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Total Coliform Rule Issue Paper

Invalidation of Total Coliform Positive Samples

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Background and Disclaimer

The USEPA is revising the Total Coliform Rule (TCR) and is considering collecting data on distribution as part of these revisions. As part of this process, the USEPA is publishing a series of issue papers to present available information on topics relevant to possible TCR revisions. This paper was developed as part of that effort.

The objectives of the issue papers are to review the available data, information and research regarding the potential public health risks associated with the distribution system issues, and where relevant identify areas in which additional research may be warranted. The issue papers will serve as background material for EPA, expert and stakeholder discussions. The papers only present available information and do not represent Agency policy. Some of the papers were prepared by parties outside of EPA; EPA does not endorse those papers, but is providing them for information and review.

Additional Information

The paper is available at the TCR web site at:

http://www.epa.gov/safewater/disinfection/tcr/regulation_revisions.html

Questions or comments regarding this paper may be directed to TCR@epa.gov.

Overview

The primary purposes of this paper on total coliform (TC) sample invalidation are to bring together pertinent information on this topic from both research studies and system surveys, to prepare issues for discussion on potential revisions to TC sample invalidation procedures, and to compile unknowns regarding this issue. This paper will identify the requirements of the TCR and EPA's rationale for including them in the rule. This paper also includes information from the states on the approximate number of positive samples being invalidated (and the justifications for these invalidations) and the approximate percentages of samples being invalidated by States due to laboratory determination (along with the analytical methods associated with interferences).

When promulgating the Total Coliform Rule (TCR), EPA recognized that there would be instances in which certain total coliform samples would need to be invalidated. Under the current Rule, the State may invalidate total coliform-positive samples only if (1) the laboratory establishes that improper sample analysis led to the positive result, (2) the State, based on repeat sample results, determines that the problem resulted from domestic or other non-distribution system plumbing problems, or (3) the State has substantial reason to believe that the positive result does not reflect the water quality in the distribution system. The state must invalidate a total coliform-negative sample if a laboratory observes interference with the test by other organisms. EPA intends to examine the appropriateness of these invalidation criteria.

I. Background

The Total Coliform Rule (TCR), published on June 29, 1989 (EPA, 1989), requires all public water systems to monitor their drinking water for total coliforms. Total coliforms are a group of closely related bacteria (family *Enterobacteriaceae*) that are generally free-living in the environment, but are also normally present in water contaminated with human and animal feces. Specifically, they are used as an indicator to determine the effectiveness of treatment and the integrity of the distribution system (e.g., cross-connection incidents, intrusions). The presence of total coliforms in finished drinking water in a distribution system indicates that the system is either fecally contaminated or vulnerable to fecal contamination (Regli et al., 2003).

The TCR requires a system to monitor its distribution system for total coliforms at sample locations consistent with a State-approved sampling plan, at a frequency that depends upon the number of people served and type of system (community water system vs. non-community water system). If a system has a total coliform-positive sample, it must (1) test that sample for the presence of fecal coliform or *E. coli*, (2) collect a set of three repeat samples (four, if the system collects one routine sample or fewer per month) within 24 hours at the same and adjacent service connections and analyze them for total coliforms; and (3) collect at least five routine samples in the next month of sampling, regardless of system size.

Under the TCR, a system that collects 40 or more samples per month (generally systems that serve more than 33,000 people) is in nonacute violation of the maximum contaminant level (MCL) for total coliforms if more than 5.0% of the samples (routine + repeat) it collects per month are total coliform-positive. A system that collects fewer than 40 samples per month

violates the MCL if two or more samples (routine or repeat samples) during the month are total coliform-positive. For any size system, if two consecutive total coliform-positive samples occur at a site during a month, and one is also fecal coliform/*E. coli*-positive, the system has an acute violation of the MCL, and must provide public notification immediately. The presence of fecal coliforms or *E. coli* indicates that recent fecal contamination is present in the drinking water.

Among its provisions, the TCR defines when a State *may* invalidate a total coliform-positive sample (141.21(c)(1)) and when a laboratory *must* invalidate a total coliform-negative sample (141.21(c)(2)). This paper will present the criteria for invalidating a sample, explain why they were included in the TCR, report comments EPA has received on the TCR invalidation criteria and procedures, and indicate how States are implementing these provisions. Outside the scope of this paper is the issue of sample rejection resulting from improper sample transport conditions to the laboratory. The information in this paper will help EPA, the water industry, and other interested parties determine the appropriateness of the current invalidation provisions in revising the TCR.

II. Invalidation Criteria for Total Coliform-Positive Samples and Rationale

Under the TCR at 40 CFR 141.21(c)(1), a State may invalidate a total coliform-positive sample only in one of the following three circumstances, designated *C1, C2, and C3.*

Improper sample analysis (C1)

An improper sample analysis may be caused by a variety of situations, such as laboratory equipment malfunction, sample container leakage or breakage, and contaminated negative-control samples. For a State to invalidate a positive sample under this criterion, the laboratory itself must indicate that the analysis was improper. It cannot be assumed by others that the laboratory erred. (The capability of the laboratory to make this decision rests upon the fact that all laboratories analyzing compliance samples under the Safe Drinking Water Act must be certified either by EPA or the State. A periodic onsite audit is a major part of the EPA and State certification programs.)

Results of a set of repeat samples suggest the problem is associated with a domestic or other non-distribution system plumbing problem (C2)

As stated in the preamble to the final rule (p. 27550), EPA's rationale was that if any repeat sample is total coliform-positive at the same tap as the original positive sample, but all repeat samples at nearby service connections (i.e., within five service connections of the positive sample) are negative, there is a reasonable probability that a domestic or other non-distribution system plumbing problem exists. However, subsequent total coliform-negative samples at both the initial tap and at nearby service connections cannot be grounds to invalidate a positive sample.

The problem illustrated in circumstance (2) is usually outside the authority and responsibility of a water system, and thus the State may invalidate the sample. If a system has a single service connection, the TCR at 141.21(c)(1)(11) prohibits the States from invalidating a total coliform-

positive sample as a plumbing system problem. The reason for this provision is the inability in such systems to distinguish between the distribution system and plumbing system.

The State has substantial grounds to believe that a total coliform-positive result is due to a circumstance or condition which does not reflect water quality in the distribution system (C3)

Examples may include sample collection from a water hose and contamination of sample by failure in the integrity of sample container. In this case, the system must still collect a set of repeat samples and use the results in determining compliance with the MCL. In addition, to invalidate a sample under this criterion, the State must document its decision in writing, along with the rationale, and have it signed by the supervisor of the State official who recommended the decision. The State must make the signed document available to EPA and the public.

The State may not invalidate a positive sample solely on the grounds that all repeat samples are negative. When publishing the TCR in 1989, EPA recognized that some States were invalidating total coliform positive samples on the basis that subsequent repeat samples were total coliform-negative. Since the distribution of coliforms within a distribution system is not uniform, if a repeat sample is taken from the same tap as the original coliform-positive sample, these results may not be representative of the water quality conditions when the original sampling occurred. For this reason, EPA believes that there is no valid explanation for invalidating an original total coliform-positive sample strictly on the basis of subsequent total coliform-negative repeat samples taken from the same tap. A further explanation of TC distribution follows.

This third category gives the State latitude to invalidate a positive sample for reasons other than the two criteria specifically defined by the Agency. The State is required to explain its rationale in writing because such decisions must be technically justifiable and supported.

Distribution of Total Coliforms in Distribution Systems

A primary reason for restricting and specifying the circumstances for invalidating a positive sample in the 1989 rule was the concern that many States were invalidating such samples solely on the basis that repeat samples were total coliform-negative. In other words, it was assumed that when the repeat samples were negative at the same or nearby taps, the original positive sample must have resulted from a non-distribution system plumbing problem or improper sample collection and handling. This view was consistent with EPA's original TCR (EPA, 1975), published in 1975, which allowed systems to invalidate a positive sample solely on the basis of two coliform-negative "check" samples, apparently on the assumption that coliforms were uniformly distributed in water.

However, research on small water systems has demonstrated that the assumption above was not appropriate. In one such study (Christian and Pipes, 1983), investigators found that coliforms were distributed according to a lognormal distribution pattern (where the logarithms for a set of values are distributed normally around a mean value), rather than a Poisson distribution (which assumes a random distribution of coliforms in water). The geometric standard deviation of the counts (mostly between 10-100) was much greater than the geometric mean (for most systems, between 10^{-1} - 10^{-4}). This demonstrated that a contaminated water system is best represented by

small patches with high coliform densities with large water volumes lacking coliforms. The calculations in this study demonstrated that even in a contaminated system, the probability that a 100-mL sample will have no coliforms present is very high. These data indicate that invalidating an initial positive sample on the sole basis that check or repeat samples are all coliform-negative cannot be technically justified.

As part of their study, the researchers concluded that errors in sample collection rarely lead to contamination of the sample (Pipes and Christian, 1982). This was based on their experiment in which water samplers from Drexel, water samplers and water department employees from four different municipalities, and Drexel staff and students tried to contaminate 111 sample bottles containing 100-mL of sterile dechlorinated tap water by placing a finger into the mouth of each bottle and shaking the bottle vigorously for about 5 seconds. Only 6 (5.4%) samples were found to contain total coliforms. Five of the six coliform-positive samples were from the 78 Drexel staff and students sampled. However, lab personnel may have had cleaner hands than those expected in the field.

The results of this investigation strongly support the view that a positive sample should be regarded as a valid sample, except when a reasonable, scientifically sound basis exists for invalidating it. To clarify when such a sound basis might exist, EPA defined those circumstances in the TCR based upon reasonable judgment. Moreover, because of concern that some systems, through ignorance of the data or natural compulsion, might invalidate a positive sample without a technically justifiable reason, as was allowed by EPA's original TCR, EPA specified that the State alone had discretion to invalidate a positive sample.

III. Invalidation Criteria for Total Coliform-Negative Samples and Rationale

Under the TCR at 40 CFR 141.21(c)(2), a laboratory must invalidate a total coliform-negative sample if interference with the test by other organisms is observed. More specifically, a laboratory must invalidate a negative sample if either of the following circumstances occurs:

(1) If an analytical method is used that depends upon the production of acid or gas from lactose to indicate the presence of total coliforms, and the resulting test is turbid without acid or gas production.

(2) If a membrane filter method is used and the resulting filter does not exhibit any total coliform-positive colonies, but is overgrown (confluent growth or too many colonies to count) with noncoliforms.

Whenever a laboratory invalidates a total coliform-negative sample, the system must collect another sample from the same location as the original sample within 24 hours of being notified of the interference problem, and have it analyzed for total coliforms. This process must be repeated until the laboratory obtains a valid result. The State may extend the time limit for collecting the sample, on a case-by-case basis. An invalidated sample, regardless of whether it was total coliform-positive or negative, does not count towards meeting the minimum monitoring requirements or in calculating compliance.

High levels of heterotrophic bacteria can interfere with the analysis of total coliforms (Seidler and Evans, 1983; EPA,1984a, b; EPA, 1985). The data in these documents clearly demonstrate that the densities of total coliforms detected can decease substantially as heterotrophic bacteria densities increase, at least with lactose-based media. Thus it is reasonable to assume that a sample containing a small number of total coliforms may fail to produce detectable acid or gas in a lactose-based medium, or any characteristic colonies on a lactose-based membrane filter medium, as a result of such interference.

In the lactose-based broth media, heterotrophic bacteria (other than coliforms) may produce a heavy, turbid growth in the media in the absence of acid or gas production (acid or gas production is indicative of coliform presence). These noncoliform heterotrophic bacteria may suppress the growth of any coliforms in the medium and thus prevent the coliforms from producing a detectable level of acid or gas. For broth media, the TCR does not define precisely how turbid (i.e., cloudiness, heavy growth) a sample result must be before it is to be invalidated. This determination is left to the discretion of the laboratory.

High levels of noncoliform heterotrophic bacteria or fungi may also interfere with membrane filter methods by yielding filters with confluent growth or too many colonies to count. The EPA Manual for the Certification of Laboratories Analyzing Drinking Water (EPA, 2005) defines confluent growth as a continuous bacterial growth covering the entire membrane filter without evidence of total coliform-type colonies. The same guidance defines "Too many colonies to count" or "Too numerous to count" (TNTC) as greater than 200 noncoliform colonies on the membrane filter in the absence of detectable coliforms. Under these circumstances, any total coliforms on the filter may be prevented from forming characteristic colonies due to competition from the heterotrophic bacteria present. Under the TCR, laboratories must not invalidate samples when the membrane filter contains at least one coliform-type colony. In addition to heterotrophic bacteria, high turbidity levels may also interfere with the analysis for total coliforms by clogging the membrane filter.

Under the TCR, if a laboratory recommends and a State invalidates a total coliform-negative sample because of confluent bacterial growth, the system must collect another sample within 24 hours of being notified from the same location as the original invalidated sample. The rationale for this is that the presence of high levels of heterotrophic bacteria or turbidity in a sample may reflect a problem in the treatment process or in the integrity of the distribution system. Consequently, the system should be expedient in determining whether a problem exists.

EPA's laboratory certification manual (EPA, 2005) notes that a laboratory may perform a total coliform confirmed/validation test and/or a fecal coliform/*E. coli* test on a total coliform-negative culture that exhibits interference. If any of these tests is positive, the sample must be reported as total coliform-positive. A fecal coliform/*E. coli*-positive result should be considered a total coliform-positive, fecal coliform/*E. coli*-positive sample, even if the original test was negative. If the confirmation/verification test(s) is negative, the sample must be invalidated because high levels of non-coliform bacteria in the presumptive tubes may have injured, killed, or suppressed the growth of any coliforms in the sample.

IV. Informal Comments Received on Invalidation since TCR Promulgation

In January 2001, the Agency initiated an informal review of the TCR by sending a memorandum to all ten EPA Regions, and through them to the States, indicating that the Agency was beginning the revision process for the TCR and requested any thoughts and suggestions for revising any aspect of the rule. The Agency sent a similar letter to the Association of State Drinking Water Administrators (ASDWA), American Water Works Association, Association of Metropolitan Water Agencies, National Rural Water Association, and American Society for Microbiology. In response to this solicitation, the Agency received a total of 16 letters (7 States, 4 Regions, 2 cities, and 4 national organizations). The Agency combined these responses with those previously received since promulgation in 1989 (more than 23 solicited and unsolicited letters, mostly from the States).

Only two recent commenters raised the issue of sample invalidation. One commenter representing a large municipal system asked EPA to spell out the sample invalidation procedures so that a system could invalidate coliform samples immediately. The commenter stated, "State regulatory data are being posted to internet sites on a faster track and these data must be kept as accurate as possible, which means early intervention of invalid samples." Another commenter, an EPA Region, suggested allowing a State to invalidate a positive sample as a plumbing system problem at a system with a single service connection, if that system had more than one tap. The Region's rationale was that a single tap might be contaminated rather than the entire plumbing system.

In the older set of comments (mostly solicited by EPA in 1993), most commenters supported sample invalidation, especially for total coliform-negative samples that exhibited interference by high levels of heterotrophic bacteria. The few States that commented on the invalidation of total coliform-positive samples indicated that improper laboratory procedure was the only criterion they used. Several States opined that total coliform-positive samples should never be invalidated. One comment from a city maintained that it was often impossible to determine the source of total coliforms by follow-up samples alone (apparently referring to the use of repeat samples to determine whether a problem was associated with the distribution system or a local plumbing system).

V. Extent to which States are Using TCR Invalidation Criteria

In July 2003, ASDWA queried the States about how many total coliform-positive coliform samples they invalidated during the last five years. Sixteen States responded.
Four States indicated that they were not tracking these data or that the data were not readily available (de-centralized offices), one State indicated that they did not invalidate samples, and one State indicated that they did not track this information but estimated that a few total coliform-positive samples were invalidated each year for each of EPA's three criteria. Another State reported that about 6,000 samples (1.7% of total) were either invalidated or rejected (apparently without analysis) because of improper sample transport conditions; however, the data were not further separated between sample invalidation and rejection except for a statement that 3813 samples were rejected for exceeding the maximum allowed transport time to the laboratory.

Data from nine other States on the number of total coliform-positive samples invalidated are provided in Table 1. Based upon the States that responded, very few total coliform-positive samples are being invalidated.

ASDWA did not report the number of total coliform-negative samples that laboratories invalidated. However, in response to an EPA inquiry in 1993, ten of 18 States responding reported that less than 1% of coliform-negative samples had been invalidated, and another six States reported that between 1-5% of the coliform-negative samples had been invalidated. Two States indicated that greater than 5% of their coliform-negative samples had been invalidated. Because EPA has approved coliform methods since 1993 that are reported to be less prone to interference by high levels of heterotrophic bacteria, the 1993 survey results may reflect a higher coliform-negative invalidation rate than is the current situation.

ASDWA also asked the States what barriers existed to invalidating a total coliform-positive sample. The States reported the following barriers:

(1) Only the State (and not the system or laboratory) can invalidate a total coliform positive sample.

(2) Process was time-consuming and resource intensive, and not the best use of State resources.

(3) Confusion as to whether a laboratory is allowed to invalidate a positive sample based upon an issue related to sample collection or handling. (Note: This issue is not valid, given that only the State can invalidate such a sample, per 141.21(c)(1)(iii).)

Table 1. Number of positive samples invalidated during five year period

State	No. samples tested (5 yrs)	TC-pos samples invalidated	FC/EC samples invalidated	Comments[1]
1	75,000	269 (0.4%)		
2	180,000	Approx. 50-100 (0.03-0.06%)		Mostly C1 and C3
3	237,000	0		
4	475,000	19 (0.004%)		Mostly C2
5	300,000	6 (0.002%)	0	
6	575,000	~ 1		
7	1,200,000	361 (0.03%)	88 (0.007%)	**TC:** C1 (69), C2 (221), C3 (71) **FC:** C2 (21) **EC:** C2 (67)
8	350,000	Approx. 125 (0.04%)		Mostly C2
9	300,000	Approx. 10 (0.003%)		

[1] Key
C1: Laboratory establishes that improper sample analysis led to the positive result
C2: The state determines the problem resulted from domestic on other non-distribution system plumbing problem
C3: State has substantial reason to believe that the positive sample result does not reflect water quality in the distribution system

VI. Summary

The Agency included constraints in the final TCR for invalidating a positive sample because of concern about the potential that any positive sample would be misinterpreted as a sample collection error if repeat samples were negative, thus leading to an invalidation of the original sample. Informal comments from some public stakeholders suggest that states rarely invalidate positive samples.

One commenter believes that a faster invalidation process would be an improvement while another suggested that allowing the systems or laboratories themselves to invalidate samples might speed up the process.

EPA believes that discussion on this issue may provide additional perspectives on this issue and may reveal any pertinent research that has been conducted in the area. Discussion may reveal issues that EPA has not considered and possibly lead to a reevaluation of TC sample invalidation standards.

VII. References

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