Violet Red Bile Glucose Agar

Intended Use

Violet Red Bile Glucose Agar is used for detecting and enumerating *Enterobacteriaceae* in food and dairy products.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

The *Enterobacteriaceae* group includes lactose-fermenting coliform bacteria, lactose-nonfermenting strains of *E. coli*, and lactose-nonfermenting species, such as *Salmonella* and *Shigella*. When examining some foods, it is desirable to detect *Enterobacteriaceae* rather than the coliform bacteria.[4]

Enterobacteriaceae are glucose-fermenting bacteria. Mossel et al.[5] modified lactose-containing Violet Red Bile Agar by adding glucose to improve the recovery of *Enterobacteriaceae*. Later work by Mossel et al.[6,7] demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar (VRBGA).

Violet Red Bile Glucose Agar is recommended for the detection and enumeration of *Enterobacteriaceae* in food and dairy products.[8,9] 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.[1]

The Violet Red Bile Glucose Agar formulation is available as a dehydrated culture medium, as prepared plated media, and in **Difco™ Hycheck™** *Enterobacteriaceae* hygiene contact slides. **Hycheck™** *Enterobacteriaceae* slides are double-sided paddles containing both Violet Red Bile Glucose Agar and Tryptic Soy Agar surfaces for immersing into fluids or for sampling surfaces.

Principles of the Procedure

Violet Red Bile Glucose Agar contains pancreatic digest of gelatin as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Glucose is a carbohydrate. Bile salts and crystal violet inhibit gram-positive bacteria. Glucose fermenters produce red colonies with red-purple halos (bile precipitation) in the presence of neutral red, a pH indicator. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent.

Formula

Difco™ Violet Red Bile Glucose Agar

Approximate Formula* Per Liter		
Yeast Extract	3.0	g
Pancreatic Digest of Gelatin	7.0	g
Bile Salts No. 3	1.5	g
Glucose	10.0	g
Sodium Chloride	5.0	g
Neutral Red	0.03	g
Crystal Violet	2.0	mg
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 41.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for no more than 2 minutes to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[8,9]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For food samples, refer to appropriate standard references for details on test methods using Violet Red Bile Glucose Agar.[8,9]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *Enterobacteriaceae* using Violet Red Bile Glucose Agar.[1]

This medium can be used in spread or pour plate procedures, with or without an overlay. In addition, this medium can be used as an overlayer for spread plates to both prevent swarming colonies and to provide semi-anaerobic conditions that suppress the growth of nonfermentative gram-negative organisms. Stab inoculation procedures can also be used with this medium.

Expected Results

Enterobacteriaceae ferment glucose, produce acid products and form red to dark purple colonies surrounded by red-purple halos.

Limitation of the Procedure

When used in the pour plate procedure, the medium should be freshly prepared, tempered to 47°C, and used within 3 hours.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Mossel. 1985. Int. J. Food Microbiol. 2:27.
5. Mossel, Mengerink and Scholts. 1962. J. Bacteriol. *84*:381.
6. Mossel, Eelderink, Koopmans and van Rossem. 1978. Lab Practice *27*:1049.
7. Mossel, Eelderink, Koopmans and van Rossem. 1979. J. Food Protect. *42*:470.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. International Organization for Standardization. 2004. Microbiology of food and animal feeding stuffs – horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony count method. ISO 21528-2, 1st ed., 2004-08-15. International Organization for Standardization, Geneva, Switzerland.

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User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

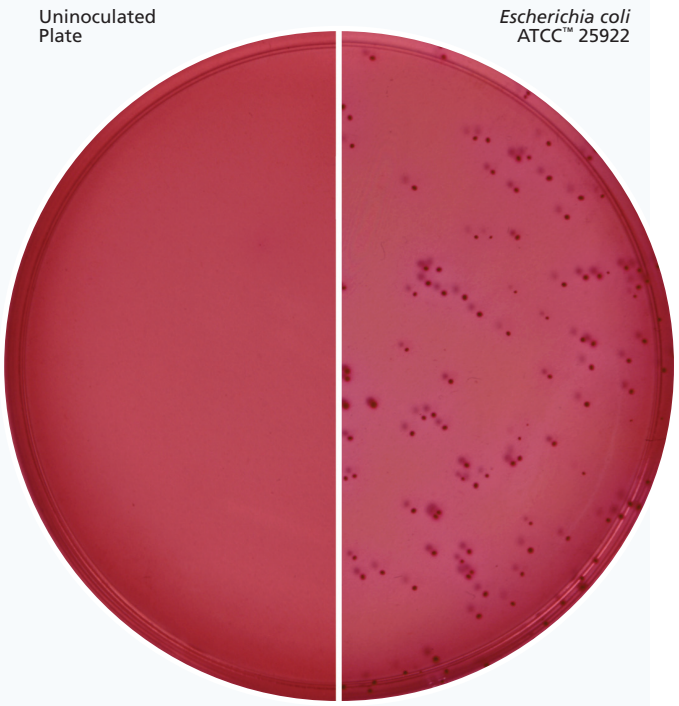
Identity Specifications

Difco™ Violet Red Bile Glucose Agar

Dehydrated Appearance:	Pink-beige to pink, free-flowing, homogeneous (may contain small dark particles).
Solution:	4.15% solution, soluble in purified water upon boiling. Solution is reddish purple, very slightly to slightly opalescent.
Prepared Appearance:	Reddish purple, very slightly to slightly opalescent.
Reaction of 4.15% Solution at 25°C:	pH 7.4 ± 0.2

BBL™ Violet Red Bile Glucose Agar (prepared)

Appearance:	Reddish to purple and opalescent.
Reaction at 25°C:	pH 7.4 ± 0.2



Cultural Response

Difco™ Violet Red Bile Glucose Agar

Prepare the medium per label directions. Using the pour plate method, inoculate and incubate at 35 ± 2°C for 18-24 hours. Using the streak plate method, inoculate and incubate (*) cultures at 30-35°C and (**) culture at 35-37°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Acinetobacter baumannii	19606	~10^3	None to poor	Colorless to red colonies, no bile ppt
Escherichia coli	25922	100-300	Good	Red to purple colonies, with bile ppt
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	100-300	Good	Red to purple colonies, with bile ppt
Staphylococcus aureus	25923	~10^3	None to poor	Colorless to red colonies, no bile ppt
*Escherichia coli**	8739	<100	Growth (30-35°C)	N/A
*Escherichia coli***	8739	<100	Growth (35-37°C)	N/A
*Pseudomonas aeruginosa**	9027	<100	Growth (30-35°C)	N/A

BBL™ Violet Red Bile Glucose Agar (prepared)

Inoculate plates and incubate *E. coli* strains and *P. aeruginosa* at 30-35°C for 18-24 hours. Incubate *S. Typhimurium* and *S. aureus* at 35-37°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Escherichia coli	25922	10-100	Good	Red to purple colonies, with bile ppt
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Red to purple colonies, with bile ppt
Staphylococcus aureus	6538	10^3-10^4	None to poor	Colorless to red colonies, no bile ppt
Escherichia coli	8739	10-100	Growth	N/A
Pseudomonas aeruginosa	9027	10-100	Growth	N/A

Availability

Difco™ Violet Red Bile Glucose Agar

CCAM COMPF EP ISO JP USP

Cat. No. 218661 Dehydrated – 500 g†

BBL™ Violet Red Bile Glucose Agar

CCAM COMPF EP ISO JP USP

United States and Canada

Cat. No. 215198 Prepared Plates – Pkg. of 20*†
215053 Prepared Plates – Ctn. of 100*†

Europe

Cat. No. 254486 Prepared Plates – Pkg. of 20*†
257042 Prepared Contact Plates – Pkg. of 33*

Difco™ Hycheck™ Enterobacteriaceae Hygiene Contact Slides

Cat. No. 290003 Violet Red Bile Glucose Agar//Tryptic Soy Agar – Box of 10 slides*

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.