IDEXX
Literature Cover Sheet

IDEXX #: 5A

Title: Federal Register June 10, 1992-National Primary Drinking Water Regulations, Analytical Techniques: Coliform Bacteria: Final Rule

Author(s):EPA

Date: June 10, 1992

Source: EPA

Topic: Colilert Final Approval

Highlights:

- EPA approves the use of Colilert(referred to as MMO-MUG) for *E. coli*.

- Colilert recovers coliforms at least as frequently MTF and exhibits greater sensitivity than MF test.

- Colilert formulated with Hepes Buffer in lieu of Phosphate Buffer is approved.

- EPA recommends the use of a 6 watt UV lamp for detecting fluorescence of *E. coli*.

* Refer to page 24745 paragraph 7 and page 24746 paragraphs 2, 3 and 4.

federal register

Wednesday
June 10, 1992

Part V

Environmental Protection Agency

40 CFR Part 141
National Primary Drinking Water Regulations, Analytical Techniques; Coli Form Bacteria; Final Rule

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 141

[WH-FRL-4108-6]

RIN: 2040-AB84

National Primary Drinking Water Regulations: Analytical Techniques; Coliform Bacteria

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: On June 19, 1989, EPA promulgated revised National Primary Drinking Water Regulations (NPDWRs) for total coliforms (54 FR 27544, June 29, 1989) pursuant to section 1412 of the Safe Drinking Water Act (SDWA). In that notice, EPA approved the use of the Minimal Medium ONPG–MUG (MMO–MUG) test for total coliform analysis for compliance with the maximum contaminant level (MCL) for total coliforms under the Safe Drinking Water Act (SDWA). (ONPG is ortho-nitrophenyl-β-D-galactopyranoside; MUG is 4-methylumbelliferyl-β-D-glucuronide.) Today's action amends 40 CFR 141.21(f) by also approving the MMO–MUG test for the detection of *Escherichia coli* (*E. coli*).

EFFECTIVE DATE: July 10, 1992.

ADDRESSES: The public comments and supporting documents cited in the reference section of this notice, the proposed notice (55 FR 22752, dated June 1, 1990), the notice of availability (56 FR 49153, dated September 27, 1991), and associated material are available for review at EPA's Drinking Water docket, 401 M Street SW., Washington, DC 20460. For access to the docket materials, call (202) 260–3027 on Monday through Friday, excluding Federal holidays, between 9 a.m. and 3:30 p.m. Eastern Time for an appointment.

FOR FURTHER INFORMATION CONTACT: The Safe Drinking Water Hotline, telephone (800) 426–4791. The Safe Drinking Water Hotline is open Monday through Friday, excluding Federal holidays, from 8:30 a.m. to 5 p.m. Eastern Time. For technical questions, contact Paul S. Berger, Ph.D., Office of Ground Water and Drinking Water (WH–550D), Environmental Protection Agency, 401 M Street SW., Washington, DC 20460, telephone (202) 260–3039.

SUPPLEMENTARY INFORMATION:

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I. Statutory Authority

The SDWA requires EPA to promulgate NPDWRs which include MCLs or treatment techniques (section 1412). NPDWRs also contain "criteria and procedures to assure a supply of drinking water which dependably complies with such maximum contaminant levels; including quality control and testing procedures to insure compliance with such levels * * *" (section 1401(1)(D)). In addition, section 1445(a) of the SDWA authorizes the Administrator to require monitoring to assist in determining whether persons are in compliance with the requirements of the SDWA. EPA's promulgation of analytical techniques is authorized under these sections of the SDWA. EPA has promulgated analytical techniques for all currently regulated drinking water contaminants; persons must use one of the approved analytical techniques for determining compliance with the MCLs (see 40 CFR 141.21–30). Today's action promulgates an additional analytical method for the detection of *E. coli*.

II. Regulatory Background

On June 19, 1989, EPA promulgated revised regulations for total coliforms (54 FR 27544, June 29, 1989), with an effective date of December 31, 1990. Paragraph 141.21(e) of those regulations requires public water systems to test all total coliform-positive cultures for the presence of either fecal coliforms or *E. coli*. Fecal coliforms and *E. coli* are both indicators of fresh sewage. The regulations specified the analytical method to test for the presence of fecal coliforms (paragraph 141.21(f)(5)), but not for the presence of *E. coli*. On June 1, 1990, EPA proposed three analytical methods for the detection of *E. coli*. On January 8, 1991, EPA promulgated two of these methods, but deferred approval of the third one, the MMO–MUG test. On September 27, 1991, the Agency published a Notice of Availability (56 FR 49153) to provide notice and an opportunity for public comment on two recently completed studies with respect to the MMO–MUG test addressing concerns regarding the ability of the method to detect environmentally stressed *E. coli*. The Notice of Availability indicated that EPA intended to approve the MMO–MUG test based on the results of these studies unless data were received to the contrary. Today's action promulgates the MMO–MUG test for *E. coli* detection.

III. Discussion of Final Rule

A. Public Comments

EPA received 29 public comments during the comment period, and three comments after the close of the comment period. Of the 32 commenters, 28 supported approval of the MMO–MUG test for *E. coli* detection, while four raised concerns. The most important of the concerns raised are addressed below. All public comments are addressed in the comment-response document for this rule, which is available in EPA's Drinking Water docket for *E. coli*.

Source of *E. coli*

Two commenters expressed concern that sewage samples were used as the source of *E. coli* in the Strandridge et al. study (one of the two recent studies cited in the Notice of Availability), as opposed to drinking water or ambient water samples. One of these commenters maintained that previous studies on the MMO–MUG test using naturally contaminated samples showed that the false-negative rate was high, implying that EPA should disapprove the MMO–MUG test.

EPA recognizes that *E. coli* in ambient water and drinking water probably have been subjected to greater environmental stress than those in sewage samples. The Agency believes, however, that sewage sources are more appropriate for determining the *E. coli* false-negative rate than other sources, primarily because (1) sewage sources have a greater diversity of *E. coli* strains than does ambient water, (2) *E. coli* density is greater in sewage than in other sources, thereby facilitating a chlorination study, and (3) drinking waters, especially if disinfected, rarely contain *E. coli*, which would make this source difficult to use as an *E. coli* source (SAB, 1991; Geldreich, 1992). Moreover, *E. coli* in the distribution system may be the result of fresh sewage directly contaminating the water supply via a cross connection or a line break, in which case sewage is a closer approximation than ambient water for these organisms.

In order to obtain low densities of stressed *E. coli* from sewage, recent investigators (Standridge et al., 1991; Covert et al., 1991; Pipes, 1991) first removed the heavier sewage particles, and then chlorinated and diluted the

sewage sample. EPA believes that *E. coli* in samples treated in this fashion adequately approximates the characteristics of those organisms in drinking water. The EPA's Science Advisory Board (SAB) reviewed the protocols employed in the investigations upon which EPA relied, and agreed that raw sewage treated in this manner was the most appropriate *E. coli* source for evaluating low densities of stressed *E. coli* (SAB, 1991).

Using these treated sewage samples, the investigators cited above found that the MMO–MUG test was sensitive to low densities of *E. coli*. Pipes, for example, found the false-negative rate to be about 9%. EPA believes this false-negative rate is satisfactory when compared to other tests. The Agency recognizes that some question still exists with regard to the most appropriate *E. coli* source, but believes this issue cannot be completely resolved without widespread comparison data using drinking water samples over a long period of time (to accumulate sufficient *E. coli* data). The Agency will continue to monitor available data periodically.

False-Positive Rate

One commenter suggested that Standridge et al. should have identified the bacteria in MMO–MUG-positive tests to ensure they were actually *E. coli*, and not false-positive. EPA disagrees. The Agency's disagreement is premised on technical literature that suggests few false-positives are associated with MUG-type tests. This was discussed in the preamble to the notices of June 1, 1990, and January 8, 1991, and in the Comment/Response document to the final rule of January 8. Although EPA is not certain why some samples were MUG-positive in MMO–MUG, but MUG-negative in EC+MUG, the Agency believes the false-positive rate for the *E. coli* portion of the MMO–MUG test is low. For this reason, this issue was not addressed in the studies by Standridge et al. and Covert et al. The Agency position is supported by Pipes (1991), who found that all MUG-positive cultures from the MMO–MUG test (total of 88) were also MUG-positive in EC Medium + Mug. Dr. Pipes used a test protocol developed by EPA and reviewed and approved by EPA's Science Advisory Board. For these reasons, EPA does not believe that the absence of false-positive data diminishes the Agency's reliance on the conclusions of Standridge et al. (1991).

Initial *E. Coli* Density

The test protocol in Standridge et al. (1991) called for use of the mTEC test to enumerate *E. coli* to determine the proper dilution for the initial test conditions. Standridge et al. found in the course of the investigation, however, that mTEC often underestimated *E. coli* density. For this reason, in order to provide more confidence that the initial *E. coli* densities were no more than five/tube, these investigators determined a Most Probable Number (MPN) from the MMO–MUG test tubes. One commenter objected to this procedure because it would have introduced a bias into the density calculation, because the variable being determined is the effectiveness of the MMO–MUG test itself. Thus, the commenter questioned whether the *E. coli* density used in the Standridge et al. study was within the range of interest (1–5 cells/100 ml).

While EPA shares the commenter's concern, the Agency notes that the MMO–MUG MPN test was only used to estimate the dilution of the chlorinated sample necessary to achieve an initial challenge dose in the range of interest. In spite of the difficulty encountered by Standridge et al. in estimating *E. coli* density by mTEC, EPA believes the initial *E. coli* density used in the analysis was generally within the range of interest. The Agency conclusion is based on two factors. First, *E. coli* densities were 5.1/100 ml or fewer in all 19 samples analyzed by EC+MUG, the Agency standard. Second, fecal coliform densities, as measured by gas production in EC+MUG, were 5.1/100 ml or fewer in 13 of 19 samples. The fecal coliform test used theoretically represents an unbiased upper boundary of *E. coli* densities, because gas production is not limited to *E. coli* strains. With the MMO–MUG test, *E. coli* densities were 5.1/100 ml of fewer in 11 of 19 samples analyzed. Thus, slightly higher densities of *E. coli* were found by the MMO–MUG test compared to the fecal coliform test, possibly as a result of statistical variation. By using the MMO–MUG test results to estimate initial *E. coli* densities, and thereby to determine needed sample dilutions, Standridge et al. used the most conservative data (i.e., greatest dilution factor) of the four tests available.

Wattage of Ultraviolet Lamp

One commenter noted that Standridge et al. had used both a 4-watt and a 6-watt ultraviolet lamp for detecting *E. coli*. The commenter requested information on whether any difference was observed.

During the public comment period, Standridge et al. provided EPA with a draft article on their comparison study that has been submitted for publication. This draft article, which the Agency has placed in its *E. coli* docket, provides additional detail on their investigation. The article states that no difference was observed between the 4-watt and 6-watt lamps with the EC+MUG test. However, the 6-watt lamp detected slightly more MUG-positive reactions (i.e., *E. coli* present) than the 4-watt lamp with the MMO–MUG test (331 vs. 321). The data indicate that difference is not statistically significant. Nevertheless, in the interest of public health, the Agency recommends the use of the 6-watt lamp.

MMO–MUG Medium Formulation

One commenter contended that the manufacturer has changed the formulation of the MMO–MUG medium by replacing the inorganic buffer with an organic buffer. The commenter argues that a change in formulation should necessarily prompt a new testing program before being approved by EPA. Apparently, the commenter is referring to the fact that the Agency approved the MMO–MUG test for total coliforms in June 1989 on the basis of test data using the earlier formulation, and that the reformulation invalidates that approval.

In investigating this comment, EPA learned that the commenter is correct that the manufacturer replaced the inorganic buffer with an organic buffer (hepes buffer) in April 1990. The Agency maintains, however, that this change is minor and should not reduce the effectiveness of the Colilert test. The rationale for this belief is based on three factors. First, the only change was in the buffer. Second, data show that hepes buffer is inert to *E. coli* (Ferguson et al., 1980). Finally, several enzymes produced by *E. coli*, though not associated with the MMO–MUG test, exhibit higher activity in a prepared test solution containing hepes buffer than in a solution containing phosphate buffer (Hulsmann et al., 1990; Good et al., 1966). Higher activity of these enzymes suggests that the hepes buffer may enhance activity (or be less inhibitory) for the two enzymes of interest in the MMO–MUG test.

The Agency's belief that the buffer change does not adversely impair MMO–MUG performance is also confirmed by several field studies. In one study of seven marine water samples, the MMO–MUG test formulation with hepes buffer (the new formulation) recovered many more *E. coli* than the old MMO–MUG formulation (average Most Probable Number was 24 vs. <2) (Ellgas et el., 1989). Although this data set is extremely limited, the Ellgas et al. study suggests that the new formulation

recovers *E. coli* more frequently than the old. In another comparison study (Layton, 1991), 143 samples were split and tested using both MMO–MUG formulations. Samples consisted of raw water and water from several different distribution systems, some of which were spiked with raw water or with laboratory strains. Of the 143 samples, 93 were total coliform-positive for both, 44 were total coliform-negative for both, one was total coliform-positive for the old formulation and not the new, and five were total coliform-positive for the new formulation and not the old. For the same sample set, 50 were *E. coli*-positive for both, 78 were *E. coli*-negative for both, four were *E. coli*-positive for the old and not the new, and 11 were *E. coli*-positive for the new and not the old. The results suggest that the new formulation is at least as good as the old one.

After learning that the MMO–MUG formulation had been changed, EPA gathered additional field date from water systems to confirm that the new MMO–MUG formulation was at least as good as the old formulation for total coliform detection. Specifically, the Agency reviewed data collected from more than 30 systems or States comparing the new formulation with one of the other three EPA-approved total coliform methods. Most of the total coliform data represented drinking water sources, although some were raw water sources. EPA evaluated only data sets in which at least one sample was total coliform-positive by at least one test (1315 such samples). By using McNemar's test (two-tailed χ^2 test with one degree of freedom and alpha of 0.05) for paired dichotomous data, EPA finds that the MMO–MUG test recovers coliforms at least as frequently as the Multiple Tube Fermentation Test, and exhibits greater sensitivity than the Membrane Filter Test and the Presence-Absence Coliform Test (USEPA, 1992). The Agency has placed this evaluation in the *E. coli* docket.

As a result of the above information and data, EPA is providing notice in today's rule that the MMO–MUG test with hepes buffer is an acceptable minor revision for the detection of total coliforms in drinking water. Ingredients per liter for the new formulation are listed below:

Ingredients (anhydrous)	Concentration
Ammonium sulfate	5 g.
Manganese sulfate	0.5 mg.
Zinc sulfate	0.5 mg.
Magnesium sulfate	100 mg.
Sodium chloride	10 g.
Calcium chloride	50 mg.
Sodium sulfite	40 mg.
Amphotericin B	1 mg.
Orthonitrophenyl-β-D-galactopyranoside (ONPG).	500 mg.
4-methylumbelliferyl-β-D-glucuronide (MUG).	75 mg.
Solanium [1]	500 mg.
Hepes buffer:	
Sodium salt	5.3 g.
Organic acid [2]	6.9 g.

[1] Solanium is a mixture of plant extracts used as a dispersant.
[2] N-2-Hydroxyethylpiperazine-N[1]-2-ethane sulphonic acid.

B. EPA's Conclusion of E. Coli Detection

After reviewing the data and public comments, EPA believes that the MMO–MUG test is satisfactory for *E. coli* detection, and is therefore approving the use of this test under the Total Coliform Rule. The Agency also believes that the benefit of approving use of a simple, rapid *E. coli* method outweighs any residual uncertainty concerning this test. However, since the use of the method for *E. coli* detection, and the modified formulation, is new and consequently has not been tested with the entire range of drinking water available in the United States, EPA encourages laboratories to perform parallel testing between the MMO–MUG test and other EPA-approved procedures for detecting *E. coli* for at least several months to assess the effectiveness of the MMO–MUG test for the specific water type being analyzed. To facilitate collection and evaluation of comparative data, EPA strongly recommends that laboratories identify which test(s) they use on the data form for each sample analyzed.

The test being promulgated today is based on the ability of *E. coli* to produce the enzyme beta-glucuronidase, which hydrolyzes 4-methylumbelliferyl-beta-D-glucuronide (MUG) contained in the test medium. This hydrolysis forms 4-methylumbelliferone, which fluoresces when exposed to ultraviolet light (366 nm). Few noncoliforms, or coliforms other than *E. coli*, produce the enzyme beta-glucuronidase. Thus, fluorescence should be a differential indicator for the presence of *E. coli* in a water sample.

IV. Regulation Assessment Requirements

A. Executive Order 12291

Executive Order 12291 requires EPA to judge whether a regulation is "major" and, if so, to prepare a regulatory impact analysis. A rule is considered major if it has an economic impact of $100 million or more, causes a significant increase in cost or prices, or any of the other adverse effects described in the Executive Order. Because the rule merely makes an additional analytical method available for use in complying with the regulation for total coliforms, EPA has determined that this action is not a major rule within the meaning of the Executive Order. Water systems/laboratories may use the new method or continue using previously-approved methods. Therefore, there will not be any adverse economic impacts.

This notice was submitted to the Office of Management and Budget for its review under the Executive Order.

B. Regulatory Flexibility Analysis

The Regulatory Flexibility Act requires EPA to explicitly consider the effect of proposed regulations on small entities. If there is a significant effect on a substantial number of small systems, means should be sought to minimize the effects. The Small Business Administration defines a small water utility as one which serves fewer than 3,300 people. Under this definition, this rule would affect about 200,000 small systems.

This final rule is consistent with the objectives of the Regulatory Flexibility Act because it will not have a significant economic impact on small entities. The rule provides laboratories with a third alternative for testing a total coliform-positive culture for *E. coli*. Because use of this method is optional, and because EPA is not promulgating any new requirement, the Agency believes that the impact of this notice does not have a significant effect on a substantial number of small entities.

C. Paperwork Reduction Act

This rule contains no information collection requirements and consequently is not covered by the Paperwork Reduction Act, 44 U.S.C. 3501 et seq.

D. Science Advisory Board, National Drinking Water Advisory Council, and Secretary of Health and Human Services

In accordance with section 1412 (d) and (e) of the Safe Drinking Water Act, the Agency consulted with the Science Advisory Board, National Drinking Water Advisory Council, and the Secretary of Health and Human Services and took their comments into account in developing this rule.

List of Subjects in 40 CFR Parts 141

Administrative practice and procedure, Analytical methods, Intergovernmental relations, Microorganisms, National Primary

Drinking Water Regulations, Total coliforms, Water supply.

Dated: May 29, 1992.

William K. Reilly,

Administrator.

V. References

Covert, T., E. Rice, S. Johnson, D. Berman, C. Johnson, P. Mason. 1991. Evaluation of the Autoanalysis Colilert Test, Coliquik Coliform Test and EC Medium with MUG for detection of *Escherichia coli* in water. (Submitted for publication).

Ellgas, W., M. Kenney, K. Osborn, A. Greenberg. 1989. Evaluation of Autoanalysis Colilert in wastewater. Proceedings of the Water Pollution Control Federation Specialty Conference on Microbial Aspects of Surface Water Quality. May 30, 1989. Chicago.

Ferguson, W., K. Braunschweiger, W. Braunschweiger, J. Smith, J. McCormick, C. Wasmann, N. Jarvis, D. Bell, and N. Good. 1980. Hydrogen ion buffers for biological research. Analytical Biochemistry 104.300–310.

Geldreich, E. 1992. Compliance concerns with the new coliform regulation. *In*: Proceedings, Water Quality Technology Conference (Part II), Orlando, Florida. American Water Works Association, Denver.

Good, N., G. Winget, W. Winter, T. Connolly, S. Izawa, R. Singh. 1966. Hydrogen ion buffers for biological research 5(2):467–477.

Hülsmann, K., A. Bergerat-Coulaud, and U. Hahn. 1990. *E. coli* Dam activity in Hepes buffer asks for a new unit definition. Nucleic Acids Research 18(23):7189.

Layton, D. 1991. Letter from Environetics to Dr. Paul Berger, U.S. Environmental Protection Agency. 12/4/91.

Pipes, W. 1991. The transferability of *Escherichia coli* from MMO–MUG media for detection in drinking water samples. [Unpublished manuscript submitted to EPA's Office of Ground Water and Drinking Water, Washington, DC)

SAB. 1991. Science Advisory Board. Microbiological testing of drinking water. EPA–SAB–DWC–91–014. U.S. Environmental Protection Agency.

Standridge, J., S. McCarty, and R. Dergrigorian. 1991. Comparison of the ability of the Autoanalysis Colilert ONPG–MUG test system to the Standard Methods lauryl tryptose broth—EC–MUG system to detect chlorine stressed *Escherichia coli*. (Submitted for publication).

USEPA. 1992. Memorandum from P. Berger, Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency, to *E. coli* Docket. 4/3/92. Statistical analysis of raw data comparing Colilert test with EPA-approved total coliform methods.

For the reasons set out in the preamble, part 141 of title 40 of the Code of Federal Regulations is amended as follows:

PART 141—NATIONAL PRIMARY DRINKING WATER REGULATIONS

1. The authority citation for part 141 continues to read as follows:

Authority: 42 U.S.C. 300f, 300g-1, 300g-2, 300g-3, 300g-4, 300g-5, 300g-6, 300j-4 and 300j-9.

2. Section 141.21 is amended by revising in paragraph (f)(3)(ii) the first word "Membrance" to read "Membrane"; by adding a sentence to the end of (f)(3)(iv), by adding paragraph (f)(6)(iii), and by revising (f)(7) to read as follows:

§ 141.21 Coliform sampling.

* * * * *

(f) * * *

(3) * * *

(iv) * * * The MMO–MUG Test with hepes buffer in lieu of phosphate buffer is an acceptable minor revision.

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(iii) Minimal Medium ONPG–MUG (MMO–MUG) Test, as set forth in the article "National Field Evaluation of a Defined Substrate Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with Presence-Absence Techniques" (Edberg et al.), Applied and Environmental Microbiology, Volume 55, pp. 1003–1008, April 1989. (Note: The Autoanalysis Colilert System is an MMO–MUG test) If the MMO–MUG test is total coliform-positive after a 24-hour incubation, test the medium for fluorescence with a 366-nm ultraviolet light (preferably with a 6-watt lamp) in the dark. If fluorescence is observed, the sample is *E. coli*-positive. If fluorescence is questionable (cannot be definitively read) after 24 hours incubation, incubate the culture for an additional four hours (but not to exceed 28 hours total), and again test the medium for fluorescence. The MMO–MUG Test with hepes buffer in lieu of phosphate buffer is the only approved formulation for the detection of *E. coli*.

(7) As an option to paragraph (f)(6)(iii) of this section, a system with a total coliform-positive, MUG-negative, MMO–MUG test may further analyze the culture for the presence of *E. coli* by transferring a 0.1 ml, 28-hour MMO–MUG culture to EC Medium + MUG with a pipet. The formulation and incubation conditions of EC Medium + MUG, and observation of the results are described in paragraph (f)(6)(i) of this section.

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[FR Doc. 92–13381 Filed 6–9–92; 8:45 am]

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