Nutrient Agar with MUG

Intended Use

Nutrient Agar with MUG is used for detecting and enumerating *Escherichia coli* in water.

Summary and Explanation

Escherichia coli is a member of the fecal coliform group of bacteria. The presence of *E. coli* is indicative of fecal contamination.[1] Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) at a final concentration of 100 µg/mL into Lauryl Tryptose Broth. Nutrient Agar is similarly modified with the addition of MUG. Rapid quantitation and verification may be achieved with the membrane filtration procedure by transferring the membrane from a total-coliform or fecal-coliform positive sample to a Nutrient Agar substrate containing 4-methylumbelliferyl-β-D-glucuronide (MUG).[1]

Mates and Shaffer[3] used the membrane filter-Endo Agar method, followed by incubation on Nutrient Agar with MUG, to detect and enumerate *E. coli* within 4 hours of membrane transfer. *E. coli* was recovered at a rate of 98% with no false-positive results.

Nutrient Agar with MUG is prepared according to the formula specified by the U.S. Environmental Protection Agency[4] and published in *Standard Methods for the Examination of Water and Wastewater*.[1]

User Quality Control

Identity Specifications

Difco™ Nutrient Agar with MUG

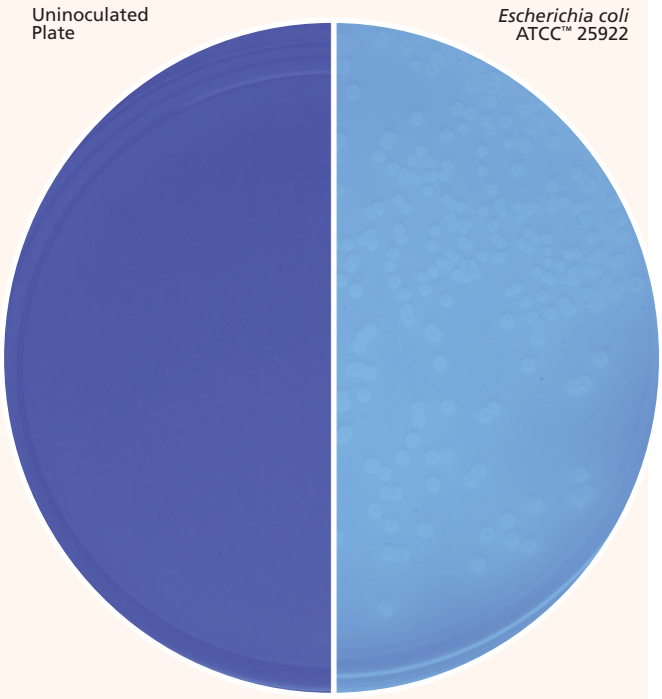
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.31% solution, soluble in purified water upon boiling. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 2.31% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Agar with MUG

Prepare the medium per label directions. After incubation on m Endo Agar LES using the membrane filter technique, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 4-24 hours at 35 ± 2°C. Examine for fluorescence under long-wave (approximately 366 nm) UV light.

ORGANISM	ATCC™	INOCULUM CFU	FLUORESCENCE
Enterobacter aerogenes	13048	30-300	–
Escherichia coli	25922	30-300	+



Principles of the Procedure

Beef extract and peptone are sources of nitrogen, vitamins, carbon and amino acids. Agar is the solidifying agent. The substrate, MUG (4-methylumbelliferyl-β-D-glucuronide), produces a blue fluorescence when hydrolyzed by the enzyme β-glucuronidase, which is produced by most *E. coli*.

Formula

Difco™ Nutrient Agar with MUG

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g
Agar	15.0	g
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 23.1 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.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Follow the methods and procedures for water testing using m Endo Agar LES in standard methods.[1] After incubation on m Endo Agar LES, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 18-24 hours at 35 ± 2°C. Expose the filter surface to long-wave UV light.

Expected Results

Observe for fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence around the periphery of the colony under long-wave (approximately 366 nm) UV light.

Typical strains of *E. coli* (red with a green metallic sheen on m Endo Agar LES) exhibit blue fluorescence on Nutrient Agar with MUG. Non-*E. coli* coliforms may produce a metallic sheen but do not fluoresce.

Limitations of the Procedure

1. Glucuronidase-negative strains of *E. coli* have been encountered.[5-7] Similarly, MUG-negative strains of *E. coli* have been reported in this assay procedure but at a very low frequency.[3]
2. Strains of *Salmonella* and *Shigella* species that produce glucuronidase may infrequently be encountered.[8] These strains must be distinguished from *E. coli* on the basis of other parameters; i.e., gas production, lactose fermentation or growth at 44.5°C.

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Feng and Hartman. 1982. Appl. Environ. Microbiol. *43*:1320.
3. Mates and Shaffer. 1989. J. Appl. Bacteriol. *67*:343.
4. Federal Register. 1991. Fed. Regist. *56*:636.
5. Chang, Brill and Lum. 1989. Appl. Environ. Microbiol. *55*:335.
6. Hansen and Yourassowsky. 1984. J. Clin. Microbiol. *20*:1177.
7. Kilian and Bulow. 1976. Acta Pathol. Microbiol. Scand. Sect. B *84*:245.
8. Damare, Campbell and Johnston. 1985. J. Food Sc. *50*:1736.

Availability

Difco™ Nutrient Agar with MUG

EPA SMWW

Cat. No.	223100	Dehydrated – 100 g
	223200	Dehydrated – 500 g