Violet Red Bile Agar with MUG (VRBA-MUG) (NCM0064)

Intended Use
Violet Red Bile Agar with MUG is used for the detection of coliforms and the fluorogenic detection of *Escherichia coli*. Violet Red Bile Agar with MUG is not intended for use in the diagnosis of disease or other conditions in humans.

Description
Violet Red Bile Agar W/ MUG is specified in many procedures to enumerate coliforms in food and dairy products. 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. Similarly, incorporating MUG into Violet Red Bile Agar permits the detection of *E. coli* among the coliform colonies. Standard methods procedures specify Violet Red Bile Agar with MUG for detecting *E. coli* in food and dairy products by fluorescence. This medium is often abbreviated as VRBA w/ MUG.

Formula / Liter

Enzymatic Digest of Gelatin	7.0 g/L
Yeast Extract	3.0 g/L
Bile Salts Mixture	1.5 g/L
Lactose	10.0 g/L
Sodium Chloride	5.0 g/L
Neutral Red	0.03 g/L
Crystal Violet	0.002 g/L
4-Methylumbelliferyl-β-D-Glucuronide	0.1 g/L
Agar	15.0 g/L

Final pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Suspend 41.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium. DO NOT AUTOCLAVE.
3. Cool to 45-50°C.

Test Procedure
1. Process each sample as appropriate by either the pour plate method (with or without overlay) or surface spread. (with or without overlay).
2. Incubate plates at 33-38°C for 18-24 hours.
3. Examine plates for growth and fluorescence.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and pinkish to reddish-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and reddish-purple.

Expected Cultural Response: Cultural response on Violet Red Bile Agar w/ MUG incubated aerobically at 33-38°C and examined for growth after 18 - 24 hours.

Effective Date: 5/15/2019
Revision: 0

620 Lesher Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodsafety@neogen.com • foodsafety.neogen.com

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Recovery	Fluorescence
Enterobacter aerogenes ATCC® 13048	50-200	>50%	Negative
Enterococcus faecalis ATCC® 29212	$>10^4$	Complete Inhibition	Negative
Escherichia coli ATCC® 25922	50-200	>50%	Positive
Pseudomonas aeruginosa ATCC® 27853	$>10^3$	Growth	Negative
Salmonella typhimurium ATCC® 14028	50-200	>50%	Negative
Staphylococcus aureus ATCC® 25923	$>10^4$	Complete Inhibition	Negative

The organisms listed are the minimum that should be used for quality control testing.

Results

Coliform organisms form purple-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.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Glucuronidase-negative strains of *E. coli* have been encountered. Similarly, glucuronidase-positive strains of *E. coli* that do not fluoresce have been reported. Strains of *Salmonella* and *Shigella* that produce glucuronidase may infrequently be encountered. 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.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Marshall, R. T. (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
2. Vanderzant, C. and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.
4. Feng, P. C. S., and P. A. Hartman. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl Environ. Microbiol. 43:1320-1329.
5. Chang, G. W., J. Brill, and R. Lum. 1989. Proportion of β-D-glucuronidase negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. 55:335-339.

Effective Date: 5/15/2019

Revision: 0

620 Lesher Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodsafety@neogen.com • foodsafety.neogen.com

6. Hansen, W., and E. Yourassowsky. 1984. Detection of β-D-glucuronidase in lactose fermenting members of the family *Enterobacteriaceae* and its presence in bacterial urine cultures. J. Clin. Microbiol. 20:1177-1179.
7. Kilian, M., and P. Bulow. 1976. Rapid diagnosis of *Enterobacteriaceae.* Acta. Pathol. Microbiol. Scand. Sect. B, 84:245-251.
8. Mates, A., and M. Shaffer. 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriol. 67:343-346.
9. Damare, J. M., D. F. Campbell, and R. W. Johnston. 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. J. of Food Sci. 50:1736-1746.

Effective Date: 5/15/2019

Revision: 0

NEOGEN®
620 Lesher Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodsafety@neogen.com • foodsafety.neogen.com