

Principles of the Procedure

EC Medium contains peptone as a source of nutrients. Lactose provides fermentable carbohydrate for the growth of coliforms. Bile salts are inhibitory for gram-positive bacteria, particularly bacilli and fecal streptococci. The medium has a strong potassium phosphate buffering system to control the pH in the presence of considerable fermentative action. Sodium chloride maintains the osmotic balance of the medium.

Formula

Difco™ EC Medium

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

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

Directions for Preparation from Dehydrated Product

1. Dissolve 37 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense into 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.

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 Perry¹ 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 methylumbelliferyl-β-D-glucuronide.

Feng and Hartman² 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. Robison³ compared the fluorogenic assay with present methodology and found that total agreement between the two methods was 94.8%. Moburg⁴ determined the amount of MUG could be reduced to a final concentration of 50 µg/mL without adversely affecting results. Koburger and Miller⁵ recommended the incorporation of MUG into EC Broth for use in testing shellfish.

Procedure

Refer to the various compendia for the specific procedures employing EC Medium.⁴⁻⁸

Expected Results

Refer to the compendia for the results expected when using this medium for the detection of coliforms and *E. coli*.⁴⁻⁸

Limitation of the Procedure

False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents.⁹

References

1. Hajna and Perry. 1943. Am. J. Public Health 33:550.
2. Tennant, Reid, Rockwell and Bynoe. 1961. Can. J. Microbiol. 1:733.
3. Fishbein and Surkiewicz. 1964. Appl. Microbiol. 12:127.
4. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
8. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. 1. AOAC International, Gaithersburg, Md.
9. Ray. 1986. J. Food. Prot. 49:651.

Availability

Difco™ EC Medium

	AOAC	BAM	CCAM	COMPF	EPA	ISO	SMD	SMWW	USDA
Cat No.	231420								
	231430								
	231410								

Dehydrated – 100 g

Dehydrated – 500 g

Dehydrated – 10 kg

EC Medium with MUG is prepared according to the formula specified by the U.S. Environmental Protection Agency⁶ 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.

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. Koberger 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. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
8. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.

Availability

Difco™ EC Medium with MUG

BAM **CCAM** **EPA** **SMWW**

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

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
<i>Enterobacter aerogenes</i>	13048	Good/±	Inhibition to good/–	–
<i>Enterococcus faecalis</i>	19433	Inhibition/–	Inhibition to good/–	–
<i>Escherichia coli</i>	25922	Good/+	Good/+	+



Escherichia coli
ATCC™ 25922