

Violet Red Bile Agar with MUG

Intended Use

Violet Red Bile Agar with MUG is used for enumerating *Escherichia coli* and total coliform bacteria in food and dairy products.

Summary and Explanation

Violet Red Bile Agar is specified in standard methods procedures to enumerate coliforms in food and dairy products.¹⁻³ In 1982, Feng and Hartman developed a rapid fluorogenic assay for *Escherichia coli* by incorporating 4-methylumbelliferyl- β -D-glucuronide (MUG) into Lauryl Tryptose Broth.⁴ Incorporating MUG into Violet Red Bile Agar permits the detection of *E. coli* among the coliform colonies.^{2,3}

Standard methods procedures include Violet Red Bile Agar with MUG for detecting *E. coli* in food and dairy products by fluorescence.¹⁻³

Principles of the Procedure

Violet Red Bile Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Bile salts and crystal violet inhibit gram-positive bacteria. Lactose is a carbohydrate source. Neutral red is a pH indicator. MUG (4-methylumbelliferyl- β -D-glucuronide) is a substrate used for detecting glucuronidase activity. Agar is the solidifying agent.

E. coli produces the enzyme glucuronidase which hydrolyzes MUG to yield a fluorogenic compound detectable with long-wave UV light (366 nm). Typical strains of *E. coli* (red colonies surrounded by a bile precipitate) exhibit blue fluorescence. Non-*E. coli* coliforms may produce red colonies with zones of precipitated bile but they are MUG negative.

User Quality Control

Identity Specifications

Difco™ Violet Red Bile Agar with MUG

Dehydrated Appearance: Reddish beige, free-flowing, homogeneous.

Solution: 4.16% solution, soluble in purified water upon boiling. Solution is reddish-purple, clear to slightly opalescent, without significant precipitate.

Prepared Appearance: Reddish-purple, clear to slightly opalescent, no significant precipitate.

Reaction of 4.16% Solution at 25°C: pH 7.4 \pm 0.2

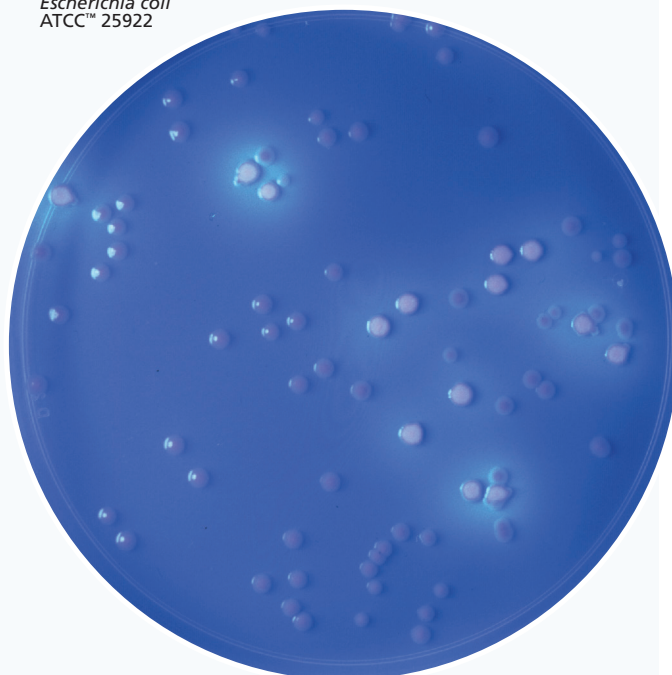
Cultural Response

Difco™ Violet Red Bile Agar with MUG

Prepare the medium per label directions. Inoculate and incubate at 32 \pm 2°C for 22-26 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	FLUORESCENCE
<i>Enterobacter aerogenes</i>	13048	30-300	Good	Pink, may have bile ppt.	–
<i>Escherichia coli</i>	25922	30-300	Good	Deep red, with bile ppt.	+
<i>Staphylococcus aureus</i>	25923	3 \times 10 ² -10 ³	Marked to complete inhibition	–	–

Escherichia coli
ATCC™ 25922



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.

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