

Formula

Difco™ Violet Red Bile Agar with MUG

Approximate Formula* Per Liter		
Yeast Extract	3.0	g
Peptone	7.0	g
Bile Salts No. 3	1.5	g
Lactose	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Neutral Red.....	0.03	g
Crystal Violet.....	2.0	mg
MUG (4-methylumbelliferyl-β-D-glucuronide)	0.1	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 41.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Process each specimen as appropriate for that specimen.¹⁻³
2. Incubate plates at 35°C for 22-26 hours.
3. Examine plates for growth and fluorescence.

Expected Results

Coliform organisms form purplish-red colonies that are generally surrounded by a reddish zone of precipitated bile. When examined under long-wave fluorescent light, MUG-positive colonies are surrounded by a bluish fluorescent halo. MUG-negative colonies lack the fluorescent halo.

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

E. coli colonies are red surrounded by a zone of precipitated bile and fluoresce blue under long-wave UV light.

Salmonella and *Shigella* strains that produce glucuronidase may be encountered infrequently but these are generally lactose negative and appear as colorless colonies which may fluoresce.

Limitations of the Procedure

1. Glucuronidase-negative strains of *E. coli* have been encountered.⁵⁻⁷ Similarly, glucuronidase-positive strains of *E. coli* that do not fluoresce have been reported.⁸
2. Strains of *Salmonella* and *Shigella* that produce glucuronidase may infrequently be encountered.⁹ These strains must be distinguished from *E. coli* on the basis of other parameters; e. g., gas production, lactose fermentation or growth at 44.5°C.

References

1. Davidson, Roth, and Gambrel-Lenarz. 2004. In Wehr and Frank (ed.). Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
2. Kornacki and Johnson. 2001. In Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Feng and Hartman. 1982. Appl. Environ. Microbiol. 43:1320.
5. Chang, Brill and Lum. 1989. Appl. Environ. Microbiol. 55:335.
6. Hansen and Yourassowsky. 1984. J. Clinical Microbiol. 20:1177.
7. Kilian and Bulow. 1976. Acta Pathol. Microbiol. Scand. Sect. B 84:245.
8. Mates and Shaffer. 1989. J. Appl. Bacteriology 67:343.
9. Damare, Campbell and Johnston. 1985. J. Food Sci. 50:1736.

Availability

Difco™ Violet Red Bile Agar with MUG

BAM COMPF

Cat. No. 229100 Dehydrated –500 g

BBL™ Violet Red Bile Agar with MUG

BAM COMPF

Cat. No. 299128 Prepared Bottle (200 mL) – Pkg. of 10

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.

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

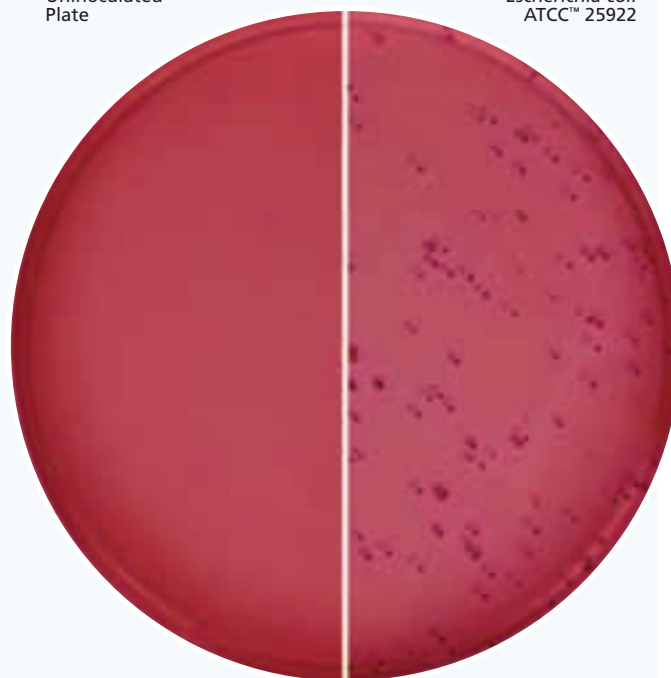
BBL™ Violet Red Bile Glucose Agar (prepared)

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
<i>Escherichia coli</i>	25922	10-100	Good	Red to purple colonies, with bile ppt
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	Red to purple colonies, with bile ppt
<i>Staphylococcus aureus</i>	6538	10 ³ -10 ⁴	None to poor	Colorless to red colonies, no bile ppt
<i>Escherichia coli</i>	8739	10-100	Growth	N/A
<i>Pseudomonas aeruginosa</i>	9027	10-100	Growth	N/A

Uninoculated Plate

Escherichia coli
ATCC™ 25922



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 overlay 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.

Vitamin B₁₂ Assay Medium

Intended Use

Vitamin B₁₂ Assay Medium is used for determining vitamin B₁₂ concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;

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 pharmacopeia, 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.

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.

3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Vitamin B₁₂ Assay Medium is prepared according to the formula described by Capp, Hobbs and Fox.¹ This medium is used in the microbiological assay of vitamin B₁₂ using *Lactobacillus delbrueckii* subsp. *lactis* (*Lactobacillus leichmannii*) ATCC™ 4797 or 7830.

Principles of the Procedure

Vitamin B₁₂ Assay Medium is a vitamin B₁₂-free medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis* ATCC 4797 or 7830. To obtain