EC Medium with MUG

Intended Use

EC Medium with MUG is used for detecting *Escherichia coli* in water, food and milk.

Summary and Explanation

EC Medium was developed by Hajna and Perry[1] to improve the methods for the detection of coliforms and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts No. 3. Growth of sporeformers and fecal streptococci is inhibited by the bile salts, while growth of *E. coli* is enhanced. EC Medium with MUG is the same formula as EC Medium with the addition of 4 methyl-umbelliferyl-β-D-glucuronide.

Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) into Lauryl Tryptose Broth at a final concentration of 100 µg/mL. Robison[3] compared the fluorogenic assay with present methodology and found that total agreement between the two methods was 94.8%. Moburg[4] determined the amount of MUG could be reduced to a final concentration of 50 µg/mL without adversely affecting results. Koburger and Miller[5] recommended the incorporation of MUG into EC Broth for use in testing shellfish.

EC Medium with MUG is prepared according to the formula specified by the U.S. Environmental Protection Agency[6] and standard methods for water and food testing.[7,8]

Principles of the Procedure

Peptone provides the nitrogen, vitamins and amino acids in EC Medium with MUG. Lactose is the carbon source in this medium. Bile Salts No. 3 is the selective agent against gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium phosphate and monopotassium phosphate are buffering agents. Sodium chloride maintains the osmotic balance of the medium.

E. coli produces the enzyme glucuronidase that hydrolyzes MUG to yield a fluorogenic product that is detectable under long wave (366 nm) UV light. The addition of MUG to EC Medium provides another criterion, in addition to growth response and gas production, to determine the presence of *E. coli* in food and environmental samples.

User Quality Control

Identity Specifications

Difco™ EC Medium with MUG

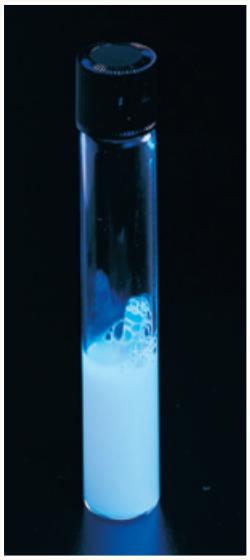
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.71% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 3.71% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ EC Medium with MUG

Prepare the medium per label directions. Inoculate tubes in duplicate with fresh 18-24 hour cultures. Incubate the first set at 35 ± 2°C for 24 ± 2 hours and the second set at 44.5 ± 0.2°C for 24 ± 2 hours. Read fluorescence under a long-wave UV light.

ORGANISM	ATCC™	RECOVERY AT 35°C/GAS	RECOVERY AT 44.5°C/GAS	FLUORESCENCE
Enterobacter aerogenes	13048	Good/±	Inhibition to good/–	–
Enterococcus faecalis	19433	Inhibition/–	Inhibition to good/–	–
Escherichia coli	25922	Good/+	Good/+	+



Escherichia coli ATCC™ 25922

Formula

Difco™ EC Medium with MUG

Approximate Formula* Per Liter		
Tryptose	20.0	g
Lactose	5.0	g
Bile Salts No. 3	1.5	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g
MUG (4-methylumbelliferyl-β-D-glucuronide)	0.05	g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 37.1 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense into test tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Follow the methods and procedures as stated in appropriate references.[6-8]

Expected Results

Following incubation, observe tubes for growth, production of gas and fluorescence. Positive gas production is demonstrated by displacement of the medium from the fermentation vial. Positive MUG reactions exhibit a bluish fluorescence under long-wave (approximately 366 nm) UV light. Typical strains of *E. coli* are positive for both gas production and fluorescence. Non-*E. coli* coliforms that grow may exhibit fluorescence but will not produce gas.

Strains of *Salmonella*, *Shigella* and *Yersinia* that produce glucuronidase may be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters; i.e., gas production, growth at 44.5°C.

Limitations of the Procedure

1. Strains of *E. coli* that fail to grow in EC Medium with MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered.
2. The presence of endogenous glucuronidase in shellfish samples may cause false positive fluorescent reactions at the presumptive stage. To prevent this problem, the use of EC Medium with MUG in the confirmatory stage has been recommended.[5]

References

1. Hajna and Perry. 1943. Am. J. Public Health *33*:550.
2. Feng and Hartman. 1982. Appl. Environ. Microbiol. *43*:1320.
3. Robison. 1984. App. Environ. Microbiol. *48*:285.
4. Moberg. 1985. Appl. Environ. Microbiol. *50*:1383.
5. Koburger and Miller. 1985. J. Food Prot. *48*:244.
6. Federal Register. 1991. National primary drinking water regulation; analytical techniques; coliform bacteria. Fed. Regist. *56*:636.
7. 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.
8. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ EC Medium with MUG

BAM CCAM EPA SMWW

Cat. No.	222100	Dehydrated – 100 g
	222200	Dehydrated – 500 g