ENCYCLOPEDIA OF

# ENVIRONMENTAL ANALYSIS AND REMEDIATION

**Robert A. Meyers**

*Editor*



**John Wiley & Sons, Inc.**

ISBN 0-471-11708-0

# MICROBIAL QUALITY OF DRINKING WATER

ANNE CAMPER
MARTIN HAMILTON
Montana State University
Bozeman, Montana

The public reasonably expects that their drinking water is safe and palatable. Drinking water suppliers, in cooperation with government regulators, strive to ensure high quality potable water. Current regulations address the public health concern by setting maximum contaminant levels (MCLs) allowed for potential contaminants in distributed water. There are MCLs for synthetic organic chemicals, lead and copper levels, disinfection by-products, and microorganisms. The regulatory strategy is to provide standardized detection and monitoring criteria to the utilities for use in demonstrating that the water is safe.

Microbiological pollution of potable water is one of the most prominent issues. According to a public communication from the American Society for Microbiology, U.S. National Center for Health Statistics data indicate that as much as $300 million to $3 billion may be spent annually on hospital costs associated with diseases caused by waterborne microbes in the United States (1). The causative agents of waterborne disease are enteric bacteria, protozoans, and viruses. It has been estimated that annual disease rates in the United States attributable to bacterial pathogens (*Salmonella, Shigella, Campylobacter,* and *Escherichia coli*) are 550,000 cases per year (2). There are also an estimated 260,000 cases per year of symptomatic giardiasis (due to the parasite *Giardia lamblia*), 420,000 cases of cryptosporidiosis (due to the parasite *Cryptosporidium parvum*), and 6.5 million cases of viral gastrointestinal infections (2). These figures indicate that, although most systems are performing extensive monitoring, there are still instances where the current approach fails to adequately protect public health.

Historically, waterborne disease was associated with fecal contamination and the presence of enteric bacterial pathogens. The source of the bacteria was assumed to be either breakthrough from the treatment plant due to inadequate treatment or contamination in the distribution system from open reservoirs, cross-connections, breaches in distribution system integrity, or back-siphoning. To detect these contamination events, it was necessary to devise a system of monitoring for quickly detecting the presence of fecal or pathogenic bacteria in drinking water and for identifying the source of contamination.

The intent of monitoring for the presence of microbes in distribution system water is to assess the water's potential for causing adverse health effects. If too many indicator or pathogenic microorganisms are found in the water, remedial action can take place before there is an epidemic of waterborne disease. One conventional remedial action is advising customers to boil water before consumption until such time as the cause of elevated microbial counts can be discovered and corrected.

In the United States, current regulations, as stipulated by the Safe Drinking Water Act (SDWA), set standards for the presence of indicator and pathogenic microorganisms in drinking water. There are separate regulations for each class of infective agents. For example, the virus/protozoan regulations are included in the Surface Water Treatment Rule (SWTR). Detection of these etiological agents is very difficult; therefore, effective monitoring methods are currently unavailable. For this reason, regulatory focus is placed on effective filtration and other barriers to entry into the distribution system. Standardized disinfection tables have been developed to show the required concentration and time of exposure of a specific disinfectant for *Giardia* and the viruses. The rules require that removal and disinfection should reduce the number of these microbes by 99.9% (conventionally known as a three log reduction). Monitoring in the distribution system is not required by the SWTR.

In contrast, the Total Coliform Rule (TCR) uses the presence of indicator organisms, the coliforms, to indicate the potential presence of enteric bacterial pathogens. The methods for detection of these organisms, although always subject to improvement, are relatively well established. Monitoring strategies involve the collection of samples from treatment plant effluents and various locations in the distribution system.

The absence of etiological agents or their surrogates is only one component in determining the public health of distributed water. The essential question is whether the customer population is experiencing disease due to waterborne microbes. It is advisable, therefore, to establish a surveillance program to watch for an increased incidence of people experiencing symptoms suggestive of waterborne infections. A special survey of physicians or households is probably required to provide reliable surveillance data. Morbidity rates based on routine public health reporting are not satisfactory for this purpose. Those rates are not sensitive measures because most waterborne illness goes unreported, the disease is often misdiagnosed, and the symptoms (diarrhea, cramps, nausea, jaundice, headaches, fatigue) may not appear significant even to some sufferers. In contrast to morbidity, mortality is more completely and accurately recorded. The occurrence of mortalities attributable to waterborne disease however, signifies a serious breakdown in the drinking water treatment, delivery, and monitoring systems. A good monitoring system should detect contamination before many people are exposed, not after it is revealed by excess mortality. Morbidity surveillance is an extremely important activity, but it is a topic beyond the scope of this article.

The goals of this article are to provide background information and the rationale behind the Total Coliform Rule for monitoring finished drinking water and to evaluate that rule. The TCR is an acceptance sampling scheme; it is similar to sampling protocols commonly used in other industries as a component of statistical process control. Evaluation of the TCR comprises a variety of issues that would arise with any microbial monitoring program for drinking water distribution systems. The issues include the choice of indicator organisms (total coliforms), sensitivity of methods for measuring concentrations of organisms, difficulties in interpreting the results, and potential conflicts with other regulations. The statistical characteristics of the rule are also assessed. Finally, in an attempt to anticipate future monitoring regulations, some alternative approaches to system monitoring are listed.

## CURRENT REGULATIONS

### The TCR Compliance Criterion

The United States Environmental Protection Agency (U.S. EPA) established the following compliance criterion based on samples taken from a drinking water distribution system (3):

*For a system which collects at least 40 samples per month, if no more than 5.0 percent of the samples collected during a month are total coliform-positive, the system is in compliance with the MCL for total coliforms.*

There are special compliance levels for small systems (<10,000 population served) that sample fewer than 40 times per month.

The monitoring regulations state that public water systems need only determine the presence or absence of total coliforms in each sample volume of 100 milliliters (mL); a determination of total coliform density is not required. This compliance rule, first published by the U.S. EPA in 1989 (4), is in contrast to the previous rule based on the density of total coliforms. The previous compliance rule required that the average density of coliforms per sample could be no greater than 1 per 100 mL in a month and that all samples must show a density of no greater than 4 per 100 mL.

Monitoring costs for the TCR in the U.S. are $139 millions (February, 1994 U.S. dollars) per year (5). This is about 9% of the estimated total annual cost of compliance with regulations under the SDWA and its amendments, which are implemented and enforced by the EPA and the individual states.

### Choice of Coliform Bacteria as Indicators of Fecal Contamination

Because of the low numbers of potential enteric pathogens and difficulties in detection, it is necessary to use indicator organisms as surrogates. Total coliforms or fecal coliforms are used worldwide as indicator organisms. Total coliforms include most species of the genera *Enterobacter, Klebsiella, Citrobacter,* and *Escherichia* and are defined by their ability to produce acid and gas from lactose on selective culture media within 24 h at 35°C. Fecal coliforms are a subset of the total coliform group, primarily including *E. coli* and thermotolerant strains of *Klebsiella* (6).

The use of these organisms as indicators for fecal contamination is based on several criteria. Indicator organisms should be present in higher numbers than the pathogens, be present whenever pathogens are found, be at least as robust as the pathogen in the environment, be relatively easily detected, and be present only when fecal contamination is found. Although there are circumstances where one or more of these criteria are not met, the coliforms have yet to be replaced by a more reliable indicator.

### Coliform Detection Methods

Under previous regulations, the presence of coliforms was reported as the number of organisms per 100 mL as detected using either a most-probable number fermentation tube (MPN or FT) dilution technique with lauryl sulfate broth or with a membrane filtration test (MF) on LES Endo or m-Endo medium.

In the case of the MPN or FT method, it was conventional to use five replicate tubes per dilution. The original positive tubes (gas production) were considered presumptive, and further testing was required to verify the presence of coliforms. The number of organisms per 100 mL original sample was then determined using standard MPN tables (7).

If the organisms were enumerated using the membrane filtration test, 100 mL was filtered through a 0.45 $\mu$m pore-sized gridded filter. The filter was then placed on an agar surface or broth-soaked absorbent pad. Confirmed colonies were reported per 100 mL of original sample.

The current compliance standard, however, is based on the proportion of 100 mL samples that are positive for coliforms, not on the density of coliforms. Although the density may be useful for other purposes, the regulations take into account only presence/absence (P–A test) in 100 mL samples. For this reason, it is sufficient to innoculate commercially available P–A broth with the 100 mL water sample and incubate. A color change and/or gas production is considered to be a presumptive positive test. If confirmation is established, the sample is positive for coliforms. An alternative method is to use a chromogenic substrate test such as *ortho*-nitrophenyl-$\beta$-D–galactopyranoside-4–methylumbelli-feryl-$\beta$-D-glucoronide (ONPG-MUG) medium which simultaneously detects the presence/absence of total coliforms and *E. coli.*

### Coliform Detection Methods May Not Be Sensitive

Whenever a subset of the heterotrophic bacterial population (here, coliforms) is to be detected and enumerated, a selective medium is required. These media utilize selective agents that are inhibitory to most of the organisms present. As a result, the growth conditions for the target organisms may not be optimal, which in turn underestimates the number and/or presence of the indicator bacteria. In the case of waterborne coliforms, it is known that environmental conditions can lead to reduced recovery on selective media (8–10). This phenomenon has been termed *injury* and the bacteria are defined as *injured* or *stressed.*

Methods have been developed to increase the ability of the bacteria to recover from injury and to replicate sufficiently to give a positive test result. The methods include short-term preincubation on nonselective media prior to transfer to the required selective media or use of a less stressful medium (7). However, the regulations do not require that these approaches be taken, and utilities probably will not voluntarily use enhanced recovery approaches that will increase the chance of noncompliance.

### Errors in Sample Collection and Handling May Bias Data

Although the proper method for sampling is stated in Standard Methods for the Examination of Water and Wastewater (7), there are published reports of inappropriate sample collection and handling (11,12). Depending on the transgression, the bacterial counts from these samples may either underestimate or overestimate actual conditions. On one hand, if a neutralizing agent is not added to a sample bottle for disinfected water, there may be a subsequent decline in the bacteria during storage and shipment. On the other hand, errors such as improper flushing of the sampling port, unsanitary handling of the sample bottle, keeping faucet screens in place during sample collection, and sampling from dirty kitchen sinks contaminated during food preparation may lead to exaggerated bacterial counts. Excessive holding times between sample collection and culturing may either increase or decrease the bacterial numbers depending on available nutrients in the water and temperature. When such procedural errors occur, the data may not represent the quality of water in the distribution system and its compliance with standards.

### Biofilm Sloughing May Confound Monitoring Data

A drinking water treatment system can experience microbial contamination even though that system is properly designed,

constructed, and operated. The phenomenon has been termed *regrowth* by the drinking water community. Regrowth is presumed to be the result of the growth and subsequent detachment of coliforms and other bacteria from biofilms on pipe surfaces, sediments, inorganic tubercles on a pipe surface, suspended particles, or virtually any other surface immersed in the aquatic environment (13,14). Biofilms are defined as microorganisms attached to a surface in an aquatic environment, with the associated extracellular polymers and inorganic particulates (15). Coliforms grow in mixed population biofilms on distribution system surfaces utilizing biodegradable organic nutrients in the water under ill-defined environmental conditions. Regrowth events are detected when the indicator bacteria leave the surface and enter the bulk water. It has been documented that surface-associated bacteria are much less susceptible to disinfection than suspended organisms (16–20). In actual distribution systems, there are many instances where an increase in chlorine concentration has failed to control biofilms or the presence of indicator bacteria (21–26). Although breakthrough and breaches in integrity in treatment or distribution may well constitute health threatening episodes, detached or sloughed biofilm microorganisms may be benign to the vast majority of consumers.

In recognition of these facts, the EPA allows a variance from the regulations (3):

> *...for systems that demonstrate to the State that the violation of the total coliform MCL is due to a persistent growth of total coliforms in the distribution system rather than fecal or pathogenic contamination, a treatment lapse or deficiency, or a problem in the operation or maintenance of the distribution system....*

It is not obvious how to demonstrate that the apparent violation is due to biofilms. Because coliform bacteria occur at specific, discrete locations within the system, a random sampling of pipe surfaces may not detect these sites (13). Monitoring for coliforms on surfaces in actual distribution systems is currently difficult, if not impossible.

There is always the possibility that coliforms from a regrowth event may occur simultaneously with fecal contamination. No definitive process currently exists to differentiate between coliforms associated with biofilms and the penetration of coliforms due to breakthrough or cross-contamination (6).

A system that seeks a variance must submit a biofilm control program plan to the particular State within 12 months of granting the first request for a variance. Although there are no guidelines for a biofilm control program, it appears that one of the most effective strategies may be to remove organic nutrients from water. In the absence of such nutrients, coliforms are not as likely to grow. Some treatment plants in Europe and Canada have been able to control organic substrate concentrations by adding a biological filtration step at the treatment plant. Biological filtration has the added advantage of reducing organic carbon that may react with disinfectants and form potentially carcinogenic disinfection by-products (27–29).

### TCR May Conflict with Other Drinking Water Regulations

Because the biofilm bacteria are less susceptible to disinfection than suspended cells, it is not generally feasible to attack those bacteria by increasing the chlorine residual in the system. There are reported instances where the free chlorine residual has been increased to levels in excess of 12 mg/L, without subsequent elimination of coliforms in the distribution system (30,31). If the source water is high in natural organic matter, elevating the free chlorine concentration may cause the system to exceed the allowable levels of disinfection by-products. Utilities may choose to use ozone as a primary disinfectant to meet requirements for *Giardia* and *Cryptosporidium* removal or for oxidation of taste/odor/color problems. In some systems, it is known that the lower molecular weight organic constituents created by ozonation support the growth of biofilm organisms (28). If biological filtration does not follow ozonation, the potential exists for stimulating heterotrophic bacterial regrowth in the distribution system. Mechanical means of removing biofilm and corrosion may well increase the particulate counts and metal concentrations in the water to levels exceeding regulations. It is a severe challenge to eliminate existing biofilm coliforms from the system without exceeding the allowable concentration of some other regulated constituent.

### TCR, Statistical Process Control, and Acceptance Sampling Plans

Since the early 1900s, public health officers in the United States have set upper limits on bacterial counts in drinking water. They originally attempted to choose compliance levels that were attainable and judged to pose no risk to public health as determined by a historical review of data from New York state relating waterborne disease outbreaks and bacterial counts. Drinking water standards have been extensively revised since then. The chain of reasoning that led to the present TCR is obviously very complicated. Nevertheless, after reading the EPA-sponsored studies that led to the TCR, the TCR was visualized by its creators to be an acceptance sampling plan, such as is used in the manufacturing industries.

Acceptance sampling was originally devised to provide quality control to a manufacturing process. It relies on taking a sample of produced units from each lot leaving the assembly line, then inspecting each unit and classifying it as acceptable or unacceptable. If the proportion of unacceptable units is sufficiently small, the lot is accepted. In the modern manufacturing world, less reliance is placed on acceptance sampling and more reliance on upstream Statistical Process Control (32,33). There are some conditions, however, where acceptance sampling is necessary; specifically, when there is a need to protect against gross system failures, safety is of foremost concern, the process fluctuates so the product is of variable quality, the cause of an unacceptable product is not always knowable, and the specified sampling error rates (discussed later) are not too small. Because all of these conditions apply to drinking water, it is reasonable to require an acceptance sampling plan for produced drinking water.

The TCR is based on the following conceptualization. For a specific time interval (eg, a calendar month), suppose that all of the water passing through the system can be partitioned into distinct 100-mL units of water. In each unit, coliform bacteria are either absent (acceptable) or present (unacceptable). Each 100-mL unit is viewed as a finished product; the totality of units for a month is in essence a lot, although the word "lot" is not used in the regulations. If a large proportion of the units are coliform-free, then the lot is acceptable. This artificial discretization of drinking water into 100-mL units allowed the EPA's analysts to calculate statistical properties of the TCR using the same formulas as used in acceptance sampling.

### Sampling Sites and Frequency of Sampling

In acceptance sampling, the units are usually drawn following stratified random sampling rules or systematic sampling rules. To sample from drinking water distribution systems, one might stratify according to several different criteria. One potential criterion is the importance of a location to public health. Perhaps a greater sampling intensity should be used for sites that are judged to be critical; that is, serving many people, serving sensitive (eg, immunosuppressed) people, schools, hospitals, important industrial sites, etc.

Another criterion might be distance from the source. It makes sense to sample so that breakthrough is detected as soon as possible. This does not necessarily imply that one should sample water as it leaves the treatment plant because, as was discussed earlier, breakthrough organisms may be injured and not detectable by standard assays. Thus sampling may be done near the treatment plant or at first customer locations using the most sensitive detection methods possible. If cross-contamination occurs in the system, then that contamination may be evident in samples at locations reached by the longest flow path or locations receiving water that has the longest residence time in the distribution system. The sampling plan should therefore include samples from some remote locations.

It is important to have some sampling locations at intermediate points in the distribution system because cross-contamination can potentially occur anywhere. Such contamination could pollute nearby water taps, but because residual chlorine may eventually kill the contaminating coliforms, the bacteria may not be detected in samples further down the system.

It is possible that contamination events are due to cyclical or transient factors such as flow reversals on a diurnal basis resulting in back-siphoning or the emptying of a contaminated tank due to unusual water demand. In such circumstances, the system will be affected by pulses of bacteria that may be missed if samples are not taken often enough. To detect such events, it is prudent to sample at regularly spaced times during the month.

Clearly, the method by which sample locations and times are chosen is more problematic for drinking water systems than for manufactured product applications. Every distribution system is unique, so some flexibility in site selection is essential. The EPA guidelines acknowledge these issues, and do not require any specific sampling scheme (3):

> *Public water systems must collect total coliform samples at sites which are representative of water throughout the distribution system according to a written sample siting plan. These plans are subject to State review and revision.... The public water system must collect samples at regular time intervals throughout the month,....*

### Statistical Properties of the TCR: Notation and Number of Samples

Some notation is necessary to discuss the statistical reasoning that underlies the EPA compliance rules. Let $\theta$ denote the (unknown) proportion of coliform-contaminated units among all 100-mL units in the system. The parameter $\theta$ is the fraction of water contaminated. It is the focus of the compliance rule. Let $n$ denote the number of units sampled during a calendar month and let the random variable $X$ denote the contaminated units among the $n$. The statistical properties of the USEPA compliance rule shall be analyzed using the same reasoning as in EPA documents, reasoning identical to acceptance sampling methodologies. The calculations require a probability model for $X$. Following convention in analyses of the TCR, the binomial probability model will be used (34,35). Specifically, assume that the random variable $X$ follows a binomial probability distribution with mean $n \cdot \theta$.

Let the sample statistic $p = X/n$ denote the fraction of contaminated units among the $n$; the statistic $p$ is an estimator of $\theta$. If the observed value of $p$ is small, one may conclude that $\theta$ is small. That conclusion is reasonable if $p$ is a reliable estimator of $\theta$. Statistical calculations can show how large $n$ must be to attain a specified reliability.

Based on statistical calculations and the premise that greater reliability is required for systems that serve more people, the EPA has published a table showing how the minimum required $n$ increases with the size of the population served (3). For example, a utility serving 35,000 people must sample at least $n = 40$ units per month, and a utility serving 1,000,000 must sample at least $n = 300$ units per month. The required $n$ is actually a minimum sample size. The regulations state that, when coliforms are found in a sample, repeat samples are required. The specific details of repeat sampling are not be discussed here; see the *Code of Federal Regulations* (3).

In acceptance sampling, a manufactured lot is accepted if the observed fraction of defective units is sufficiently small. For drinking water, the threshold of acceptability is called the Maximum Contaminant Level (MCL). Except for small systems where $n \leq 39$, the EPA has set the MCL at 0.05; that is, the system is compliant according to current EPA regulations if and only if $p \leq 0.05$.

### Statistical Properties of the TCR: Goal of TCR Compliance Rule

The goal of the compliance rule is not stated in any official regulatory publications, but it can be established by inference. One can reasonably infer that the compliance rule specifies 0.05 as the maximum fraction of water contaminated (35). Justification for this inference is that, if one actually sampled all 100-mL units in the system and 5% or fewer contained coliforms, then the system would be in compliance.

The goal may be interpreted as more stringent because the EPA proclaimed a Maximum Contaminant Level Goal (MCLG) of zero; ie, no coliforms in the system. The MCLG is the "maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health of persons would occur, and which allows an adequate margin of safety ... a nonenforceable health goal" (3). The justification for MCLG = 0 is that, "conceptually, coliforms should not be present in drinking water, because they may indicate the presence of pathogenic organisms in the water" (4). Although the target $\theta = 0$, may be appropriate as an ideal, it is not a practical target for compliance because a system would be compliant with such a stringent target only if *all* sampled units were coliform-free ($p = 0$).

Let $\theta_0$ denote the largest value of $\theta$ consistent with compliance. Then the EPA compliance rule implies a *de facto* acceptance value of $\theta_0 = 0.05$. Suppose that a drinking water system had a fraction of water contaminated that is slightly greater than 0.05; eg, suppose $\theta = 0.05001$. Is that system sufficiently above the target to be called unacceptable? The conventional answer is probably not, but there is some value greater than

0.05 which is of practical importance. Let $\theta_1$ denote that value. The EPA, after seeking the advise of experts, determined that $\theta_1 = 0.1$: "The expert panel recommended this level, at which at least 90 percent of the water is coliform-free, be accepted as a 'Protection Reliability Standard'" (36).

To summarize our understanding of EPA targets,

- If the fraction of water contaminated is 0.05 or less ($\theta \leq 0.05$), then the water is definitely acceptable.
- If the fraction of water contaminated is 0.10 or more ($\theta \geq 0.10$), the water is definitely unacceptable.
- A system for which the fraction of water contaminated is between 0.05 and 0.10 ($0.05 < \theta < 0.10$) is not sufficiently above the target to be of practical concern even though it is not strictly acceptable.

It has been calculated that the current acceptance target of $\theta_0 = 0.05$ is equivalent to an average total coliform density of 0.1 per 100 mL, perhaps depending less on the assumptions entering the calculations (37). The protection reliability standard of $\theta_1 = 0.1$ is equivalent to an average total coliform density of 0.4 per 100 mL. The target level of contamination for the new rule is more stringent than for the previous rule that required an average density less than 1 per 100 mL.

### Statistical Properties of the TCR: Error Rates for the Compliance Rule

Because of chance events in the sampling process, it is possible that coliforms could be absent from many of the $n$ sampled units, even when $\theta$ is not small (eg, $n = 0.10$). It is also possible that coliforms could be present in many of the sampled units, even when $\theta$ is small (eg, $n = 0.05$). Although misleading samples are improbable, they prevent the compliance rule from being perfect. It is of interest to calculate (*1*) the chance that $p > 0.05$ although $\theta \leq 0.05$, which is the chance that the sample data incorrectly indicate a noncompliant water, and (2) the chance that $p \leq 0.05$ although $\theta \geq 0.10$, which is the chance that the sample data incorrectly indicate a compliant water. Calculation (*1*) yields the false fail rate at $\theta$, for $\theta \leq 0.05$, denoted by $R_{ff}(\theta)$; calculation (2) yields the false pass rate at $\theta$, for $\theta \geq 0.10$, denoted by $R_{fp}(\theta)$. If the false pass rate is large, then the compliance rule often fails to discover coliform contamination. If the false fail rate is large, then the compliance rule is expensive to implement because producers may expend time and resources mistakenly doing repeat monitoring of water that actually is already in compliance, or mistakenly alarming and inconveniencing the public by issuing warnings to boil the water before consumption. Obviously, $R_{ff}$ and $R_{fp}$ are important quantities to regulators, producers, and consumers. The choice of threshold for compliance requires counterbalancing the two rates; decreasing one increases the other. For example, if the regulatory agency imposed a more stringent compliance criterion (eg, by requiring $p \leq 0.01$), the effect would be to reduce $R_{fp}$ but increase $R_{ff}$.

For the EPA compliance rule, $R_{ff}$ and $R_{fp}$ have been calculated (35,37). Those calculations have been briefly summarized. For given $n$, let square brackets denote the largest integer function; that is, $[0.05n]$ denotes "the largest integer $\leq 0.05n$." Because $X$ follows a binomial distribution and the observations show compliance if and only if $X \leq 0.05n$, the error rates are given by equations 1 and 2. These equations are the foundation upon which the present compliance levels were constructed.

$$R_{fp}(\theta) = \sum_{i=0}^{[0.05n]} \binom{n}{i} \theta^i (1-\theta)^{n-i}, \text{for } 0.10 \leq \theta < 1 \quad (1)$$

$$R_{ff}(\theta) = \int_{i=[0.05n]+1}^{n} \binom{n}{i} \theta^i (1-\theta)^{n-i} \text{ for } 0 < \theta \leq 0.05 \quad (2)$$

Figure 1 is based on equations 1 and 2. It shows the error rates associated with the EPA compliance rule as $\theta$ varies from 0.01 to 0.2. When $\theta \leq 0.05 = \theta_0$, the error is a false fail; when $\theta \geq 0.10$, the error is a false pass. The error rates generally become smaller as $n$ increases. The only exception is that, when $n$ is large, $R_{ff}(0.05) = 0.5$, so the curve for large $n$ climbs steeply from near zero to 0.5 as $\theta$ approaches 0.05. The samples per month shown in the plot, $n = 40$, 100, and 400, are the sample numbers required for water systems that serve populations of 40,000, 100,000, and 2,000,000 people, respectively.

The compliance rule was intended to achieve $R_{fp}(0.1) = 0.05$ when $n = 60$; but, because there were some inaccuracies in the original EPA calculations, $R_{fp}$ is larger than intended. In fact, at $n = 60$, $R_{fp}(0.1) = 0.14$ (37). In other words, if the fraction of water contaminated in the system is 0.1 ($= \theta_1$) and $n = 60$ samples are taken from the system during this month, then there is a 14% chance that the water will incorrectly pass the compliance criterion. Figure 1 shows that approximately $n = 100$ samples per month are required to achieve $R_{fp}(0.1) = 0.05$. It also shows for a given $n$ how small $\theta$ would have to be in order for $R_{ff}(\theta)$ to be less than 0.05.

### Statistical Properties of the TCR: Evaluating Assumptions that Underlie Statistical Calculations

The binomial distribution for $X$ and the associated statistical error rate calculations using equations 1 and 2 are appropriate only if the system conforms to the following assumptions:

- $n$, the number of samples per month is fixed prior to the commencement of sampling,

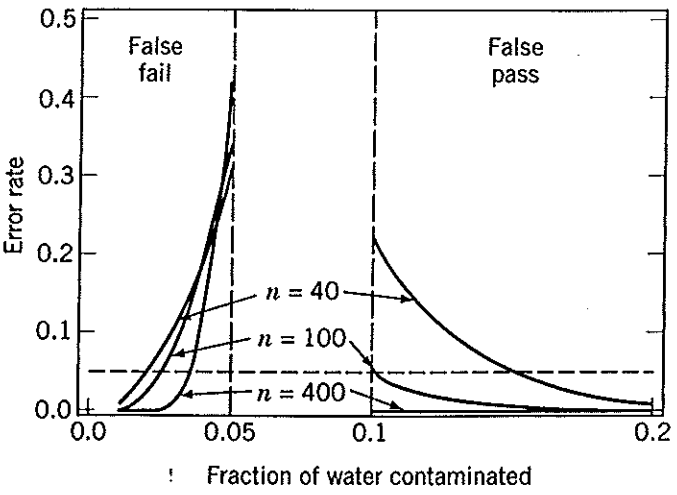


**Figure 1.** Error rates associated with the EPA compliance rule; specifically, the false pass rate $R_{fp}(\theta)$, $0.10 \leq \theta \leq 0.2$, according to equation 1 and the false fail rate $R_{ff}(\theta)$, $0.01 \leq \theta \leq 0.05$, according to equation 2 for $n = 40$, 100, or 400. The dashed horizontal line is at an error rate of 0.05. The dashed vertical lines are at $\theta = 0.05$ and $\theta = 0.10$ from left to right.

- each sample is correctly classified as either contaminated (coliforms present) or not contaminated (coliforms absent),
- each of the 100 mL samples has the same probability $\theta$ of being positive for coliforms, and
- the samples are statistically independent.

None of these assumptions hold exactly; therefore, the statistical error rate calculations of the previous section must be treated as approximations. The appropriateness of each assumption in the context of drinking water monitoring is now evaluated with the goal of deciding whether equations 1 and 2 are good approximations.

**Number of Samples Per Month is Fixed.** Formulas 1 and 2 are for specific values of $n$. Because the EPA regulations require repeat sampling when coliforms are found, the number of samples in a month may end up being larger than the initial $n$. In fact, two people independently monitoring the same system during the same month might end up with different $n$ values even though both followed the regulations exactly. This would happen, for example, if all of person A's samples were uncontaminated, but person B drew one or more contaminated samples and had to do some resampling. Computer simulation was used to account for variable $n$ when error rates were calculated (35). These results do not significantly differ from those found assuming fixed $n$. Equations 1 and 2 could be reasonable approximations to the true error rates even though the fixed $n$ assumption is not strictly true for monitoring data.

**Each Sample is Correctly Classified as Either Contaminated (Coliforms Present) or Not Contaminated (Coliforms Absent).** This assumption is problematic because there is no accepted "gold standard" method for detecting coliform contamination. The EPA regulations allow any of four analytical methods for deciding whether coliforms are present in a 100 mL sample: (*1*) the FT technique, (*2*) the MF technique, (*3*) the PA coliform test, and (*4*) the minimal media ONPG-MUG test (Colilert system). These methods do not give the same answers when testing the same water. For example, 500 mL samples were taken from a drinking water system on 1483 different occasions (38). Each sample was analyzed by each of FT, MF, and PA using 100 mL portions: 336 (23%) confirmed positive samples by one, two, or all three of the techniques were found. Only 185 (12.5%) were positive by all three techniques.

All of the assay techniques depend on the ability of the coliform cells to divide and create detectable numbers of cells. As indicated earlier, viable, but injured coliform bacteria may not be discovered by conventional methods. There are various other uncertainties associated with assaying water samples, including variations in sample gathering techniques, transport, and storage prior to analysis. Little is known about the between-lab variabilities of the methods, but significant variability is expected. For these reasons, the error rates produced by equations 1 and 2 are probably smaller than the actual error rates. It is not obvious how the equations should be adjusted to provide more accurate error rates.

**Each of the $n$ 100 mL Samples has the Same Probability $\theta$ of Being Positive for Coliforms.** Because the samples are taken at different locations and at different times during the month, this assumption is never strictly true. The density of coliforms in the system differ from time to time due to variation among and within source waters, especially seasonal and diurnal variation of surface water, variation in treatment plant operations, variation in consumer usage patterns, including seasonal variation (more flow during gardening or irrigation season), and diurnal variation (more flow in morning and evening for residential areas, more flow during business hours in commercial areas), variation in the chemical and physical profile of the water (eg, temperature, different residual chlorine concentrations depending on source, residence time in pipeline, etc), variation in proportion of coliforms that are injured but not killed, and variation among and within storage locations (reservoirs and tanks). The water at a certain sampling site may well be a mixture of different waters because of interconnections between various sources, treatment plants, and storage locations. The components of that mixture vary in time, especially at sites where the direction of flow is occasionally reversed. The density of detectable coliforms may vary from site to site because of many of the same reasons that cause variation in time. In systems where there is a significant biofilm, the coliform density at a sampling site can be influenced by the pipe properties along routes from sources to the site. Relevant properties of the pipes are materials, age, diameter, and corrosion products on the pipe wall.

Although $\theta$, the fraction of water contaminated, can fluctuate in time and location, it is possible that when no contamination event has occurred, $\theta$ stays within an interval of small values, well below $\theta_0$. For this situation, if would be reasonable to compute $R_{ff}(\theta)$ with equation 2, where $\theta$ is at the upper end of the interval. If, however, $\theta$ varies from sample to sample over an interval around $\theta_0$, then error rates computed by equation 2 are suspect.

When a contamination event occurs, the elevated fraction of water contaminated varies among sampling sites, possibly exceeding $\theta_1$ at only a few sites. In this case the error rate computed by equation 1 should not be trusted.

**The Samples are Statistically Independent.** This would be a more plausible assumption if sampling locations were chosen at random from all locations where water exits the system. Instead, sampling locations are chosen systematically to cover the system. Moreover, the sampling sites are usually restricted to convenient, public sites where the cost is minimal and there is no potential invasion of privacy.

The statistical independence assumption implies that samples adjacent in time or space are not correlated. Samples will always be temporally and spatially correlated. The influence of such correlations may be negligible when there are no contamination events and there is a low fraction of water contaminated throughout the system. In this case, one could reasonably compute $R_{ff}(\theta)$ with equation 2. When contamination events occur, however, the temporal and spatial correlations are significant, making the conventional error rate calculations based on equation 1 meaningless.

## FUTURE MONITORING AND REGULATIONS

Due to increased concern about public health and more stringent water quality regulations, the drinking water industry has been interested in finding quicker, less expensive, and more reliable monitoring techniques. In many cases, the existing techniques are known to be inadequate, but no better

alternatives exist. As in the past, the development of new monitoring techniques will be driven by the regulations. There is an obvious need to couple occurrence and numbers of indicators with numbers of pathogens and with subsequent risk of waterborne disease. Some possibilities are listed below.

### Modeling

Because water distribution systems have complicated flow patterns, sporadic cross-contamination may go undetected or produce a confusing set of monitoring data. The flow patterns should be taken into account when choosing sampling sites or when investigating a noncompliant system. For these reasons, a valid computer model of the flow in a water distribution network could be very useful. In the future, more extensive use of computer models in the design of monitoring systems and the interpretation of monitoring data can be expected.

Computer models are presently expensive to develop initially; each network requires a unique model. The model must be revised and reevaluated when the system is significantly altered or expanded. Tools have been created to make the construction of such models easier. To cite one effort, the EPA has created a software package called EPANET to perform simulation of hydraulic and water quality conditions within pressurized pipe networks (39). EPANET was designed to run on a modern desktop computer. It is currently being used to study such water quality problems as chlorine decay dynamics, source blending, effects of altered tank operation on water age, and control of total dissolved solids in reclaimed water. The model can be calibrated and evaluated for a network using tracers (eg, fluoride).

### Biofilm Regrowth

Researchers are attempting to determine methods for reducing biofilms in drinking water distribution systems. Preliminary results indicate that biofilm growth is impeded if biodegradable organic matter levels are controlled and that pipe materials may be an important consideration (40,41). As progress is made in understanding factors leading to biofilm accumulation, it is more likely that regrowth can be identified and potentially controlled.

### New Monitoring Techniques and Devices

New methods, based on biotechnology, for direct detection of waterborne contaminants are becoming available. Ideally, the method should preclude the isolation and growth of the bacteria on culture media. Although the new methods are currently impractical due to cost, problems in interpretation, or ease of application, they show the potential for rapid, automated, and accurate procedures.

For example, the biomass of complex microbial consortia can be determined by measuring the concentrations of components of cell membranes. The detailed analysis of universal components, such as the lipids found in all cell membranes, yields detailed patterns that have been established as biomarker "signatures" for specific groups of microbes. Fatty acid methyl esters (FAME) can be extracted, measured, and the profiles compared with those of known organisms to identify bacteria in the environment (42,43); the detection method has been standardized and is marketed by Microbial ID, Inc. (MIDI). Phospholipids can also be extracted and used as an identification tool. The signature lipid biomarker technology has been applied to monitoring biofilms in drinking water distribution systems. One limitation of this approach at this time is that a sufficiently large amount of membrane lipids must be extractable from the sample to allow detection. Consequently, culturing of the waterborne bacteria is often necessary prior to identification. Nevertheless, this approach may eventually be able to discriminate between biofilm coliforms and fecal or pathogenic coliforms in a timely fashion (see BIOMARKERS).

It may be feasible to monitor using the small ribosomal subunit RNA molecule, a cell component with specificity for each species of importance in drinking water. Associations between ribosomal RNA "fingerprints" and microbial species are being collected by the Ribosomal Database Project at the University of Illinois (Urbana, Illinois). There are a variety of other RNA and DNA fingerprinting techniques being used or under development, any one of which may prove useful for monitoring drinking water. Again, the method requires a sufficiently large sample so that detection of a few cells in water is not yet feasible.

The NASA Mid-Continent Technology Transfer Center (College Station, Texas) has described a novel variation on current oligodeoxynucleotide tests, using chemiluminescence to identify the target microorganisms. It detects and quantifies the microorganism by measuring the total amount of light emitted. Oligodeoxynucleotide probes have been developed for *E. coli,* coliform bacteria, *Vibrio cholerae, Burkholderia cepacia, Staphylococcus aureus, Staphylococcus epidermidis,* and *Salmonella* spp. The probe system can be designed to detect almost any group, genus, and in many cases, species of microorganism. If the light intensity is great enough, preculturing of the bacteria may not be necessary.

Another method is the use of specific fluorescent antibodies to target indicator bacteria. A water sample is collected, the bacteria concentrated on a membrane filter, and an appropriate antibody applied. A method has been described wherein a monoclonal antibody specific for an enterobacteria common antigen was used to detect coliforms in a variety of drinking water samples (44). Fluorescently tagged cells are then counted via a microscope. An automated approach may be possible if flow cytometry is used for detection (45,46). An advantage of the antibody method is that no culturing is required, but conversely, no information can be attained about the viability of the cells.

A recent development has been the application of direct impedance methods (47,48). Water samples are filtered, immersed in a medium containing trimethylamine oxide (TMAO), and the impedance measured over time. *Escherichia coli* produces a TMAO reductase; the resulting product causes a measurable change in impedance. Although culturing is required, detection times are reduced from nearly 24 hours (with plate counts) to a few hours.

Another method that has received increased attention is the amplification of specific regions of the target cells' genetic information using the polymerase chain reaction (PCR). The method has been applied to water samples for the detection of coliforms, *E. coli,* and *Shigella* spp. (49,50). PCR does not require that the cells be precultured, although there is some question about the sensitivity of the method when cell counts are extremely low. There is also the possibility that nonviable cells can be detected (51).

These are just a few representative technologies among many being investigated. Although they are presently expen-

sive and too difficult to implement on a routine basis, these sophisticated techniques will eventually be simplified to a point where they can replace conventional culture techniques for identification of waterborne pathogens.

Viruses and protozoan parasites are also important to the health of consumers. New indicators for viruses and protozoa will be developed and used for monitoring. *E. coli* may remain a useful indicator of pollution by fecal bacteria. New biotechnology may allow for rapid, inexpensive detection of *E. coli* and other fecal bacteria. In the future public health authorities may place less reliance on total coliforms for detecting breaches in integrity, although total coliforms will remain useful as surrogate indicators of treatment failure or breakthrough.

It is also possible that the density of heterotrophic plate count bacteria determined using optimal recovery techniques may be an early warning of potential regrowth events or failure in system integrity. There is also the potential for the use of *Clostridium* spores to serve as surrogates for pathogenic protozoans, although more evidence for similar behavior is required.

### Risk Assessment

No scientific, risk-based approach has been used to prioritize microbial contaminants, nor has a formal risk analysis been done for a single microbial contaminant among the hundreds that can be found in drinking water. Risk assessment (qv) for pathogens in drinking water is expensive and time-consuming and only a few initial steps have been taken so far. A serious effort has been launched to do risk assessments, however. The results of that effort will provide the basis for future regulations concerning waterborne pathogens.

The risk of disease depends on factors pertaining to the infectious agent, the human host, and the drinking water system environment. Relevant characteristics of the agent include infectivity, virulence, and pathogenicity. Important characteristics of the potential host population include age distribution, susceptible subpopulations and water consumption patterns. Characteristics of the drinking water environment include the density of viable pathogens in the source water, the quality of water treatment, and the age and design of the drinking water distribution network. Coordinated epidemiological studies and laboratory experiments will be required to elucidate the relationships between these characteristics and disease rates.

## CONCLUSIONS

Total coliforms are important because they are easily detected indicators of fecal contamination. The current U.S. Total Coliform Rule is based on the proportion of 100 mL samples from the drinking water distribution system that are positive for coliforms during a calendar month. The goal of the monitoring rule is to differentiate systems for which the fraction of water contaminated is 0.05 or less from systems for which the fraction of water contaminated is 0.10 or more. Drinking water monitoring regulations commonly used in the United States and other countries are in essence an acceptance sampling plan such as commonly used in manufacturing plants. Conventional acceptance sampling statistical methods were used by EPA analysts to calculate the false fail error rate and the false pass error rate for the compliance rule, but the data characteristics required to assure the accuracy of these calculations may well be incorrect for drinking water data. To calculate the false pass error rate accurately, it is probably necessary to analyze each system separately using a valid model for the flow characteristics of that system.

Because of the inherent variability in the collection, handling, and analysis of samples and in the assay method, the presence/absence result for each sample is not perfectly accurate; for example, a sample may incorrectly be classified as negative for total coliforms because the coliform bacteria are injured and therefore difficult to detect with standard assays. Biofilm sloughing can cause a violation of the compliance standard even though contamination of the system has not occurred.

Future rules for monitoring to detect fecal contamination of drinking water will take advantage of advances in biotechnology, biofilm control, mathematical modeling, and risk assessment. Even with modern technology, however, there may be a relatively high likelihood that significant contamination is not discovered quickly by the microbial monitoring system. Communities should consider exploring the feasibility of an epidemiological morbidity monitoring system designed to detect incident cases of waterborne disease before many are exposed. Such epidemiological monitoring would also provide important risk assessment data.

The principles applied in evaluating the Total Coliform Rule can be also be applied when evaluating other regulations for detecting harmful microbes in drinking water.

## ACKNOWLEDGMENT

This work was supported by the Center for Biofilm Engineering at Montana State University, a National Science Foundation supported Engineering Research Center (cooperative agreement EEC-8907039), and by the Center's Industrial Associates. Thanks to an anonymous referee whose suggestions led to improved exposition.

## BIBLIOGRAPHY

1. American Society for Microbiology, *American Society for Microbiology Endorses Safe Drinking Water Act,* unpublished public communication, 1996.
2. R. D. Morris and R. Levin, in E. G. Reichard and G. A. Zapponi, eds., *Assessing and Managing Health Risks from Drinking Water Contamination: Approaches and Applications,* National Water Research Institute, Fountain Valley, Calif., 1995, p. 75.
3. 40 CFR, *Code of Federal Regulations,* U.S. National Archives and Records Administration, Washington, D.C., 1993.
4. *Fed. Reg.* **54,** 27544 (1989).
5. G. W. Lykins, Jr., R. M. Clark, *J. Environ. Eng.* **120,** 783 (1994).
6. P. S. Berger, R. M. Clark, and D. J. Reasoner, in J. Lederberg, ed., *Encyclopedia of Microbiology,* Vol. 4, Academic Press, Inc., San Diego, Calif., 1992, p. 385.
7. *Standard Methods for the Examination of Water and Wastewater,* American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D.C., 1995.
8. A. K. Camper and G. A. McFeters, in A.I. Laskin, ed., *Advances in Applied Microbiology,* Academic Press, Inc., San Diego, Calif., 1983, p. 177.
9. M. J. Domek, M. W. LeChevallier, S. C. Cameron, and G. A. McFeters, *Appl. Environ. Microbiol.* **48,** 289 (1984).
10. M. W. LeChevallier and G. A. McFeters, *J. Amer. Water Works Assoc.* **77,** 81 (1985).

11. Public Health Laboratory Service Water Sub-Committee, *J. Hygiene* **51,** 472 (1953).
12. A. E. McDaniels and R. H. Bordner, *J. Amer. Water Works Assoc.* **75,** 458 (1983).
13. M. W. LeChevallier, *J. AWWA* **82,** 74 (1990).
14. A. K. Camper, in G.G. Geesey, Z. Lewandowski, and H.-C. Flemming, eds., *Biofouling and Biocorrosion in Industrial Water Systems,* Lewis Publishers, Boca Raton, Fla., 1993, p. 91.
15. *Biofilms,* John Wiley & Sons, Inc., New York, 1990.
16. M. W. LeChevallier, C. D. Cawthon, and R. G. Lee, *Appl. Environ. Microbiol.* **54,** 2492 (1988).
17. A. K. Camper, M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters, *Appl. Environ. Microbiol.* **52,** 434 (1986).
18. J. W. Costeron and co-workers, *Ann. Rev. Microbiol.* **41,** 435 (1987).
19. D. S. Herson, B. McGonigle, M. A. Payer, and K. H. Baker, *Appl. Environ. Microbiol.* **53,** 1178 (1987).
20. H. F. Ridgway and B. H. Olson, *Appl. Environ. Microbiol.* **44,** 972 (1982).
21. M. W. LeChevallier, T. M. Babcock, and R. G. Lee, *Appl. Environ. Microbiol.* **53,** 2714 (1987).
22. R. S. Martin and co-workers, *J. Amer. Water Works Assoc.* **74,** 34 (1982).
23. K. J. Reilly and J. S. Kippen, *J. Amer. Water Works Assoc.* **75,** 309 (1983).
24. V. P. Oliveri, A. E. Kakalian, K. W. Bossung, and E. D. Lowther, in R.L. Jolley, R.J. Bull, W.P. Davis, S. Katz, and V.A. Jacobs, eds., *Water Chlorination, Chemistry, Environmental Impact, and Health Effects,* Lewis Publishers, New York, 1985, p. 651.
25. Centers for Disease Control, *Morbid. Mortal. Weekly Rep.* **34,** 142 (1985).
26. L. D. Hudson, J. W. Hankins, and M. Battaglia, *J. Amer. Water Works Assoc.* **75,** 564 (1983).
27. E. J. Bouwer and P. B. Corwe, *J. Amer. Water Works Assoc.* **80,** 82 (1988).
28. P. A. Huck, P. M. Fedorak, and W. A. Anderson, *J. Amer. Water Works Assoc.* **83,** 69 (1991).
29. B. E. Rittman and V. L. Snoeyink, *J. Amer. Water Works Assoc.* **76,** 106 (1984).
30. K. B. Earnhardt, *Proceedings, American Water Works Association Water Quality Technology Conference, Miami Beach, Fla.,* American Water Works Association, Denver, Colo., 1980.
31. E. D. Lowther and R. H. Moser, *Proceedings, American Water Works Association Water Quality Technology Conference, Denver, Colo.,* American Water Works Association, Denver, Colo., 1984, p. 323.
32. D. C. Montgomery, *Introduction to Statistical Quality Control,* John Wiley & Sons, Inc., New York, 1991.
33. N. R. Farnum, *Modern Statistical Quality Control and Improvement,* Duxbury, Belmont, Calif., 1994.
34. W. O. Pipes, in P. Berger and Y. Argaman, eds., *Assessment of Microbiology and Turbidity Standards for Drinking Water* EPA/570/9-83-001, U.S. Government Printing Office, Washington, D.C., 1983, p. 1.
35. M. B. Borup, *J. Amer. Water Works Assoc.* **84,** 66 (1992).
36. *Fed. Reg.* **52,** 42224 (1987).
37. M. A. Hamilton, *Environ. Sci. Technol.* **28,** 1808 (1994).
38. N. J. Jacobs, W. L. Zeiglen, F. C. Reed, T. A. Stukel, and E. W. Rice, *Appl. Environ. Microbiol.* **51,** 1007 (1986).
39. EPA Risk Reduction Engineering Laboratory, *EPANET Users Manual (Order No. PB94-165610AS),* National Technical Information Service, Springfield, Va., 1994.
40. A. K. Camper, W. L. Jones, L. Goodrum, and J. Borkowski, *Factors Limiting Microbial Growth in Distribution Systems: Laboratory and Pilot-Scale Results,* American Water Works Association, Denver, Co., 1996, Chapt. 5.
41. M. W. LeChevallier, C. D. Lowry, and R. G. Lee, *J. Amer. Water Works Assoc.* **82,** 87 (1990).
42. R. J. Bobbie and D. C. White, *Appl. Environ. Microbiol.* **39,** 1212 (1980).
43. S. K. Haack, H. Garchow, D. A. Odelson, L. Forney, and M. J. Klug, *Appl. Environ. Microbiol.* **60,** 2483 (1994).
44. I. Hubner, I. Steinmetz, U. Obst, D. Giebel, and D. Bitter-Suermann, *Appl. Environ. Microbiol.* **58,** 3187 (1992).
45. G. Vesey and co-workers, *J. Appl. Bacteriol.* **75,** 87 (1993).
46. R. L. Tyndall, R. E. J. Hand, R. C. Mann, C. Evans, and R. Jernigan, *Appl. Environ. Microbiol.* **49,** 852 (1985).
47. K. O. Colquhoun, S. Timms, and C. R. Fricker, *J. Appl. Bacteriol.* **79,** 635 (1995).
48. P. Silley, and S. Forsythe, *J. Appl. Bacteriol.* **80,** 233 (1996).
49. A. K. Bej, J. L. DiCesare, L. Haff, and R. M. Atlas, *Appl. Environ. Microbiol.* **57,** 1013 (1991).
50. A. K. Bej, S. C. McCarty, and R. M. Atlas, *Appl. Environ. Microbiol.* **57,** 2429 (1991).
51. K. L. Josephson, C. P. Gerba, and I. L. Pepper, *Appl. Environ. Microbiol.* **59,** 3513 (1993).