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## **VRBG Partially Prepared Media**

For the detection and enumeration of enterobacteriaceae

**Intended Use** - VRBG Agar (Bile Glucose Agar containing crystal violet and neutral red) is used for the detection and enumeration of *enterobacteria* in dairy products, meat, prepared pork products and other food products.

**Principles of the Procedure** - Violet Red Bile Glucose Agar is recommended for the detection and enumeration of Enterobacteriaceae in food and dairy products. Violet Red Bile Glucose Agar is also listed in the USP as the recommended solid medium for use in the isolation of bile-tolerant gram-negative bacteria from nonsterile pharmaceutical products. Pancreatic digest of gelatin provides carbon, nitrogen, vitamins, and minerals. Yeast extract supplies B-complex vitamins. Glucose is a carbohydrate. Bile salts and crystal violet inhibit gram-positive bacteria. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent. Compliance: CCAM, COMPF, EP, ISO, JP, USP

**Formula / Liter** - Formulation: Yeast extract 3g/L, Pancreatic digest of Gelatin 7g/L, Bile Salts No. 3 1.5g/L, Glucose 10g/L, Sodium Chloride 5g/L, Neutral red 0.03g/L, Crystal Violet 2g/L, Agar 15g/L

Physical Appearance - Clear, slightly opalescent, and reddish-purple in color

**Storage** − 2 − 25 °C

**pH at RT -** 7.4 ± 0.2

**Warning and Precautions** - For laboratory use only. Do not use bottles if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration. After use and prior to discarding, specimen containers and all contaminated material, including the used culture media and contaminated culture containers, must be autoclaved for 20 to 30 min at 121 °C or higher (if large volumes of disposed materials must be sterilized), or incinerated by validated procedures. Liquefy the bottled medium only once. Do not allow any leftovers to solidify and liquefy thereafter for a second time as repeated heating will damage the ingredients of the medium, leading to unsatisfactory microbiological performance.

**PROCEDURE** – Place the bottle into a jar containing water, which is placed on a hot plate and brought to boiling or use a microwave oven, sufficiently long to dissolve the whole medium. Slightly loosen the cap before heating to allow pressure exchange. Do not place media bottles with metal closures into a microwave oven.

The time needed for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device before use, its wattage, size, and the volume and temperature of the medium in the container. It is recommended to test and record the time needed for liquefaction after the first use.

After complete liquefaction, remove the container from the heating device and place into a water bath set at 48–50 °C. Warning: Wear heat-protective gloves. Do not place the hot container into an ice bath or in cold

	Page 1 of 2	G:\FLYERS\MICRO\MICRO-PHL\Update\PHL-MI- 214-01-VRBG
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water to accelerate cooling as this might cause cracks in the glass. Risk of severe scald. Leave the container in the water bath sufficiently long to allow cooling of the complete medium to the set temperature.

Pour the medium into the dishes if surface inoculation is desired. For a normal 90 to 100 mm dish, 19 to 21 mL is an appropriate volume. Allow the completed medium to solidify, invert the plates and allow to dry at room temperature for an adequate time (for complete solidification, store overnight at 18–23 °C). Wrap in fresh plastic bags and store at 2–8 °C. Prepared plates of this medium may be used for 5 to 7 days. If the pour plate method shall be applied, add the material to be tested or its dilution into the empty dish, overlay with the medium, rotate the dish gently to mix, and allow to solidify completely.

Productivity Test (from 10-100 cfu)				
Strains	Glucose Fermentation	Recovery		
E. coli ATCC 25922	Positive: pink colonies	≥ 0.5		
Salmonella typhymurium	Negative: colorless with	≥ 0.5		
ATCC 14028	black shadow colonies			
Selectivity (10 <sup>4</sup> cfu)				
Enterococcus faecalis	Inhibited			
ATCC 19433				

Page 1 of 2 G:\FLYERS\MICRO\MICRO-PHL\Update\PHL-MI- 214-01-VRBG
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