Use of EC-MUG Media to Confirm Escherichia coli Contamination in Water Samples Protocol

Created: Monday, 23 August 2010

Author

- Naowarat Cheeptham
- Archana Lal

Information

History

Presence of coliforms and fecal coliforms in food and water samples is considered an indicator of fecal contamination. *Escherichia coli* is a member of the fecal coliform bacteria, and traditionally the presence of *E. coli* in any tested sample is taken as indication of fecal contamination.

Escherichia coli (EC) medium was first introduced by Hajna and Perry (12). EC medium consisted of a buffered lactose broth with 0.15% bile salts no. 3 and was used for testing water, milk, shellfish, and other material for evidence of fecal contamination. EC medium was shown to be excellent for the isolation of coliform bacteria at 37°C and of *E. coli* at 45.5°C (19). Feng and Hartman (9) developed EC medium with 4-methylumbelliferyl-β-D-glucuronide (MUG) for rapid screening of *Escherichia coli*detection. They incorporated MUG in lauryl tryptose broth (LTB) at a final concentration of 100 µg/ml. LTB is very similar to EC medium in terms of its components, however, LTB was developed by Mallmann and Darby to have sodium lauryl sulfate added to it for the purpose of detecting coliform organisms in food and dairy since this chemical is known to be selective but not inhibitory for coliforms (5). Moberg (18) reported that a MUG concentration of 50 µg/ml provided the same intensity of blue fluorescence as the 100 µg/ml MUG levels. Koburger and Miller (15) recommended EC broth with MUG to test contamination in shellfish.

Approved by the U.S. Environmental Protection Agency (24), 4-methylumbelliferyl-β-D-glucuronide*Escherichia coli* broth medium (EC-MUG) is an effective and rapid method for detection and verification of *E. coli* in food, water, and environmental samples.

Purpose

In this protocol, we will emphasize using EC broth and EC agar media with MUG for *E. coli* detection in water samples. Conventionally and still practiced in many places, the Standard Analysis of Waterwhich includes the Most Probable Number (MPN) has to date dominated the scene of coliform and fecal coliform bacterial tests in water samples (3, 16). The Most Probable Number test is quantitative and can be

determined from the result of the presumptive test (16).

EC broth and agar media with MUG is best suited for confirmatory testing of the presence of *E. coli*after a presumptive positive result for fecal coliform bacteria.

Theory

The enzyme ß-glucuronidase (GUD) was first recognized in *E. coli* by Buehler et al. (1). About 96 to 97% of *E. coli* strains contain GUD enzyme (13) that is capable of hydrolyzing the colorless substrate MUG to yield a bluish fluorogenic product 4-methylumbelliferone (MU) that fluoresces under long wave UV light (366 nm) and can be easily visualized in the medium or around the colonies. MUG is neither inhibitory nor stimulatory to the growth of *E. coli* (22).

EC medium contains tryptose as a source of nutrients and lactose as the carbon source (fermentable carbohydrate for coliforms). Bile salts no. 3 is the selective agent against gram-positive bacteria, especially bacilli and fecal streptococci. Dipotassium phosphate and monopotassium phosphate are used as buffering agents to control the pH in the medium. Sodium chloride helps to preserve the osmotic balance of the medium (4).

In addition to growth response and acid and gas production, addition of MUG to the EC medium provides another criterion to determine the presence of *E. coli* in water samples. The presence of fluorescence is considered to be a positive *E. coli* test (9, 21). *E. coli*-negative samples are identified by lack of fluorescence within 24 hours and require no further testing (18). About 5% of the known strains of *E. coli* that are anaerogenic (do not produce gas) (6) can be detected due to the bluish fluorescence. Fluorescence observation is more sensitive and efficient than gas production for detection of *E. coli*.

Some other enteric bacteria, e.g., a few species of *Salmonella*, *Shigella*, and *Yersinia*, are also known to have the GUD enzyme (7, 8, 17, 18, 20, 21) and therefore the ability to produce fluoresecence, but none resulted in the production of blue fluorescence in more than 1,400 samples evaluated in a study by Moberg (18).

RECIPES (4, 5)

EC broth medium with MUG (g/liter)

Tryptose or trypticase	20.0
Lactose	5.0
Sodium chloride	5.0
Bile salts mixture or bile salts no. 3	1.5
Dipotassium hydrogen phosphate (K_2HPO_4)	4.0
Potassium dihydrogen phosphate (KH_2PO_4)	1.4

4-methylumbelliferyl-ß-D-glucuronide (MUG)	0.1

Dissolve dehydrated powder of the EC broth medium with MUG per label directions (37.1 g in 1 liter of purified water). Mix thoroughly, warm slightly to dissolve completely. Transfer 10 ml of the medium into each fermentation tube, which contains a small inverted Durham tube inside it (10). Close all the fermentation tubes with heat resistant caps. Autoclave all the tubes at 121°C for 15 minutes at 15 psi. After sterilization, the pH of the solution should be 6.9 $\pm$ 0.2 at 25°C. The inverted Durham tubes inside the fermentation tubes should be free of any trapped air bubbles. The prepared broth is clear and can be light to medium gold in color.

The prepared EC broth medium with MUG can be stored in the refrigerator for up to 3 months in screw cap tubes (24). The stored medium should be incubated overnight at 35°C before use.

EC agar medium with MUG (g/liter)

Tryptose or trypticase	20.0
Lactose	5.0
Sodium chloride	5.0
Bile salts mixture or bile salts no. 3	1.5
Dipotassium hydrogen phosphate (K_2HPO_4)	4.0
Potassium dihydrogen phosphate (KH_2PO_4)	1.4
4-methylumbelliferyl-ß-D-glucuronide (MUG)	0.1
Agar	15.0

Dissolve dehydrated powder of the EC agar medium with MUG per label directions (23.1 g in 1 liter of purified water). Mix thoroughly, warm slightly to dissolve completely. The pH of the solution should be 6.9 $\pm$ 0.2 at 25°C. Autoclave the medium at 121°C for 15 minutes at 15 psi. After sterilization, fillpetri plates with the sterile liquid agar medium (approximately 20 ml per plate) and let agar medium properly solidify before use. The prepared agar in each plate is clear and can be light to medium gold in color.

The prepared media can be stored in the refrigerator for about 2 weeks (24). The stored medium should be incubated overnight at 35°C before use.

PROTOCOL (4, 23)

When drinking water samples are tested, EC broth and agar media with MUG are typically used as verification means to indicate whether there is any *E. coli* contamination in the samples (whether the positive results showing coliforms and fecal coliforms are confirmably *E. coli*).

EC broth medium with MUG

Inoculation. Aseptically transfer 1 ml of the liquid from the positive presumptive tube to the fermentation tube containing EC broth medium with MUG. In the case of positive presumptive growth on the surface of an agar plate, transfer the distinct colony (demonstrating green metallic sheen on M-Endo medium or on eosin-methylene blue plates) to the EC broth medium with MUG. Prior to incubation, Durham tubes should be checked for any trapped gas bubbles, since trapped bubbles may lead to false-positive readings.

Incubation. Within 30 minutes of inoculation, incubate the inoculated EC-MUG broth tube(s) in a water bath at 44.5°C ± 0.2°C for 24 hours ± 2 hours. The water level in the water bath should be maintained to cover the uppermost level of the broth medium in the fermentation tubes. Please note that incubation temperature can be set either at 35°C ± 2°C or 44.5°C ± 0.2°C, however, 44.5°C ±0.2°C is recommended for detection of thermophilic fecal coliforms and should be done in a water bath since it is recommended in the standard protocol to be done in a high humidity environment. A dry incubator may be used with a wet towel or cotton as a source of humidity but there could be a wide temperature swing in the dry incubator.

Interpretation of Results. Development of turbidity in the fermentation tubes and presence of gas in the Durham tubes within 24 hours ± 2 hours of incubation at 44.5°C ± 0.2°C are considered positive evidence of fecal coliforms in water samples. Presence of growth (turbidity) and a bright blue fluorescence under a long-wave (366 nm) UV light (with or without the production of gas) are considered confirmatory for the presence of *E. coli* (11) (Fig. 1 , 2, and 3).

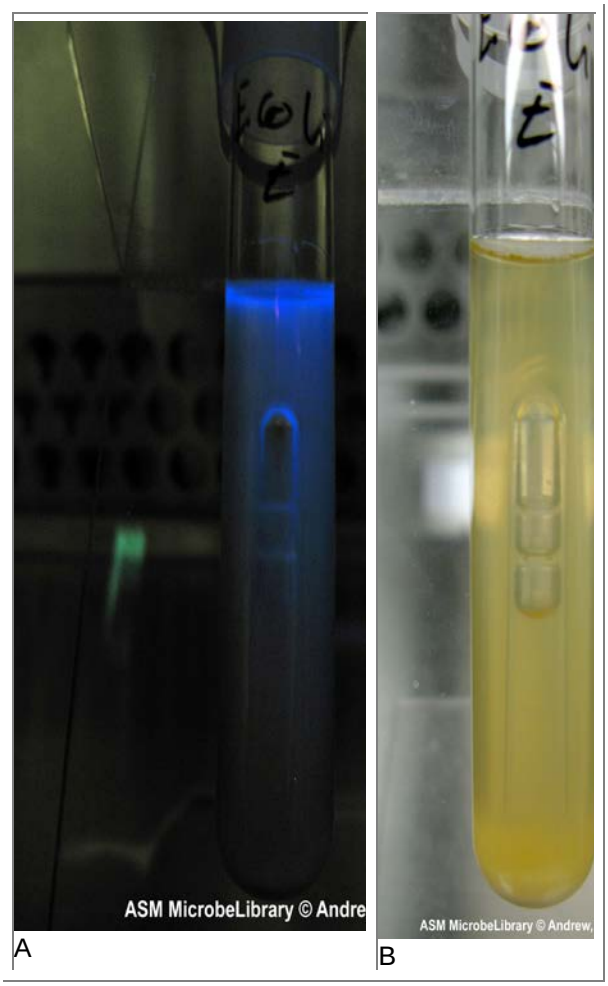
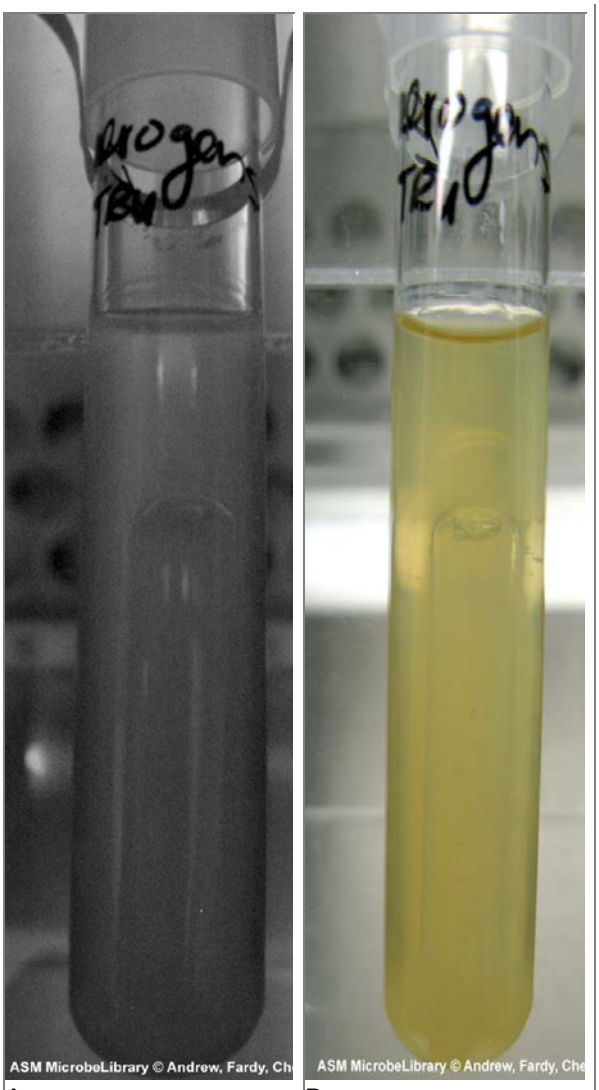


FIG. 1. Growth of *E. coli* in EC-MUG broth observed (A) under UV light and (B) without UV light. (A)under UV light and (B) without UV light.

FIG. 2. Growth of *Bacillus* sp. in EC-MUG broth observed



A B

FIG. 3. Growth of *Enterobacter aerogenes* in EC-MUG broth observed (A) under UV light and (B)without UV light.

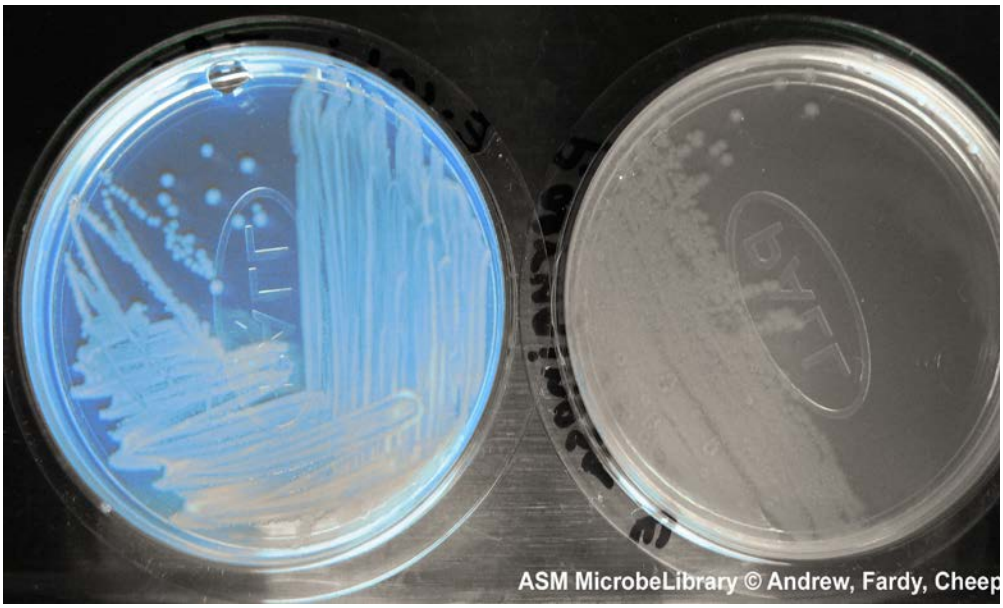
EC agar medium with MUG

Inoculation and incubation. Aseptically transfer 1 loopful of the liquid from the positive presumptive tube to the EC agar with MUG plate. Use the four-way streaking technique to inoculate a loopful of the liquid onto the prepared plate of EC agar media with MUG. Incubate the plate for 18 to 24 hours at 35°C ± 2°C.

Interpretation of results. To observe for fluorescence following incubation, the agar surface or agar plate is exposed under the long-wave (approximately 366 nm) UV light. Positive MUG reactions exhibit a bluish fluorescence around the periphery of the colony. Typical strains of *E. coli* exhibit blue fluorescence on the EC agar medium with MUG, while non-*E. coli* coliforms do not fluoresce (Fig. 4 and 5).

A **B**

FIG. 4. (A) *E. coli* and (B) *P. aeruginosa* on EC-MUG agar medium observed without UV light.



A **B**

FIG. 5.
(A) *E. coli* and (B) *P. aeruginosa* on EC-MUG agar medium observed under UV light.

Inoculated EC-MUG broth tubes and agar media should be examined

under long-wave UV light in the dark. A 6-watt hand-held UV lamp should work well but if using a more powerful UV lamp (such as a 15-watt fluorescent lamp), protective glasses or goggles should be worn (11).

Apart from gas production, growth at 44.5°C can be used to distinguish *E. coli* from some strains of*Salmonella*, *Shigella,* and *Yersinia* that produce GUD. With the possibilities of false-negatives and false-positives, further biochemical tests such as the indole test and ortho-nitrophenyl-β-D-galactopyranoside test are recommended for complete identification.

False-positive results by some strains of *Salmonella*, *Shigella*, and *Yersinia* in *E. coli* analysis may be easily eliminated by an indole test. While *E. coli* is indole positive, other enterics are indole negative (2). To prevent the indole false-positive results with *Yersinia enterocolitica* with 50% of the strains being indole positive (25), eosin-methylene blue agar can be used to distinguish between *E. coli* and*Yersinia enterocolitica*.

To ensure correct results interpretation, it is recommended to use *E. coli* control cultures to confirm a positive result and use the negative ATCC control culture of *Enterobacter aerogenes* and an uninoculated control to confirm a negative result (23).

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

1. Prior to use in MUG assays, all glassware should be checked for autofluorescence. Some glassware contain cerium oxide for quality control measures and fluoresce under UV light and interfere with the MUG test (13). To prevent false-positive results, nonfluorescent borosilicate glassware should be used.

2. When it comes to UV light penetration either through the glass tubes or plastic plates, levels of penetration totally depend on types of glass and plastics, thickness of the glass and plastics, and also wavelength and length of exposure. In this study, we used typical glass test tubes and clear plastic petri plates when photos were taken.

REFERENCES

1. **Buehler, H. J., P. A. Katzman, and E. A. Doisey.** 1949. Bacterial glucuronidase. Fed. Proc. **8:**189.
2. **Cakir, I., H. B. Dogan, E. Baspinar, F. Keven, and A. K. Halkman.** 2001. The need for confirmation in coliform and *E.*

coli enumeration in foods. Turkish J. Vet. Anim. Sci. **26:**1049–1053.
3. **Cheeptham, N., J. Van Hamme, C. Fardy, and J. Urban.** 2004. BIOL 220 lab manual: introductory microbiology laboratory manual; lab 9, environmental microbiology. Department of Biological Sciences, Faculty of Science, Thompson Rivers University, Kamloops, British Columbia, Canada.
4. **Clesceri, L. S., A. E. Greenburg, A. D. Eaton.** 1998. Standard methods for the examination of water and wastewater, APHA, AWA, WEF, 20th ed. Part 9000: microbiology examination; part 9221: multiple-tube fermentation technique for members of the coliform group; part 9222: membrane filter technique for members of the coliform group; and part 9223: enzyme substrate coliform test. Joint publication of American Public Health Association, American Waterworks Assocation, and Water Environment Federation, Washington, DC.
5. **Difco.** 2009. Difco manual. EC medium with MUG formula, p. 196–197.http://www.bd.com/ds/technicalCenter/inserts/EC_Medium_with_MUG.pdf.
6. **Difco.** 2009. Difco manual. Lauryl tryptose broth formula, p. 296–297. http://www.bd.com/ds/technicalCenter/inserts/Lauryl_Tryptose_Broth.pdf.
7. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of the *Enterobacteriaceae*, 3rd ed. Burgess Publishers Company, Minneapolis, MN.
8. **Entis, P., and P. Boleszcsuk.** 1990. Direct enumeration of coliform and *Escherichia coli* by hydrophobic grid membrane filter in 24 hours using MUG. J. Food Prot. **53:**948–952.
9. **Farnleitner, A. H., L. Hocke, C. Beiwl, et al.** 2001. Rapid enzymatic detection of*Escherichia coli* contamination in polluted river water. Lett. Appl. Microbiol. **33:**246–250.
10. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assay for immediate confirmation of*Escherichia coli.* Appl. Environ. Microbiol. **43:**1320–1329.
11. **Feng, P., S. D. Weagant, M. A. Grant.** 2002. Bacteriological analytical manual. Enumeration of *Escherichia coli* and the coliform bacteria. U.S. Food and Drug Administration, Washington, DC. http://www.fda.gov/Food/ScienceResearch /LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm064948.htm.
12. Hardy Diagnostics. 1996. EC broth with MUG and Durham tube manual. **Hardy Diagnostics, Santa Maria, CA.** http://www.hardydiagnostics.com/catalog2/hugo/ECBrothMUG.htm.

13. **Hajna, A. A., and C. A. Perry.** 1943. Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am. J. Public Health **33:**550–558.
14. **Hartman, P. A.** 1989. The MUG (glucuronidase) test for *Escherichia coli* in food and water, p. 290–308. In A. Balows, R. C. Tilton, and A. Turano (ed.), Rapid methods and automation in microbiology and immunology. Brixia Academic Press, Brescia, Italy.
15. **Killian, M., and P. Bulow.** 1976. Rapid diagnosis of Enterobacteriaceae. I. Dectection of bacteria glycosidases. Acta Pathol.

Microbiol. Scand. Sect. B. **84:**245–251.
16. **Koburger, J. A., and M. L. Miller.** 1985. Evaluation of a fluorogenic MPN procedure for determining *Escherichia coli* in oysters. J. Food Prot. **48:**244–245.
17. **Madigan, M. T., J. M. Martinko, and J. Parker.** 2006. Brock biology of microorganisms: an international edition, 11th ed. Prentice-Hall International, Inc., Upper Saddle River, NJ.
18. **Matner, R. R., T. L. Fox, D. E. Emciver, and M. S. Curiale.** 1990. Efficacy of petrifilm *E. coli*count plates for *E. coli* and coliform enumeration. J. Food Prot. **53:**145–150.
19. **Moberg, L. J.** 1985. Fluorogenic assay for rapid detection of *Escherichia coli* in food. Appl. Environ. Microbiol. **50:**1383–1387.
20. **Perry, C. A., and A. A. Hajna.** 1944. Further evaluation of EC medium for the isolation of coliform bacteria and *Escherichia coli*. Am. J. Public Health **34:**735–738.
21. **Peterson, E. H., M. L. Nierman, R. A. Rude, and J. T. Peeler.** 1987. Comparison of AOAC method and fluorogenic method (MUG) assay for enumeration of *E. coli* in foods. J. Food Sci.**52:**409–410.
22. **Robison, B.** 1984. Evaluation of a fluorogenic assay for detection of *Escherichia coli* in foods. Appl. Environ. Microbiol. **48:**285–288.
23. **Sarhan, H. R., and H. A. Foster.** 1991. A rapid fluorogenic method for the detection of*Escherichia coli* by the production of β-glucuronidase. J. Appl. Bacteriol. **70:**394–400.
24. **United States Environmental Protection Agency, Office of Water.** 1991. Test methods for Escherichia coli in drinking water. EC medium with Mug tube procedure and Nutrient agar with Mug membrane filter procedure. U.S. Environmental Protection Agency, Washington, DC. http://nepis.epa.gov/
25. **Winn, W. C., S. D. Allen, W. M. Janda, E. W. Koneman, G. W. Procop, P. C. Schreckenberger, and G. L. Woods.** 2006. Koneman's color atlas and textbook of diagnostic microbiology, 6th ed., p. 244–245. Lippincott Williams & Willkins, Philadelphia, PA.

REVIEWERS

This source was peer-reviewed at the ASM Conference for Undergraduate Educators 2009.

Participating reviewers:

Jason Baker
Missouri Western State University, St. Joseph, MO

Carolyn Bouma
West Texas A & M University, Canyon, TX

David Brooks
East Central College, Union, MO

Richard Coppings
Jackson State Community College, Jackson, TN

Sylvia F. McDevitt
Skidmore College, Saratoga Springs, NY

Jessica Thibeat
Western CT State University, Bristol, CT