IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

VIOLET RED BILE (VRB) AGAR

Cat. no. G78	Violet Red Bile Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. U96	Violet Red Bile Agar, 125ml Polycarbonate Bottle, 100ml Deep	20 bottles/box
Cat. no. U296	Violet Red Bile Agar, 8oz. Glass Bottle, 200ml	12 bottles/box
Cat. no. J132	Violet Red Bile Agar with MUG / Violet Red Bile Agar with MUG, 15x100mm Biplate, 18ml/18ml	10 plates/bag

INTENDED USE

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

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

SUMMARY

Violet Red Bile 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.

The addition of MUG (Cat. no. J132), a fluorogenic compound, allows for the rapid detection of *E. coli* when the medium is observed for fluorescence using a long-wave (366nm) UV light source. See Interpetation of Results below.

FORMULA

Ingredients per liter of deionized water:*

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	15.0gm

In addition, Cat. no. J132 also contain 0.1g of MUG (4-methylumbelliferyl-ß-D-glucuronide)

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 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.

PROCEDURE

Sample Collection: Consult listed references for information on sample collection.[1,2]

Method of Use: Allow medium to warm to room temperature prior to inoculation. Consult listed references for information concerning inoculation procedures.[1,2]

For melting bottled media: Autoclave at 121ºC. for 1-3 minutes or until melted. Alternatively, a covered, boiling waterbath (100 degrees C.) can be used. There should be enough water in the waterbath to reach the media line. A covered waterbath will help to reach and maintain the temperature. Heat in waterbath until melted.

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 45-50º 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: After autoclaving, do not heat 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

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. If the media contains MUG, then the colonies of *E. coli* will fluoresce blue.

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

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

Non-lactose-fermenting microorganisms produce colorless colonies.

For products with MUG:

Use a 365nm wavelength handheld UV Lamp (Cat. no. UVL56 or LSS3) to detect colony fluorescence. These handheld lamps require that the room lights be turned off, since ambient light will interfere with fluorescence detection. Alternatively, a dark viewing box (Cat. no. CM10A) with its companion UV lamp (Cat. no. EA160) may be used so that the room lights will not need to be turned off.

CAUTION: Not all UV wavelengths are capable of producing sufficient fluorescence effects. It is important to use a UV light with a wavelength at or near 365nm, one with higher power (in watts, not lumens), and one that is high efficiency. Use of UV lights not meeting these criteria will fail to produce sufficient fluorescence. Most inexpensive battery operated LED UV lights produce light at multiple wavelengths, use less watts, and/or low power, and are thus **not acceptable** and will produce erroneous results. Cat. no. LSS3 is an exception and has been verified to work well. Please do not use cheaper versions.

Tips for using fluorescence

1. Use a 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect colony fluorescence. See

'CAUTION' above regarding inexpensive handheld UV lights. Alternatively, a dark viewing box with its compatible UV lamp may be used as described above. Viewing must be done in the dark.
2. Hold the lamp directly over isolated colonies on the plate, approximately 3 to 4 inches (7 to 10cm) away.
3. Isolated colonies of *E. coli* will fluoresce a blue glow.
4. Only well isolated colonies will fluoresce. Colonies in areas of confluent growth will not.
5. Fluorescence will fade over time.

LIMITATIONS

Products with MUG the Fluorescence must be read in a darkened environment with a 365nm wavelength UV lamp of adequate power (see “Tips for Using Fluorescence” above).

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

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.

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, slides, staining supplies, Lauryl Sulfate Broth, other culture media, microscopes, incinerators, and incubators, as well as handheld UV lamp (Cat. no. UVL56 or LSS3) or dark viewing box (Cat. no. CM10A) with compatible UV lamp (Cat. no. EA160), and 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 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
Violet Red Bile Agar with MUG (Cat. no. J132):					
Escherichia coli ATCC® 25922	A	18-24 hrs	35°C	Aerobic	Growth; pink-red colonies, fluoresces bluish halo under long-wave UV light

Salmonella enterica ATCC® 14028	A	18-24 hrs	35°C	Aerobic	Growth; colorless colonies, no fluorescence under long-wave UV light
Enterococcus faecalis ATCC® 29212	B	18-24 hrs	35°C	Aerobic	Partial to complete inhibition

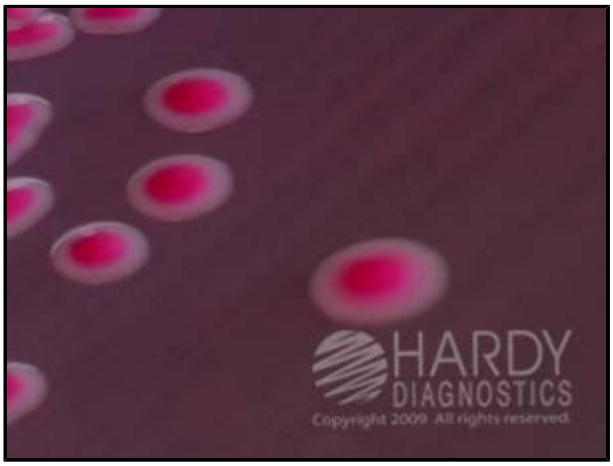
* Refer to the document "Inoculation Procedures for Media QC" for more information.

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 certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

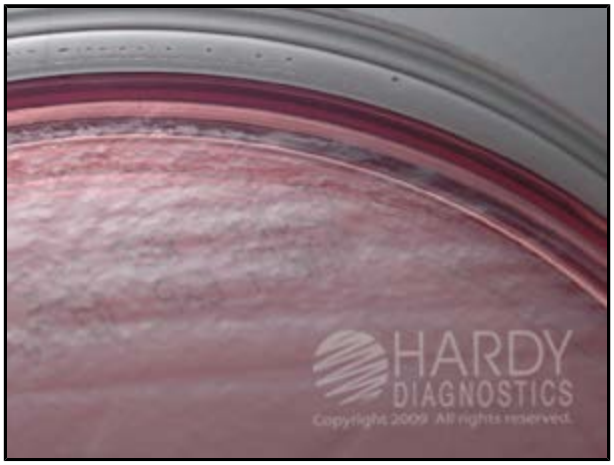
PHYSICAL APPEARANCE

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



Enterobacter aerogenes (ATCC® 13048) colonies growing on Violet Red Bile Agar (Cat no. G78). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC® 25922) colonies growing on Violet Red Bile Agar (Cat no. G78). Incubated aerobically for 24 hours at 35ºC.



Salmonella enteritidis (ATCC® 13076) colonies growing on Violet Red Bile Agar (Cat no. G78). Incubated aerobically for 24 hours at 35ºC.

Staphylococcus aureus (ATCC® 25923) growth inhibited on Violet Red Bile Agar (Cat no. G78). Incubated aerobically for 24 hours at 35ºC.

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.

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

HDQA 2207F [D]