

The provisions of the Total Coliform Rule provide the basic mechanism through which all public water systems determine the microbial quality of their water. It is one of the most important rules promulgated as part of the SDWA because it targets commonly occurring microbial contaminants that may cause acute health effects.



This presentation on the Total Coliform Rule addresses the history and development of the TCR and provides a detailed overview of the rule's requirements. It also touches on related PN requirements, SDWIS reporting and Special Primacy Requirements mandated for adoption of the rule by State primacy agencies.

This training session was developed in conjunction with a session titled, "Regulating Microbial Contaminants".



When the NIPDWRs were promulgated in 1975 (following passage of the SDWA in 1974), the provisions were based on 1962 Public Health Service Regulations. For the period from 1975 to 1990, total coliform bacteria were considered to be one of two microbial indicators of water quality – turbidity being the second indicator. Analytical methods used to detect coliforms in drinking water were based on the organism's ability to ferment the sugar lactose under prescribed conditions of incubation time and temperature.

TCR monitoring requirements were relatively simple, with two consecutive daily check samples required for each total coliform-positive sample. If neither of the check samples were positive, the system incurred no violation and additional activity was not required.

The total coliform provisions remained essentially unchanged until the Total Coliform Rule became effective in 1990.



Total Coliform Bacteria are used as indicator organisms. An indicator organism is one that does not typically cause disease but is a good indicator that disease-causing organisms may also be present.

Criteria preferred for an indicator organism are listed. They describe an organism that responds to environmental conditions in a manner similar to the pathogens they represent. The use of indicator organisms is preferred to analyses for individual pathogens because of the large number of discrete test methods that would be required to determine if specific pathogens are present.

Total coliforms meet several of these criteria. However, they are imperfect indictors for the following reasons:

•Total coliform bacteria are adequate indicators of other bacteria and of viruses, but are much less resistant to disinfection practices than the protozoans *Giardia* and *Cryptosporidium*.

•Some species included in the total coliform group are environmental bacteria and their presence does not necessarily mean water was contaminated with sewage or fecal material so they may be present in pathogen-free water.



While the NIPDWRs were in effect, waterborne disease outbreaks occurred in public water systems. Many of these outbreaks were caused by the protozoan *Giardia*, which is more resistant to disinfection than are total coliform bacteria. *Giardia* could therefore be present and viable in finished water with no total coliform bacteria present.

Turbidity standards in place at the time were not adequate to ensure high-quality raw water received filtration treatment adequate for the control of this organism. For example, turbidity standards for a conventional filtration plant required a monthly average of 1 NTU in the finished water and at no time could the finished water turbidity exceed 5 NTU. Turbidity standards have since been lowered by the 1998 IESWTR and 2002 LT1ESWTR. The new LT1ESWTR limits will apply January 14, 2005.

The NIPDWRs did not address Heterotrophic Bacteria (also referred to as heterotrophic plate count or HPC). These bacteria require complex organic compounds of nitrogen and carbon for growth. They are typically harmless organisms, but can substantially interfere with the analytical methods for total coliforms. Intereference means HPC can overwhelm any total coliforms present in a water sample and cause a false-negative test result.

Interference in total coliform test methods occurs because:

- •Growth media used for total coliforms contains complex carbon and nitrogen;
- •HPC organisms can multiply in warm water samples of high organic content; and
- •HPC organisms can multiply rapidly in total coliform growth media.

When the NIPDWRs were in place, if interference was detected a replacement sample was required. Systems could collect replacement samples repeatedly with no violations incurred even though the coliform analysis was unknown.



A significant detail of the proposed Total Coliform Rule was the requirement for all public water systems (including community and non-community systems) to collect a minimum of 5 water samples for coliform analysis each month. Systems serving 300 or fewer persons could reduce to 1/month, and systems serving 301-500 persons could reduce to 3/month if the system filters and disinfects surface water or disinfects ground water and has a sanitary survey.

The minimum number of samples was based on a statistical analysis which indicates that if 60 samples are collected and 95 percent are negative for coliforms, then there is a 95 percent confidence level that the fraction of water with coliforms present is less than 10 percent. (Pipes, 1983). The proposed minimum sampling frequencies were a significant increase in monitoring requirements for small systems.

The final rule was passed on the same date as the Surface Water Treatment Rule. The intent at that time was for the SWTR to address the treatment of surface water sources, the upcoming ground water rule would address disinfection of ground water sources and the total coliform rule would apply to all systems and provide information on their distribution system integrity.



Traditionally, the total coliform group has been defined as described on this slide. These criteria have been in use since the 1920's.

The metallic green sheen on the membrane filter plate is characteristic of coliform growth, although this slide does not show distinct colonies because of the high concentration of coliforms present. The green sheen is caused by a byproduct of lactose fermentation reacting with a chemical added to the growth media.

The test tubes contain growth media (Lauryl Tryptose Broth - LTB) with inverted glass tubes. As the total coliforms ferment lactose they produce carbon dioxide. The gas is trapped in the inverted tube and provides a visible bubble. The change in color from yellow to pink is the indicator of acid production.



Traditionally, fecal coliforms are defined as described. They are incubated at a constant 44.5 degrees C to mimic the body temperature of warm-blooded animals. This is intended to eliminate all but those organisms coming from sewage or a fecal source. Fecal coliforms are a sub-group of total coliforms. *E. coli* is a fecal coliform.

A total coliform-positive water sample may be tested to see if fecal coliforms are also present by transferring some of the total-coliform positive culture to EC broth. The liquid is incubated at the elevated temperature and observed for the production of gas. If gas is produced, the sample is fecal coliform positive. The fecal-coliform organism may be *E. coli*, *Klebsiella* or another type of bacteria.



Because the test tube and membrane filter methods require confirmation steps which may require up to 5 days for a positive test result, the water industry sought more rapid alternative test methods.

Enzyme-based indicators were developed which enabled test results to be obtained within 24-48 hours. These tests rely on the presence of specific enzymes to cause the color reactions. The enzymes play a role in lactose fermentation.

While these methods reduced the time required for analytical results, they also provide a positive result for total coliforms from a wider varie ty of organisms. Some bacteria which were unable to create gas or acid from their lactose fermentation could cause the color reaction with the pertinent enzyme.



*E. coli* is promoted by some as a better indictor of sewage or fecal contamination than are total or fecal coliforms. *E. coli* is known to be commonly found in the intestines of warm blooded animals and is not a general environmental bacteria. Most strains are harmless.

*E. coli* 0157:H7 has been identified as the cause of several waterborne disease outbreaks in recent years. It is especially significant in that it has killed people who became infected through drinking contaminated water.



*E. coli* 0157:H7 is a strain of *Escherichia coli* and, like other *E. coli*, inhabits the intestines of warm blooded animals. Contamination of meat products likely occurs during slaughter or meat processing, and excretion of the bacteria can contaminate raw vegetables and sources of drinking water.

In September, 1999, one of the largest waterborne outbreaks occurred in New York State with over 1,000 people affected, > 60 hospitalized and 2 deaths (a 3-year-old girl and a 79-year old man). The source was identified as water contaminated by cattle manure which seeped into a non-chlorinated well at a County Fair following heavy rains. The well is 20-feet deep and 83 feet from the edge of a barn where cows on exhibit are housed.

In May 2000, a waterborne outbreak sickened 2,300 and killed 6 in Walkerton, Ontario. Heavy rainfall that washed cattle manure into a shallow well is believed to be the source of *E. coli* 0157:H7 and *Campylobacter*.



According to a September, 1999, Journal AWWA Article (Vol 91, No 9: Pgs 101-109), *E. coli* 0157:7 is detected using typical total coliform media. However, it does not grow well at the temperature used for fecal coliform analysis (44.5 C), and does not cause a positive reaction (fluorescence) with UV light using MUG.

The point here being none of the available test methods are ideal and public water systems must rely on sanitary surveys, routine inspections and daily perseverance to prevent contamination of their water systems since reliance on indicator organisms is imperfect.



This slide shows the various *Federal Register* notices which have had fairly significant changes to the original Total Coliform Rule of 1989.

The variance criteria addressed the concerns of some States and water systems that the new rule could be violated by the growth of total coliform organisms in the distribution system, which did not create a public health threat or indicate treatment failure or a sewage or fecal contamination incident at the source.



These sections of the 40 CFR are related to the Total Coliform Rule and are provided as reference. The presentation does not follow this organizational outline.



**"Confluent Growth":** means a continuous bacterial growth covering the entire filtration area of a membrane filter, or a portion thereof, in which bacterial colonies are not discrete.

**"Domestic or other non-distribution system plumbing problem":** means a coliform contamination problem in a public water system with more than one service connection that is limited to the specific service connection from which the coliform-positive sample was taken.

**"Near the first service connection":** means at one of the 20 percent of all service connections in the entire system that are nearest the water supply treatment facility as measured by water transport time within the distribution system.

**"System with a single service connection":** means a system which supplies drinking water to consumers via a single service line.

**"Too numerous to count":** means that the total number of bacterial colonies exceeds 200 on a 47-mm diameter membrane filter used for coliform detection.



There are three violation categories for the Total Coliform Rule. They will be discussed in more detail in later slides.

For determining compliance with the Monthly MCL, a calendar month is used.

If routine samples are collected at the end of one calendar month and any related repeat samples are collected in the following calendar month, compliance with the Monthly MCL is still based on results obtained in each of the two separate months. Therefore, sample collection is recommended well before the end of the month.

Calculating Monthly MCL compliance is based on 5.0 percent of the samples to clarify rounding issues. For example, a system collecting 75 samples per month has 4 total-coliform positive samples. 4/75 = 5.3%, which would be a violation.

Even though a monthly MCL violation is incurred, the system must proceed with all repeat samples to determine whether the system has any Acute MCL violations. An Acute MCL violation may also be incurred and is calculated based on sample results, regardless of the month(s) in which the sample was collected.



Routine and repeat sample cultures which are total coliform positive must be also tested for the presence of fecal coliforms or *E. coli*. This information is used in determining compliance with the Acute MCL.

If a sample result is fecal coliform or E. coli positive, the system must notify the State as follows:

- By the end of the day the system is notified, or
- By the end of the next business day if the State office is closed when the system is notified of the result.



The language of the Total Coliform Rule specifies only two situations where an Acute MCL violation is incurred.

1. A Routine Total Coliform Sample Is Positive; the fecal coliform or *E. coli* test of that sample is positive, and one or more of the three or four repeat samples is total coliform-positive.

2. A Routine Total Coliform Sample Is Positive; the fecal coliform or *E. coli* test of that sample is negative, and one or more of the repeat samples is total coliform-positive and is also fecal coliform or *E. coli* – positive.

Example Acut	e MCL Viol	ation	
	Total Coliform	Fecal Coliform	
Routine	+	-	
Repeat	+	+	
	Total Coliform	Fecal Coliform	
Routine	+	+	
Repeat	+	-	

These two scenarios are the only ones specified in the current Total Coliform Rule as constituting an Acute Violation of the MCL for Total Coliforms. This boils down to the requirement that in a set of routine and related- repeat samples, both the routine and at least one repeat sample must be total coliform-positive <u>and</u> one of the two must also have either fecal coliforms or *E. coli* detected.

If all three of a system's Routine Samples are fecal coliform-positive, but none of the repeat samples are total coliform positive (and therefore not fecal coliform positive either) the system incurs a Monthly MCL violation but *not* an Acute MCL violation.





The final rule lists the items noted as Best Available Technologies for compliance with the TCR.



An **Exemption** is not allowed for the MCL for Total Coliforms. Exemptions are allowed for some contaminants when a disadvantaged system is unable to comply with an MCL or treatment technique or to develop an alternative source of supply. A compliance schedule and public hearings are required, and there cannot be any unreasonable risk to public health caused by operating under the exemption.

A **Variance** is allowed for the TCR under certain conditions. A variance means that because of the raw water quality a system cannot meet an MCL <u>despite</u> using BAT, treatment techniques or other means (taking costs into consideration). There also cannot be an unreasonable risk to health.



Persistent Bacterial Regrowth Variance Guidance Criteria:

In the Past 30 Days...

•The system was free of FC or EC based on at least daily sampling;

•Less than 1 coliform/100 ml influent water in at least 95% of samples taken daily;

•Source water treatment meets turbidity requirements;

-Continuous disinfection residual of 0.2 ppm is maintained;

-No waterborne disease outbreak history in present system configuration;

-System maintains bi-weekly contact with the State and Local health officials to assess illness

possibly attributable to microbial occurrence in water;

•No EC+ compliance sample in last 6 months, unless State convinced it was not an influent water contamination event;

•Sanitary survey within past 12 months;

•State-accepted X-connection control program and system performs an audit of its effectiveness;

•System submits a biofilm control plan;

•Weekly HPC at least at 10% of number of routine coliform sample sites; and,

•Daily monitoring and maintenance of disinfection residual (at least 95% of sites and HPC count of less than 500 colonies/ml at sites without disinfectant residual).



The titles of the sections pertaining to sampling for the TCR.



Within the section on Routine Monitoring, several specific requirements are described.



States must indicate in their Special Primacy Provisions for adoption of the TCR the method by which they will determine whether system sample siting plans are acceptable. The submission must include provision for periodic reviews. It is acceptable for the system to develop and use the plan, place it in its records, and have the State review the plan during sanitary surveys.

The sample siting plan should be designed to use sites representative of conditions within the distribution system. The proposed rule specified the number of sites used over the course of a year should be at least 3-times the number of monthly samples required. Although this provision was not incorporated into the final rule, its intent was to ensure sites are continuously moved around to represent all water, not just that used in high-population or high water- use areas. This proposal was based on a study which showed differences in the frequency of coliform occurrence could be substantial among the different sections of a single distribution system.



Given a system of this size, use the example diagram to indicate where samples might be collected and the issues that should be considered when developing a plan.

- Dead Ends
- Sources
- Tanks
- Known areas of deteriorating water mains
- variable pressure areas
- etc...

A workshop to have participants develop a plan is provided later in the session.

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- 1997	,e.i.e.y		
[	Population	Samples/Month	
	25 - 1,000		
[	1,001 - 2,500		
	2,500 - 3,300		
	3,301 - 4,100		
	4,101 - 4,900		
	4,901 - 5,800		
	>3,960,000	480	
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Note there are a total of 34 population categories.

The population served by a system is determined by the State, although there is federal guidance available on how it could be determined. This guidance is found in Section 66A of the January 2000 "Water Supply Guidance Manual".

www.epa.gov/safewater/wsg/wsg\_66a.pdf



Routine monitoring frequencies may be reduced to no less that one sample per quarter at State discretion.

States must document these decisions and several criteria must be met by the system.

The system may not have had a history of coliform contamination of treated water, and must have had a sanitary survey within the previous 5 years which showed the source is a protected ground water source (as described in special Primacy Provisions) and the system is free of sanitary defects.



Prior to this rule some States did not have NCWS monitoring for coliforms at all and some were less than one sample per year.



This provision was intended to capture seasonal systems serving significant populations on a periodic basis such as ski areas and campgrounds. During off-season months, the State may reduce the sample frequency for ground water systems to account for the decrease in population served.



Because of the acute health threats inherent to using a surface water source, the TCR requires these systems to monitor the same as a community water system.



In an effort to provide a more accurate picture of the quality of water throughout a month, water samples must be collected at regular time intervals (such as 2 per week for the first 4 weeks of a month).

Smaller systems are allowed to sample all on one day to accommodate their reduced staff availability and resources.

All systems must sample according to their written sample siting plan – whether community or non-community systems.



This provision only applies to surface water systems or GWUDI systems meeting filtration avoidance criteria.

The photo is of a distribution system sample tap. They are used for routine sample locations, not just for this application at unfiltered surface supplies. Many systems prefer to use them as they remove any in-home plumbing or treatment system affects on the quality of the water.



Water systems may submit samples for coliform analysis that would not be included in Monthly or Acute MCL violation calculations. These are special purpose samples and are limited to those collected from water which is not reaching customers.

Examples are samples taken from new or repaired water mains to check the quality of the water <u>before</u> the water in the main is served to the public, or source water samples if the water is treated after the point at which the special sample is collected.



This slide outlines the provisions addressing Repeat Samples.



The number of repeat samples required attempts to bring the system to the minimum of 60 samples collected in a year (five per month for 12 months) per the statistical analysis described under the proposed TCR.

Systems are encouraged to collect Routine Samples early in the calendar month to allow repeat or replacement sample collection within the same month. Laboratory workloads should be considered to avoid over- and under- scheduling sample analyses.

Systems may need to have a set of repeat sample containers on hand to enable compliance with the 24-hour time limit for repeat sample collection.

Notification of systems of the results of their routine sample analysis may be by telephone, internet or through the mail. The time lag between obtaining the results and notification of the system may therefore range from a few hours for a phone call to several days before the mail is received and opened.



Repeat sample locations are specified in the rule and the repeat sample locations should be specified on the sample siting plan.



Real World implementation issues faced by regulators include the following:

•Systems with typically satisfactory sample results may view a unsatisfactory result as due to sample collection error. System operators should be trained to conclude that any positive sample warrants a complete review of their system's sanitary integrity. Sporadic, low levels of contamination are frequently encountered and may reflect a serious public health threat.



While routine samples may be required to be collected throughout each calendar month, repeat samples for all systems with more than one service connection must be collected on the same day.

If samples are collected over a 4-day period, each sample must still be delivered to the laboratory in enough time to allow analysis to begin within 30 hours of sample collection.



Systems must continue to sample until either an MCL violation is triggered or a complete set of total coliform-negative repeat samples is obtained.

Even if the Monthly MCL is triggered based on routine samples alone, repeat samples must be collected for each of the positive routine samples. This is required so that compliance with the Acute MCL can be determined.



Criteria included in the rule through which a State may waive the requirement that 5 Routine samples be collected the next month:

•State, or agent approved by State, performs a site visit before the end of the next month that determines whether additional monitoring and/or any corrective action is needed.

•State has determined why the sample was total-coliform positive and established that the problem has been corrected or will be corrected before the end of the next month the system serves water to the public.

•State must document in writing

The decision to waive the 5 routines

Causes of the TC+ sample

Corrective actions taken by the system

•The document is signed by the supervisor of the State official making the finding.

•The documentation is made available to EPA and the Public.

The System must take at least one routine sample before the end of the next month it serves water to the pubic unless:

•The State determined the system corrected the problem before it took the samples, and

•All repeat samples were TC-

# Routine Samples/Month	# Repeat Samples	# Routine Samples Next Month
1 or Fewer	4	5
2, 3 or 4	3	5
5 or More	3	Per Routine Requirements

Again, requiring 5 samples the next month is based on the argument that if 60 samples are collected in a year a 95 percent confidence is obtained that all but 10 percent of the water is satisfactory if all of the samples are negative.



This slides shows the significance of a single positive routine sample on a small system, even if all repeat samples are total coliform negative. The total cost is an additional ~\$160 in laboratory fees, excluding labor or shipping costs.



The rule includes a provision that a routine sample can be used as a repeat sample if certain criteria are met as follows:

•If the System collects a Routine Sample(s) before learning the results of the analysis of an initial routine sample collected previously and in the same month, and

•The results of the initial routine samples are TC+, and

•The routine samples were collected within 5 adjacent service connections of the initial sample.

•The system must still collect the required number of routine samples in the month.

Sample designations may become confusing for data entry and system personnel. On the slide an example is provided where information submitted on the sample form to the laboratory may indicate R for Routine and 1 for sample siting plan location 1. UR and DR apply to upstream and downstream locations, respectively.



The preamble to the final TCR specifies that a set of total coliform-negative repeat samples is not sufficient to invalidate an initial routine sample result.

HPC Interference may be identified by the lab in any of the following ways:

- Multiple Tube Fermentation: Turbid Culture in Absence of Gas

- Turbid Culture in Absence of an Acid Reaction in Presence/Absence Test

-Confluent Growth or a Colony Number that is too numerous to count using membrane filtration techniques

### AND

- No coliforms are detected

If a replacement sample is required it must be collected from the same location within 24 hours of notification of the result.

The laboratory may analyze the replacement sample with a different test method t han was used for the original sample. The presence/absence tube fermentation techniques are less sensitive to HPC interference than are membrane filter methods.



The final rule prescribed a time period by which initial sanitary surveys must be completed.

The requirement that sanitary surveys be performed for systems collecting fewer than 5 samples per month targeted limited resources on those systems doing less coliform monitoring.

Note: The IESWTR requires the State to conduct sanitary surveys for all surface water and GWUDI systems no less frequently than every 3 years for community systems and no less frequently that every 5 years for non-community systems.





Using this diagram and the instructions on the following slide, workshop participants are asked to develop and explain a sample siting plan for this example system.



Have each group label and explain their plan on a flip chart at the front of the class.





The system was an untreated ground water supply. Water samples were collected at the Superintendent's home. The well pumped from  $\sim$ 6:00-7:00 am until noon, at which time the tanks were full and the system used stored water for the rest of the day.

On Thursday, a sample was FC+. On Friday, repeat samples were TC-The next Monday, samples were FC+ The next Tuesday, samples were FC+

Children at the school had cramps and bloody diarrhea; 7 Nursing home residents died.

A model of the system and investigation determined the water served by the wells was routinely sampled as TCR samples were collected before noon. Water serving the system from the tanks was contaminated by *E. Coli*.

Samples would only be + if they were collected at a time and location representative of water from the tanks.

Sample siting plans should incorporate time of day, source of water, water use and other considerations into their design.



The video on microbiological test methods is not a training video on sterile technique. It is intended to provide an overview of methods and the significance of test results.



40CFR 141.21 includes all approved test methods. The methods included in the rule range from the traditional membrane filtration or test tube methods to the newer enzyme-based methods. Ultimately, the test method approved for use by the State in their certification of analytical laboratories will determine options available to water systems.



## Left:

•Lauryl Tryptose Broth

•Initial step in Presence/Absence test for Total Coliforms; verification step for green-sheen colonies on membrane filters.

•Gas indicated by bubble of carbon dioxide; acid indicated by color change from yellow to red.

# Center:

- •Brilliant Green Lactose Broth
- •Confirms total coliforms in Lauryl Tryptose Broth
- •Look for gas production in up to 48 hours

### **Right:**

- •E.C. Broth
- •Incubate in water bath at 44.5 degrees C for 24 hours
- •Gas formation indicates fecal coliform-positive.



# **Colilert (left):**

- •Incubated 35 degrees C for 24-28 hours
- •MMO-MUG based method
- •Yellow = Total Coliform-Positive
- •Fluoresce under UV = *E. coli* positive

# Center:

•*E. coli*-positive tests showing positive fluorescence

# **Colisure (Right):**

- •Incubated 35 degrees C for 24-48 hours
- •Yellow = media with water sample added
- •Red = Total Coliform-Positive
- •Fluoresce under UV = *E*. *coli* positive



#### Top:

Fecal coliform membrane filtration method Fecal coliform colonies are blue

#### **Bottom:**

Total coliform membrane filtration method Total coliform colonies have a green metallic sheen



The test method chosen will affect how quickly positive test results are obtained. While negative test results are all obtained within 48 hours, and some in 24 hours, confirmation steps required for total coliform membrane filtration analysis may extend the time delay to 4 days.



Although it is the system's responsibility to report violations to the State within 48 hours, systems must notify states of a fecal coliform or *E. coli*-positive test result (even if no violation is incurred) within 24 hours of being notified of the test result.



This session only touches on the requirements for public notification.

Speakers are encouraged to distribute the fact sheet prepared for the PN rule to provide a more clear reference to the Tiers and minimum notification requirements.

Speakers should also be cognizant of the revised PN rule and the time period on which they become effective for all systems.









Records required to be kept by States are specified. The content of the record (signature required, etc) are specified in the rule for each item.





For SDWIS-FED data records, the type of violations are specified. For SDWIS reporting, violations are categorized as described on the next slide.





Special primacy requirements are those requirements a State must meet to be granted primary enforcement authority for a rule. Although most States adopted the TCR over 10 years ago, reviewing the primacy application and related special primacy requirement responses provides insight into the methods the State indicated they would use to implement the rule.



Responses to special primacy requirements are only required for those discretionary provisions the State chooses to adopt.

