VIOLET RED BILE WITH GLUCOSE AGAR (VRBGA), USP

Cat. no. G178	Violet Red Bile with Glucose Agar, USP, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G297	Violet Red Bile Glucose Agar with Lactose, USP, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Violet Red Bile with Glucose Agar is recommended for the detection and enumeration of *Enterobacteriaceae* in food and dairy products.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Enterobacteriaceae include lactose-fermenting coliform bacteria, non-lactose-fermenting strains of *Escherichia coli*, and other non-lactose-fermenting species of *Salmonella* and *Shigella* involved in food spoilage. Because of their potential contamination of food and dairy products, it is important to detect members of the *Enterobacteriaceae*, rather than traditional coliform bacteria.[1-4]

All species in the *Enterobacteriaceae* family ferment glucose. Mossel et al. modified traditional Violet Red Bile Agar, adding glucose, resulting in the formulation now known as Violet Red Bile Glucose Agar.[5-7]

Violet Red Bile with Glucose Agar contains peptones and yeast extract to supply carbon, nitrogen, essential minerals, and B-complex vitamins to stimulate the growth of bacteria. Glucose supplies energy for growth and metabolism. Bile salts and crystal violet inhibit the growth of gram-positive bacteria. Neutral red is added as a pH indicator. Agar is the solidifying agent. Organisms that ferment glucose will produce red to purple colonies with red-purple halos, demonstrating bile precipitation in the presence of neutral red.

FORMULA

Ingredients per liter of deionized water:*

Glucose	10.0gm
Enzymatic Digest of Gelatin	7.0gm
Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Bile Salts	1.5gm
Neutral Red	0.03gm
Crystal Violet	2.0mg

Agar	15.0gm

Final pH 7.4 +/- 0.2 at 25ºC.

In addition,

Violet Red Bile Glucose Agar with Lactose also contains 10g/L of lactose.
Final pH 7.4 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Consult listed references for information on sample collection and procedures for use.[1-8]

Spread Plate Method:

1. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

2. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.

3. Spread the dilution evenly over the surface of the medium.

4. Using a sterile spreader device, distribute the inoculum evenly over the agar surface.

5. Incubate plates aerobically for 48 +/- 2.0 hours at 35ºC.

INTERPRETATION OF RESULTS

Enterobacteriaceae ferment glucose, thereby producing acid by-products, and form red to dark purple colonies surrounded by a reddish zone, or halo, of bile precipitate.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Due to a variation in nutritional requirements, some strains encountered may grow poorly or fail to grow at all on this medium.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, spreaders, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 8739	J	18-24hr	30-35°C	Aerobic	Growth; pink to red colonies with bile precipitate
Pseudomonas aeruginosa ATCC® 9027	J	18-24hr	30-35°C	Aerobic	Growth
In addition to the above, Cat. no. G297 also include the following:					
Staphylococcus aureus ATCC® 6538	J	24-48 hrs	30-35°C	Aerobic	Partial to complete inhibition

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Tested in accordance with USP <62>.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or

regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Violet Red Bile with Glucose Agar should appear clear, slightly opalescent, and reddish-purple in color.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Dairy Products,* APHA, Washington, D.C.

2. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods,* APHA, Washington, D.C.

3. Draft Standard Methods for Microbiological Examination of Meat Products. 1977. Part 3: *Detection and enumeration of Enterobacteriaceae*. BS5393: Part 3, ISO/DIS 5552.

4. Mossel, D.A.A. 1985. Media for *Enterobacteriaceae*. *Int. J. Food. Microbiol.*; 2:27.

5. Mossel, D.A.A., W.H.J. Mengerink, and H.H. Scholts. 1962. Use of a modified MacConkey agar medium for the selective growth and enumeration of *Enterobacteriaceae*. *J. Bacteriol*.; 84:381.

6. Mossel, D.A.A., I. Eelderink, M. Koopmans, and F. van Rossem. 1978. *Lab Practice*; 27:1049-1050.

7. Mossel, D.A.A., I. Eelderink, M. Koopmans, and F. van Rossem. 1979. Influence of carbon source, bile salts and incubation temperature on recovery of *Enterobacteriaceae* from food using MacConkey-type agars. *J. Food Protect.*; 42:470.

8. The Official Compendia of Standards. USP General Chapter <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms. *USP-NF*. United States Pharmacopeial Convention Inc., Rockville, MD.

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The Hardy Diagnostics manufacturing facility and quality

management system is certified to ISO 13485.