0 g
Pancreatic Digest of Casein .....	5.0
Yeast Extract .....	3.0
Lactose.....	10.0
Sucrose .....	10.0
Sodium Chloride.....	5.0
Agar.....	20.0
Brilliant Green .....	12.5 mg
Phenol Red .....	80.0
Novobiocin.....	20.0 mg

Final pH 6.9 ± 0.2 at 25°C

**XLT4 agar medium:**

Proteose Peptone .....	1.6 g
Yeast Extract .....	3.0
Lysine .....	5.0
Xylose .....	3.75
Sucrose .....	7.5
Lactose.....	7.5
Ferric ammonium .....	0.8
Sodium Chloride.....	5.0
Sodium thiosulfate.....	6.8
Agar.....	18.0 g
Phenol Red .....	0.08
XLT4 supplement .....	4.6 ml

Final pH 7.4 ± 0.2 at 25°C

**PRECAUTIONS:\***

For *in vitro* diagnostic use. Observe approved biohazard precautions.

**Storage:** Upon receipt, store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

**Limitations:** Processing delays in excess of 2-3 hours (see "Specimen Collection") of an unpreserved stool specimen greatly jeopardizes the recovery of many salmonellas. These organisms are very susceptible to the acidic changes which occur with a drop in temperature of the feces.<sup>3</sup>

Colonies of *Salmonella* species may vary in color from red to pink-white on Brilliant Green agar medium depending on the length of incubation and the strain. *Salmonella* may vary in color from red-pink with black centers or red to black colonies on XLT4 agar medium. Colonial morphology is a presumptive aid only and does not provide information for speciation. Further biochemical and serological tests must be performed to complete the identification.

Other nonlactose-fermenting or slow lactose-fermenting colonies may grow on Brilliant Green with novobiocin agar medium and mimic the enteric pathogens.<sup>4</sup>

*Salmonella typhi*, *Salmonella paratyphi*, and *Shigella* species do not grow adequately on Brilliant Green with novobiocin agar medium, limiting its effectiveness as a screening medium for stool cultures.

Upon incubation, Brilliant Green medium can become bright red, but returns to normal color at room temperature. Discoloration of the medium will occur if exposed to light.

Multicolored, metallic looking crystals/flecks may develop on the surface on freshly inoculated XLT4 agar medium or on medium held over several days. These crystals/flecks do not interfere with the performance of the medium.

This bi-plate agar medium is highly selective. It is recommended to inoculate this media in combination with a less selective medium such as MacConkey, Hektoen, or other suitable media, as well as an enrichment broth, for best results in isolating all enteric pathogens.

#### PROCEDURE:\*

**Specimen Collection:** Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and delivered to the laboratory within 2-3 hours. If there is a delay, suitable transport media such as Cary-Blair or Enteric Pathogen Transport must be used to maintain the viability of the organisms.

**Method of Use:** Prior to inoculation, the medium should be brought to room temperature. Inoculate the infectious material in such a manner as to facilitate isolation of individual colonies. Incubate aerobically at 35°C and examine plates at 18-24 hours and 48 hours of incubation.

**Interpretation:** Non-lactose fermenting gram-negative bacteria appear as red-pink-white-colored, opaque colonies surrounded by brilliant red zones in the Brilliant Green with Novobiocin agar medium. Organisms producing this effect are possible enteric pathogens and should have further biochemical and/or serological tests performed. Lactose-fermenting or sucrose-fermenting colonies appear as yellow to greenish-yellow colonies surrounded by yellow-green zones in the medium.

*Salmonella* appear red to pink with black centers in the XLT4 agar medium. Most gram-negative species such as *Enterobacter aerogenes*, *Escherichia coli*, and *Pseudomonas* spp. are markedly to completely inhibited. Non-lactose fermenters, such as *Shigellae*, will appear as clear red colonies with no blackening. *Citrobacter* species will appear as yellow colonies with no blackening.

**Materials Required but Not Provided:** Transport media and standard microbiological supplies and equipment such as those products commonly used in a microbiological laboratory are not provided.

#### QUALITY CONTROL:\*

##### Brilliant Green with Novobiocin agar medium:

###### Microorganisms Used (ATCC #):

*Salmonella typhimurium* (14028)

*Salmonella enteritidis* (13076)

*Escherichia coli* (25922)

*Staphylococcus aureus* (25923)

###### Expected Results:

Growth; reddish-pink colonies with red diffusion

Growth; white-pink colonies, red diffusion

Inhibition, partial (yellow colonies) to complete

Inhibition, partial to complete

**XLT4 agar medium:**

**Microorganisms Used (ATCC #):**

*Salmonella typhimurium* (14028)  
*Proteus mirabilis* (43071)  
*Escherichia coli* (25922)  
*Enterococcus faecalis* (29212)

**Expected Results:**

Growth; red to pink colonies with black centers  
Inhibition, partial (no swarming colonies) to complete  
Inhibition, partial (yellow colonies) to complete  
Inhibition

**User Quality Control:** Check for signs of contamination and deterioration. The Brilliant Green with Novobiocin agar medium should appear slightly opalescent and is normally reddish-brown to green in color. The XLT4 agar medium should appear translucent, and orange-red in color.

**BIBLIOGRAPHY:**

1. Kauffmann, F., *Z. Hyg. Infektionskr.*, 117:26, 1935.
2. Kristensen, M., V. Lester, and A. Jurgens, *Br. J. Exp. Pathol.*, 6:291, 1925.
3. Miller, R.G. and C.R. Tate. 1990. *Maryland Poultryman*. April:2-8.
4. Sack, R. B., et al., *Cumitech 12*, American Society for Microbiology, Washington, D. C., 1980.
5. Taylor, W. I., *Am. J. Clin. Pathol.*, 44:471, 1965.

\*For more detailed information, consult appropriate references.

PML Microbiologicals, Inc.

Data #201

Copyright 1989 by PML Microbiologicals, Inc.

Revision Date: August 2007