IDEXX
Literature Cover Sheet

IDEXX #: 5F

Topic: Colilert MPN Approval

Title: Federal Register - National Primary Drinking Water Regulations: Analytical Techniques; Coliform Bacteria; Final Rule

Date: July 17, 1989

Source: EPA

Highlights:

- EPA approves the use of Colilert (referred to as MMO-MUG) for total coliforms

- Membrane filtration's false positive rate (22%) is 69% higher than Colilert's (13%).

- Favorable comments endorsing the use of Colilert:
  - reliable
  - accurate
  - rapid
  - easy to use
  - not effected by large numbers of heterotrophic bacteria
  - save time and cost

* See page 2998, "I. Summary of Todays Action"& page 2999, " A. Favorable Comments"

federal register

Monday
July 17, 1989

Part V

Environmental Protection Agency

40 CFR Part 141

National Primary Drinking Water Regulations: Analytical Techniques; Coliform Bacteria; Final Rule

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 141

[FR 3555-8]

National Primary Drinking Water Regulations: Analytical Techniques; Coliform Bacteria

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This action amends the National Primary Drinking Water Regulations (NPDWRs) promulgated pursuant to section 1412 of the Safe Drinking Water Act (SDWA) to specify an additional analytical method to enumerate total coliforms for the purpose of determining compliance with the current maximum contaminant levels (MCLs) for coliform bacteria in 40 CFR 141.14. Only approved analytical techniques may be used for determining compliance with MCLs. The two methods currently approved in 40 CFR 141.21 are: (1) The Multiple-Tube Fermentation (MTF) technique, and (2) the Membrane Filter (MF) technique. The third alternate procedure which EPA is approving today is the Minimal Medium ONPG-MUG test.

EPA requested comment on whether it should approve the Autoanalysis Colilert test (henceforth called the Minimal Medium ONPG-MUG test, or MMO-MUG test, a more specific generic name) for determining compliance with the current MCLs for total coliforms on May 6, 1988 (53 FR 16352). The Agency has determined that the proposed technique is substantially equivalent in both precision and accuracy to the techniques already approved.

EFFECTIVE DATE: The rule is effective 30 days from publication. In accordance with 40 CFR 23.7, this regulation shall be considered final Agency action for the purposes of judicial review at 1 p.m. Eastern Standard Time on July 31, 1989. The incorporation by reference of the publication listed in the regulation is approved by the Director of the Federal Register as of August 16, 1989.

ADDRESSES: The public comments and supporting documents cited in the reference section of this notice are available for review at EPA's Drinking Water Docket, 401 M Street SW., Washington, DC 20460. For access to Docket material, call (202) 382-3027 between 9 a.m. and 3:30 p.m. for an appointment.

FOR FURTHER INFORMATION CONTACT: The Safe Drinking Water Hotline, telephone (800) 426-4791 or (202) 382-5533 for callers in the Washington, DC area and Alaska. The Safe Drinking Water Hotline is open Monday through Friday, excluding Federal holidays, from 8:30 a.m. to 4:00 p.m. Eastern time.

I. Summary of Today's Action

Today's action makes available an additional analytical method for determining compliance with the current MCLs for total coliform bacteria in 40 CFR 141.14. This method is the MMO-MUG test. With the promulgation of this method, three analytical procedures for total coliform analysis are available: the Membrane Filter technique, the Multiple-Tube Fermentation technique, and the MMO-MUG test. Any of these tests may be used to analyze drinking water samples for the estimation or enumeration of coliform bacteria to determine compliance with the current MCLs for this group of organisms.

II. Statutory Authority and Regulatory Background

A. 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 ensure compliance with such levels" (section 1401(1)(D)). In addition, section 1445(a) of the Act authorizes the Administrator to require monitoring to assist in determining whether persons are in compliance with the Act. EPA's promulgation of analytical techniques is authorized under these sections of the Act. 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).

B. Regulatory Background

On May 6, 1988, as part of the Notice of Availability on proposed changes to the NPDWR for total coliforms, EPA requested comment on whether it should approve the MMO-MUG test (called by its commercial name, the Autoanalysis Colilert System in that notice) as a third procedure to determine the presence or absence of total coliforms under the revised total coliform rule. Due to the significant number of individuals who expressed interest in using the MMO-MUG test as soon as possible (rather than waiting for the effective date of the revised coliform rule), and because of its simplicity and possible lower costs, EPA also requested comment on whether it should approve the five-tube MMO-MUG test to determine the mean density of total coliform bacteria in drinking water for determining compliance with the current MCLs for these organisms (53 FR 16348). This notice pertains only to testing for compliance with the current MCLs. Use of this test to determine compliance with the revised coliform rule, to be published shortly, will be addressed when EPA promulgates that rule.

The test described in the Notice of Availability was a five-tube quantification method. The test was validated as a ten-tube test by an extensive field evaluation co-sponsored by EPA and the American Water Works Association Research Foundation, which explicitly followed the protocol of the EPA Environmental Monitoring Systems Laboratory (EMSL) for certification of alternative analytical methods (1). These data on the national field evaluation were recently published (2), and are also available in the docket for the proposed changes to the current coliform rule. Other recently published information describing the MMO-MUG test are cited in Section VI below (3,4). EMSL has determined that statistical analyses confirm that the five- and ten-tube test results are comparable. Based on the validation data and EMSL's evaluation, the Agency has concluded that this method is equivalent to currently approved methods in terms of precision and accuracy. Furthermore, the false positive rate, as determined by reaction in brilliant green lactose bile broth, is 13% for both the MMO-MUG test and the currently approved MTF test. The false positive rate for the currently approved MF technique is approximately 22%. Since it is a new method and consequently has not been tested with the whole 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 enumerating total coliform bacteria for at least several months to assess the effectiveness of the MMO–MUG test for the specific water type being analyzed and to assess the impact on numbers of positive tests of the techniques. To enable and enhance collection and evaluation of comparative data from the increasing number of tests available, EPA strongly recommends that laboratories identify which test(s) they use on the data form for each sample analyzed. It is important to note that EPA has not yet determined whether this test is fully satisfacto2y for assessing water other than drinking water.

The MMO–MUG system is based on the ability of total coliform bacteria to produce the enzyme beta-galactosidase which hydrolyzes o-nitrophenyl-beta-d-galactopyranoside (ONPG) present in the chemically defined medium to form a yellow color. The formulation of the test medium poorly supports the growth of non-coliform microorganisms; the target coliform microorganisms produce the yellow color within 24 hours. The test procedure consists of aseptically adding 10 ml of the water sample to each of five tubes containing a measured amount of MMO–MUG test medium, capping the tubes, mixing vigorously by inversion, incubating at 35–37 °C for 24 hours, and observing for a yellow color. If the color is so light a yellow that a definitive reading cannot be made, the tubes are re-incubated at 35–37 °C up to, but not more than, four additional hours. Deepening of the yellow color indicates the presence of total coliforms.

Total coliform bacteria are a heterogeneous group of organisms, the definition of which is based on their ability to produce acid and gas from lactose, although a number of strains may produce no gas. Taxonomists who work with this group of bacteria use a range of physiological and biochemical tests, but do not deal with absolutes, because a certain percentage of strains recovered from nature will vary from the established norm. Therefore, methodology based on different enzymatic reactions may occasionally detect different organisms, but, if in actual field studies the percentage of coliforms detected by a new method falls within acceptable limits of comparability with an established method, EPA believes the new test can also be deemed acceptable without challenging current definitions. This being so, EPA does not believe that a new definition for coliform bacteria is required.

An additional enzyme, beta-glucuronidase, is produced by *Escherichia coli* and forms a fluorescent substance when it hydro-4-methylumbelliferyl-beta-D-glucuronide (MUG) present in the MMO–MUG medium, allowing for detection of this baceterium (2). However, studies to evaluate the efficacy of the MMO–MUG test for detection of *E. coli* are still underway. Thus EPA's promulgation of the MMO–MUG test for estimating the mean density of total coliforms should not be construed as an EPA endorsement of the effectiveness of this test for detection of *E. coli* at this point.

Ingredients per liter for the MMO–MUG test medium (2.5) are listed below:

$(NH_2)_2SO_4$, 5g
$Mn(SO_4)_2$, 50mg
$ZnSO_4$, 50mg
$MgSO_4$, 100mg
NaCl, 10g
$CaCl_2$, 50mg
KH_2PO_4, 900mg
Na_2HPO_4, 6.2g
Na_2SO_3, 40mg
amphotericin B, 1mg
ortho-nitrophenyl-beta-D-galactopyranoside (ONPG), 500mg
4-methylumbelliferyl-beta-D-glucuronide (MUG), 75mg
Solanium, 500mg

This list of ingredients is given for information purposes only; as with other media, EPA does not encourage preparation of the MMO–MUG medium by individual users because of quality assurance problems. The MMO–MUG medium can be purchased commercially in a prepackaged form, or in bulk to be dispensed as needed. Laboratories making their own MMO–MUG medium should ascertain that it is prepared with rigid quality assurance measures to ensure that results are comparable to those obtained with the commercially available media.

The interim total coliform rule defines two maximum contaminant levels (MCLs)—a "single sample violation" and a monthly average. Both MCLs are defined according to the analytical procedure used. Under this final rule, when a laboratory uses the MMO–MUG test, the MCLs are identical to those prescribed in 40 CFR 141.14(b)(1) for the five-tube fermentation tube (MTF) method and 10-ml standard portions.

III. Comments and Responses

As noted earlier, in the May 6, 1988, notice, EPA requested comment on whether it should approve the Autoanalysis Colilert System (MMO–MUG test) as a five-tube quantification method for analyzing samples to determine compliance with the current total coliform bacteria MCLs and to determine compliance with the proposed revised total coliform MCL as a qualitative test. EPA received many comments addressing this issue, the great majority supporting EPA approval. In today's notice, the Agency will only summarize and respond to those comments specific to the use of the MMO–MUG test to determine compliance with the current total coliform bacteria MCLs. EPA will identify and respond to comments on the use of the MMO–MUG test to determine compliance with the revised coliform rule when it promulgates that rule. If a comment pertained to both rules, or if the Agency could not determine which rule the comment referred to, EPA has identified and responded to the comment in this notice, and will address it in the forthcoming revised rule, as well.

A. Favorable Comments

Fifteen commenters specifically endorsed use of the MMO–MUG test. The commenters found the procedure reliable, accurate, rapid, and easy to use. They liked the additional flexibility that another alternate method would provide, and pointed out the advantage of having a procedure available for detection of coliforms that would not be affected by the presence of large numbers of heterotrophic bacteria. Some thought that use of the procedure would save time in transport of samples and would reduce laboratory cost. One commenter not only stated that the MMO–MUG test should be approved as an additional method to determine MCL compliance, but also believed that it would be of assistance in identifying "trouble spots," i.e., as a quick, simple method for coliform detection in a distribution system heavily contaminated with heterotrophic bacteria.

B. Recommended Modifications

Six of the commenters who approved the new test had suggestions or recommended limitations concerning its use.

1. Limitations Suggested

One commenter was opposed to the use of the MMO–MUG test for analysis of compliance samples by uncertified laboratories, claiming it would result in under-reporting or erroneous reporting, and have a negative effect on small laboratories dependent upon water testing for their livelihood. If these laboratories were to close for lack of

funds, suppliers would need to spend more money for transportation of samples. This commenter recommended that only State personnel, or State-recommended personnel, be allowed to use the test. Another commenter questioned whether EPA intends to require laboratories to be certified for performance of the test.

Some commenters thought that the test should be available for smaller systems and for on-site testing. Another commenter, did not consider the test to be appropriate for use in analyzing non-disinfected water, because the method would provide no indication of the number of heterotrophic bacteria present.

Under 40 CFR 141.28, a public water system must use a laboratory approved by the State (a "certified laboratory") to analyze most regulated drinking water contaminants, including total coliforms. The State certification requirement should not negatively affect small laboratories that are already certified for total coliform analyses using the currently approved methods. EPA's regulations require that all compliance analyses be conducted by laboratories approved by the State, and it is not EPA's intention that unapproved laboratories or other analysts be permitted to run the MMO–MUG test. Because the MMO–MUG is so similar to the EPA-approved Multiple Tube Fermentation Technique, EPA is merely encouraging States to allow *certified* laboratories to use the MMO–MUG test without specific initial certification. EPA is planning to develop and publish specific certification criteria for the MMO–MUG test for future use in on-site evaluations of laboratories. For the purposes of today's rule, EPA still requires that all coliform analyses be conducted in laboratories approved by the State, but the Agency is not specifying specific certification criteria for the MMO–MUG test.

Regarding the use of this test for enumerating total coliforms in undisinfected drinking water because of its inability to signal the presence of high levels of heterotrophic bacteria, the test was not designed for that purpose; the test is simply designed to detect and enumerate coliforms. If enumeration of heterotrophic bacteria is desirable, standard analytical procedures are available.

2. Comments on Procedures for the MMO–MUG Test

One commenter supported the MMO–MUG test, but stated that the sample volume should be 100 ml, rather than 50 ml.

As described in the May 6, 1988, notice (53 FR 16352), EPA has reviewed data which demonstrate that the MMO–MUG procedure, when used as a five-tube test (with 10-ml sample portions in each tube), gives results that are comparable to those achieved when using the EPA-approved five-tube MTF procedure. Thus, for consistency with the EPA-approved MTF procedure, EPA is approving the use of the five-tube MMO–MUG test, which uses a total of 50 ml of water sample. As stated above, the Agency is in the process of revising the total coliform rule; one of the proposed provisions would require systems to use a 100-ml water sample, regardless of analytical method used. Thus, EPA will probably replace the requirement to use a 50-ml water sample for the MMO–MUG test, as approved in today's rule, with a requirement to use 100 ml (in a ten-tube test or a single bottle) beginning on the effective date of the revised rule, i.e., 18 months after promulgation.

3. Other Comments

Other commenters who supported use of the MMO–MUG test suggested that it be tentatively approved until it could be compared with other tests in a greater variety of situations, and then re-evaluated after four years. Another commenter supported use of the test, but thought that EPA should not refer to the test by its trade name (i.e., Autoanalysis Colilert System).

EPA believes that existing data support approval of the MMO–MUG test at this time; however, EPA agrees that comparison studies are useful. Thus, as mentioned above, the Agency encourages laboratories to conduct multi-month studies comparing the MMO–MUG test to previously approved EPA methods to insure that results are comparable for the specific water being tested before relying completely on the new test. Water suppliers should keep in mind that, although the MMO–MUG technique is equivalent to currently approved techniques, changes in the number of positive tests could affect compliance determinations. The Agency also agrees that the test approved today should be referred to by a generic name rather than a trade name. Accordingly, EPA now refers to this method as the MMO–MUG test.

C. Comments Opposed to the MMO–MUG Test

One commenter opposed the MMO–MUG test because the medium was expensive and only available from one source. A second commenter found the test cumbersome as currently packaged as well as expensive. A third did not like the test, believing that the test does not quantify coliforms, even though it was easy to use and gave rapid results. Still another commenter believed that the availability of the test to smaller facilities would create difficulty in tracking the results of laboratory tests.

- The Agency has reviewed the costs associated with the MMO–MUG test and determined that it generally does not cost more than the current approved methods, and that, in fact, in some settings it may prove less expensive when time and labor costs are considered. Commenters provided no data which demonstrate that this conclusion is invalid. Of course, laboratories using the existing approved methods to evaluate samples are free to continue using them if they find them less costly or otherwise preferable.

EPA also has reviewed the problem of MMO–MUG medium availability. The producer of this medium has assured the medium's developer and EPA of its availability, dependent upon demand (6, 7, 8, 9). Although the medium currently can be obtained only from a single source, there are precedents for approval of media containing an ingredient available from a single source (6). The ingredients for MMO–MUG medium are all readily available commercially with the single exception of the dispersing agent, Solanium. The producer lists this ingredient as available on its current price list (7) and has stated its intention to fulfill public need (8, 9). In addition, the public listing of ingredients makes it more likely that other manufacturers will produce the same product or slightly modified versions of the product, so that equivalent media would no longer be available from only one source.

- EPA does not believe the test, as currently packaged, is more cumbersome to use than other approved tests. In fact, EPA believes that the test may be significantly less cumbersome. The five-tube test approved today is designed to estimate the coliforms present in a sample by the Most Probable Number procedure. Therefore, the MMO–MUG test is a suitable method for estimating total coliform bacteria in water samples to determine compliance with the current MCLs for total coliform bacteria.

The requirement to use a certified laboratory for performance of the MMO–MUG test should prevent any increase in difficulty of tracking test results since such laboratories are accustomed to recordkeeping required for compliance purposes.

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. EPA has determined that this regulation will not have an economic effect of $100 million or more, cause a significant increase in cost or prices, or cause any of the averse effects described in the Executive Order, and, therefore, is not a major rule. This regulation simply specifies an additional analytical technique which can be used by laboratories to enumerate coliform bacteria. Laboratories may use the new method or continue using one of the previously approved methods. Therefore, there will not be any adverse economic impacts. This rule may, in fact, reduce costs because it approves a method that is simpler than existing approved methods.

This notice was submitted to OMB for its review under the Executive Order.

B. Regulatory Flexibility Act

This amendment is consistent with the objectives of the Regulatory Flexibility Act (5 U.S.C. 602 et seq.) because it will not have a significant economic impact on a substantial number of small entities. This rule gives laboratories, including small laboratories, the latitude to use an alternate method for total coliform analysis, if they wish. As noted earlier, this method costs no more to perform than methods already approved and may, in fact, cost less.

C. Paperwork Reduction Act

This rule contains no requests for information and, therefore, is not covered by the Paperwork Reduction Act, 44 U.S.C. 3501 et seq.

V. Effective Date

This rule is issued under SDWA sections 1401, 1412, and 1445. Although section 1412(b) provides that the National Primary Drinking Water Regulations (as defined in section 1401) take effect 18 months after their promulgation, under section 1445 there is no such limitation for monitoring, reporting, and recordkeeping regulations which may be used to assist in determining compliance. To allow the monitoring methods to be used 30 days after promulgation, EPA is promulgating these regulations under section 1445. Effective 18 months after promulgation, the analytical methods will also be deemed to be promulgated under sections 1401 and 1412.

VI. References and Public Docket

The following items are included in the public docket together with other correspondence and information. The public docket is available for review in Washington, DC, at the address listed at the beginning of this notice.

- Technical reviews of the MMO-MUG test.
- Memorandum recommending approval of the MMO-MUG test (Colilert system) from the Director, Environmental Monitoring Systems Laboratory in Cincinnati to the Director, Office of Drinking Water.
- Description of the proposed analytical technique and performance data.
- Public comments.

List of Subjects in 40 CFR Part 141

National Primary Drinking Water Regulations; Total coliforms; Incorporation by reference.

William K. Reilly,

Administrator.

Dated: July 10, 1989.

References

(1) Covert, T.C. 1985. USEPA's Method Equivalency Program for Drinking Water Samples. USEPA, Cincinnati.

(2) Edberg, S.C., Allen, M.J., Smith, D.B., and the National Collaborative Study. 1988. National Field Evaluation of a Defined Substrate Method for the Simultaneous Enumeration of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with the Standard Multiple Tube Fermentation Method. Appl. Environ. Microbiol. 54:1595-1601.

(3) Edberg, S.C., Allen, M.J., and Smith, D.B. 1988. Rapid, Specific, Defined Substrate Technology for the Simultaneous Detection of Total Coliforms and *Escherichia coli*. Toxicity Assessment: An International Journal 3:565-580.

(4) Edberg, S.C., Ludwig, F., and Smith, D.B. 1989. Rapid, Simultaneous Detection and Enumeration of Total Coliforms and *Escherichia coli* by the Colilert System: A Technology Transfer. Final Report, American Water Works Association Research Foundation, in press.

(5) Edberg, S.C., Allen, M.J., Smith, D.B., and the National Collaborative Study. 1988. ERRATUM. Appl. Environ. Microbiol. 54:3197.

(6) Letter, 10/28/88, Stephen C. Edberg, Yale University, Department of Laboratory Medicine, to Joseph Cotruvo, Office of Drinking Water, U.S. Environmental Protection Agency.

(7) Price List, Drinking Water Testing, Colilert Components and Peripherals, Solarium, Catalogue Numbers RC 306 and RC 307, Access Analytical Systems, Bradford, CT.

(8) Letter, 1/13/87, Sandra Stratman, Access Analytical Systems, Bradford, CT, to Robert Bordner, U.S. Environmental Protection Agency, Cincinnati, OH.

(9) Letter, 10/6/88, Sandra Stratman, Access Analytical Systems, Bradford, CT, to Alfred DuFour, U.S. Environmental Protection Agency, Cincinnati, OH.

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. 300g-1, 300g-3, 300g-6, 300j-4, and 300j-9.

2. Section 141.14 is amended by adding a new paragraph (b)(3) to read as follows:

§ 141.14 Maximum microbiological contaminant levels.

* * * * *

(b) * * *

(3) When the Minimal Medium ONPG-MUG test described in § 141.21(a) of this part is used, coliform bacteria shall not be present at levels exceeding those specified in paragraph (b)(1) (i)-(iii) of this section.

* * * * *

3. Section 141.21 is amended by adding the following text to the end of paragraph (a):

§ 141.21 Microbiological contaminant sampling and analytical requirements.

(a) * * * Total coliform analyses may also be conducted by the Minimal Media ONPG-MUG (MMO-MUG) test described in Edberg, et al. (1988) except that 10 ml of water sample must be added to each of the five tubes. Analyses must be conducted in accordance with the analytical recommendations set forth in "National Field Evaluation of a Defined Substrate Method for the Simultaneous Enumeration of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with the Standard Multiple Tube Fermentation Method." Stephen C. Edberg, Martin J. Allen, Darwell B.

Smith, and the National Collaborative Study, Applied and Environmental Microbiology, 54:1595–1601, June 1988 (amended under Erratum, Applied and Environmental Microbiology, 54:3197, December 1988). This incorporation by reference was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51. Copies may be obtained from the American Water Works Association Research Foundation, 6666 West Quincy Avenue, Denver, CO 80235 (telephone 303–794–7711). Copies may be inspected at EPA's Drinking Water Docket, 401 M Street, SW., Washington, DC 20460, or at the Office of the Federal Register, 1100 L Street, NW., Room 8401, Washington, DC.

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[FR Doc. 89–16891 Filed 7–14–89, 8:45 am]
BILLING CODE 6560–50–M