IFU

Instructions for Use

CRITERION™ VIOLET RED BILE (VRB) AGAR

Cat. no. C7260	CRITERION™ Violet Red Bile Agar	73gm
Cat. no. C7261	CRITERION™ Violet Red Bile Agar	500gm
Cat. no. C7262	CRITERION™ Violet Red Bile Agar	2kg
Cat. no. C7263	CRITERION™ Violet Red Bile Agar	10kg
Cat. no. C7264	CRITERION™ Violet Red Bile Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Violet Red Bile Agar is recommended for the detection of coliforms in food or dairy products.

SUMMARY

Violet Red Bile (VRB) Agar is a selective medium used to detect and enumerate lactose-fermenting coliform microorganisms. The medium is recommended for use in the microbiological analysis of milk and other dairy products and for use in the examination of water.[1,2]

The medium contains bile salts and crystal violet which serve as inhibitory agents toward some gram-positive microorganisms, especially staphylococci. Neutral red is employed as the pH indicator.

Lactose-fermenting microorganisms produce pink to red colonies that are generally surrounded by a reddish zone of precipitated bile. Non-lactose-fermenting microorganisms result in colorless colonies.

FORMULA

Gram weight per liter:	36.6gm/L
Lactose	10.0gm
Pancreatic Digest of Gelatin	7.0gm
Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Bile Salts No. 3	1.5gm
Neutral Red	30.0mg
Crystal Violet	2.0mg
Agar	10.0gm

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 2-30ºC. 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 36.6gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely. Avoid overheating.

3. **Do not autoclave.**

4. Cool to 45-46ºC. and dispense 15-20ml into 100mm Petri dishes containing inoculum. Rotate the plate carefully to distribute the inoculum.

5. After solidification of the inoculated medium, evenly add a cover of 4ml of the cooled (45-46 degrees C.) agar medium.

PROCEDURE

Sample Collection: Consult listed references for information on sample collection.[1-3]

Method of Use: Allow medium to warm to room temperature prior to inoculation. Consult listed references for information concerning inoculation procedures.[1-3]

INTERPRETATION OF RESULTS

Lactose-fermenting microorganisms, including coliforms, produce pink to red colonies that are generally surrounded by a reddish zone of precipitated bile.

Surface colonies of *Escherichia coli* appear as entire-edged colonies, while deep colonies appear lens-shaped.

Colonies of *Enterobacter aerogenes* often appear mucoid and pinkish in color.

Enterococci spp. may grow, and if so, usually appear pinpoint in size and rose colored.

Non-lactose-fermenting microorganisms produce colorless colonies.

LIMITATIONS

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.

Violet Red Bile Agar contains a low concentration of bile salts, and therefore is not completely specific for enterics.

Enterococci may grow, and if so, usually appear pinpoint in size and rose colored.

In using this selective medium, best results will be obtained if it is not subjected to autoclave sterilization since organisms not killed by the boiling required to dissolve the medium, will not form colonies during the 24 hour incubation period. Following boiling to dissolve the medium completely, it is ready for use. The plates should not be incubated longer then 24 hours, inasmuch as the organisms whose growth has been suppressed, may develop and confuse the count. Best results are obtained of plates are not too heavily seeded.

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	
Enterobacter aerogenes ATCC® 13048	A	18-24hr	35°C	Aerobic	Growth; pink to red colonies, may have a slight precipitate around colonies

Escherichia coli ATCC® 25922	A	18-24hr	35°C	Aerobic	Growth; pink to red colonies with a red precipitate around colonies
Salmonella enteritidis ATCC® 13076	A	18-24hr	35°C	Aerobic	Growth; colorless colonies
Staphylococcus aureus ATCC® 25923	B	18-24hr	35°C	Aerobic	Partial to complete inhibition

* 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 Agar powder should appear homogeneous, free-flowing, and reddish-beige in color. The prepared media should appear 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. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater,* APHA, Washington, D.C.

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

4. Atlas, R.M. 1997. *Handbook of Microbiological Media,* 2nd ed. CRC Press, Boca Raton, FL.

5. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

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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]