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Instructions for Use

CRITERION™ VIOLET RED BILE GLUCOSE AGAR (VRBGA)

Cat. no. C7270	CRITERION™ Violet Red Bile Glucose Agar	71gm
Cat. no. C7271	CRITERION™ Violet Red Bile Glucose Agar	500gm
Cat. no. C7272	CRITERION™ Violet Red Bile Glucose Agar	2kg
Cat. no. C7273	CRITERION™ Violet Red Bile Glucose Agar	10kg

INTENDED USE

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

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]

CRITERION™ Violet Red Bile 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*

Gram weight per liter:	35.6gm/L
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	9.0gm

Final pH 7.4 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-3ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original reddish-beige.

Store the prepared culture media at 2-8ºC.

The expiration date 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 incubation times as stated below.

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." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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 M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

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

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 35.6gm of the dehydrated culture media in one liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat with frequent agitation and boil for one minute to dissolve completely. **Do not autoclave.**

3. Cool to 45-50ºC. and dispense desired amount into sterile containers.

PROCEDURE

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

The medium is appropriate for use with spread plate or pour plate methods, with or without an agar overlay. The

medium can also be used as an agar overlay for spread plates to prevent colony swarming and to provide semi-anaerobic conditions to suppress the growth of nonfermentative, gram-negative microorganisms. Additionally, stab inoculation procedures using agar deeps can be used with Violet Red Bile Glucose Agar.

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.

Pour Plate Method:

1. Melt agar by placing in a boiling waterbath until liquified.

2. Cool media to 45-50ºC. Maintain in a 45-50ºC waterbath until ready to pour.

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

4. Place a 1ml inoculation into a sterile petri plate.

5. Aseptically pour approximately 18ml of the cooled media (45-50ºC) over the inoculum. Carefully swirl the plate to mix the inoculum evenly.

Note: Do not keep media longer than 3 hours at 45-50ºC. Sterile solidified medium can only be remelted once.

6. Allow to solidify.

7. 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

If using the pour plate technique, the medium should be prepared fresh, tempered to 45ºC, and used within 3 hours.

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

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., 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 Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Growth; red to purple colonies, with reddish precipitate
Salmonella enterica ATCC® 14028	B	18-24hr	35°C	Aerobic	Growth; red to purple colonies, with reddish precipitate
Enterobacter aerogenes ATCC® 13048	A	18-24hr	35°C	Aerobic	Growth; red colonies, may have a slight precipitate
Staphylococcus aureus ATCC® 25923	A	18-24hr	35°C	Aerobic	Partial to complete inhibition; colorless to red colonies, no bile precipitate

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

USER QUALITY CONTROL

Users of dehydrated 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 certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Violet Red Bile Glucose Agar powder should appear homogeneous, free-flowing, and reddish-beige in color. The prepared medium 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. 2008. *USP27-NF22*. United States Pharmacopeial Convention, Rockville, MD.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207B [D]