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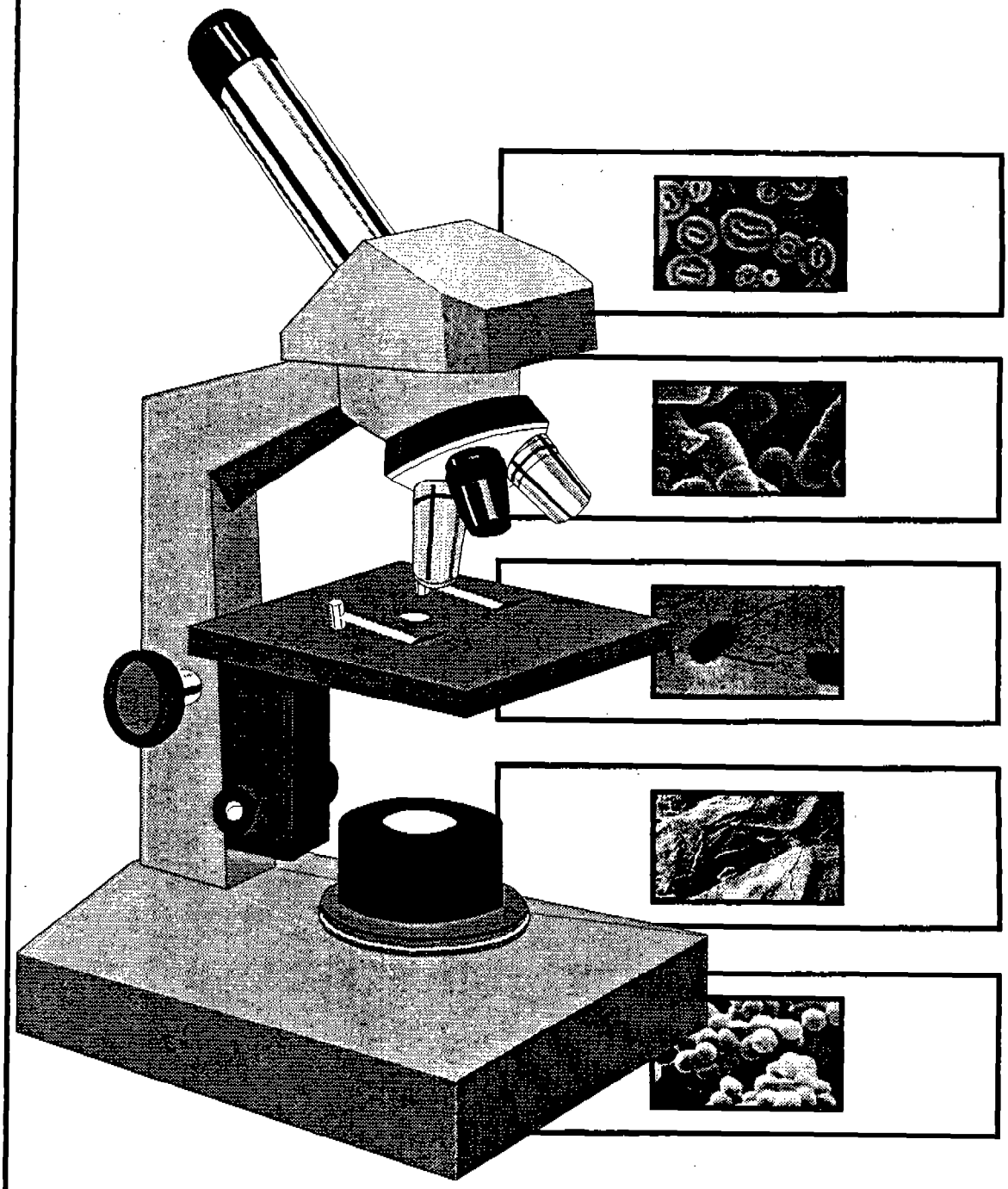
BACTERIOLOGICAL SURVEILLANCE OF DRINKING WATER

USACHPPM TECHNICAL GUIDE NO. 224



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BACTERIOLOGICAL SURVEILLANCE OF DRINKING WATER

(USACHPPM TECHNICAL GUIDE NO. 224)

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BACTERIOLOGICAL SURVEILLANCE OF DRINKING WATER
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CHAPTER 1
INTRODUCTION

1-1. Purpose

The purpose of this technical guide (TG) is to provide a general overview of current regulatory requirements and helpful guidance regarding the bacteriological surveillance and evaluation of drinking water quality. A separate information paper will address microbiological contaminants of a nonbacterial nature (e.g., *Cryptosporidium, Giardia lamblia*, and viruses).

1-2. References

Appendix A contains a list of references. Copies of U.S. Environmental Protection Agency (EPA) documents are available by contacting the Safe Drinking Water Hotline at 1-800-426-4791.

1-3. Abbreviations and Terms

The glossary found in Appendix B contains the abbreviations and definitions of key terms used in this TG.

1-4. Technical Assistance

Additional assistance regarding drinking water issues may be obtained from the Water Supply Management Program of the USACHPPM at DSN 584-3919 or commercial (410) 671-3919.

1-5. Preparation

This technical guide combines and supersedes Information Paper No. 17, "Bacteriological Surveillance of Water," written by Mr. John Brokaw, August 1989, and Information Paper No. 38, "Response Guidance for Microbiological Contamination of Potable Water Systems," written by MAJ James Evenden, 1 November 1983.

> Use of trademarked names does not imply endorsement by the U.S. Army, but is intended only to assist in identification of a specific product.

CHAPTER 2
GENERAL INFORMATION

2-1. Development of Bacteriological Criteria

a. The control of biological pathogens is one of the most significant drinking water quality goals. Disinfection using chlorine is one principal method of water treatment used to ensure the biological safety of water. However, despite many improvements in disinfection and other types of water treatment, outbreaks of waterborne disease still occur. Major causes of outbreaks in community water systems include microbiological contamination of the distribution system, and the existence of treatment deficiencies, such as inadequate filtration and interruption of disinfection (reference 1).

b. The direct measurement of many species of pathogenic organisms, often associated with fecal contamination, is extremely difficult. The density of these organisms is usually very low (even in a badly polluted water supply) and the analytical techniques used in their determination are difficult. For these reasons, indicator organisms are used to determine the presence of fecal contamination in a water supply. The most common organisms used as indicators of possible water contamination are bacteria in the coliform group of the family Enterobacteriaceae. *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, and *Enterobacter cloacae* are examples of the gram-negative, lactose-fermenting, rod-shaped bacteria. In actuality, the assay for total coliforms is designed to detect the presence of *E. coli*, which constitutes the greatest portion of the normal intestinal flora of humans and other warmblooded animals. Constant monitoring of the water supply, using methods designed primarily to detect the presence of *E. coli*, can therefore warn of dangerous levels of human pathogens.

c. Since 1880, the criterion for determining the microbiological quality of drinking water has been its coliform content. Current EPA regulations specify the method and frequency of sampling, the analytical methods of analysis, and the maximum allowable coliform content (reference 2). Standard Methods for the Examination of Water and Wastewater describes these procedures in detail (reference 3).

d. Federal or state regulatory authorities establish drinking water standards with consideration toward economically feasible treatment methods and a margin of safety to the consumer. It has been reported repeatedly in the literature that the presence of any type of coliform organism in drinking water is undesirable. The bacterial standard for drinking water now uses a determination of coliform presence or absence (i.e., a quantification of coliform level is no longer required).

2-2. Significance of Coliforms in Water Systems

a. The significance of the various coliform organisms in water is a subject of considerable study. All types of coliform organisms may occur in feces. Although *E. coli* nearly always will be found in fresh pollution derived from warmblooded animals, other coliform organisms may be found in fresh pollution in the absence of *E. coli* (such as *Enterobacter, Klebsiella, Citrobacter*, and *Escherichia*). The presence of *Enterobacter* and *Klebsiella* often indicates contamination from environmental sources. Dirt, grain, and plants may all potentially harbor *Klebsiella* (reference 3).

b. Another area of much interest and research is growth of bacteria, particularly coliforms, in distribution systems. Regrowth organisms are those cells, and their successor cells, that originated in the source water, passed through treatment processes that may have caused injury to the cells, and recovered viability in the distribution system. Aftergrowth describes contamination of the distribution water by bacteria detached from the system piping and not from the source water. Thus, water leaving the treatment plant organism-free will show increases in populations with passage through the distribution system (reference 4).

c. Biofilm refers to microbial cells immobilized on a pipe surface or on a particle (suspended in water or stationary within a filter). Typically, biofilm colonies form at rough surfaces (reference 5). Biofilms are significant since they are capable of harboring pathogenic organisms.

(1) Biofilm consists mainly of bacteria; heterotrophic bacteria are the most common. Their source, as well as the risk to public health, is unknown. Total coliforms may also be present, possibly indicating the presence of enteric pathogens. Fecal coliforms signify fresh fecal contamination and may be present in biofilms. To a smaller extent, pigmented bacteria and Actinomycetes, fungi, protozoa, and other invertebrates can be found.

(2) Certain environmental factors play a role in biofilm development. Water temperature plays a large part, affecting all factors related to microbial growth, such as rate of growth, seasonal variances in growth, and lag time (the length of time before cell division starts in a system). Hydraulic effects (i.e., flow velocity, flow reversal, and stagnation) also influence microbial growth, as well as low disinfectant levels, corrosion of distribution pipes, and sediment accumulation. The amount of nutrients available, especially carbon, nitrogen, and phosphorus, is important in limiting growth. Of these three nutrients, carbon is needed in the largest amount. When assimilable organic carbon (AOC) levels reach 50μg/L and above, bacterial growth will develop (reference 6). Maintaining AOC levels below 10 μg/L has been found to inhibit growth (reference 7).

(3) The best way to avoid biofilm problems is to create and follow a plan for limiting their growth. However, if a coliform biofilm problem surfaces, measures can be taken to control it.

(a) Increasing disinfection residuals is one means of controlling biofilm. A disinfectant must be selected carefully. It has to be able to neutralize microbial growth effectively, and stable enough to remain in the distribution system. Approximately 3 to 6 mg/L (milligrams per liter) of free chlorine residual in distribution systems has been effective in controlling biofilm growth (reference 5).

(b) Corrosion control is another means for reducing biofilm populations. This can be achieved by adjusting the pH and alkalinity and/or using chemical inhibitors. With decreased corrosion of pipes, there are fewer places for biofilm bacteria to attach and multiply.

d. Many water systems reporting coliform occurrences in their distribution networks have determined that the predominant organism was a member of the *Klebsiella* genus. *Klebsiella* can normally be controlled through adequate disinfection. If passage into the distribution system occurs, however, the bacteria may be protected by particulate matter and porous pipe sediments. The organism also can produce a capsular, slime coating under adverse conditions to protect itself against disinfectants. These mechanisms can lead to establishment of the organism in the system. This may result in periodic sloughing of cells into flowing water.

2-3. Problems in the Analysis of Coliforms

a. Coliforms meet many of the criteria for an ideal indicator organism; however, there are some deficiencies. These include atypical lactose reactions and suppression of coliforms by high populations of other organisms (especially when no free residual chlorine is present). False positives (*Aeromonas*) and false negatives from strains that are unable to ferment lactose, and the fact that coliforms do not represent a homogeneous group are further shortcomings (reference 8).

b. Problems may arise when collecting tap samples. Proper technique in collection is important. There may be some variability due to flushing the tap, contamination of the tap, sloughing of biofilm, or collector error.

c. Coliforms exposed to chlorine but not killed are defined as injured, and may require special treatment. Because coliform bacteria not killed by chlorine may not react typically, samples should be dechlorinated by the addition of sodium thiosulfate.

d. The instructions in Standard Methods (reference 3) regarding equipment and analytical procedures are very specific and detailed. It is important that they be followed carefully. A

slight variation in technique may have very significant effects on the results. Even when all possible care is taken, bacteria sometimes do not cooperate. When the membrane filter (MF) procedure is used coliforms often produce colonies that do not have the typical appearance. An experienced analyst and confirmation tests may be required to distinguish true coliform colonies.

2-4. Other Indicator Organisms

a. Microbiological examinations of potable waters are usually conducted to determine either the presence or absence of the coliform group, and the total number of bacteria present per milliliter of sample. In addition to the procedures for total coliforms and total plate count, there are methods that more specifically identify the origin of bacteriological contamination. Fecal coliform and fecal streptococci procedures are two commonly used analytical methodologies for this purpose. These testing procedures are recommended for drinking water when more generalized testing yields positive results (reference 9). Because of organism survival characteristics, it is not recommended to use only the fecal streptococci procedure when investigating or determining water quality. Other fecal indicators (fecal coliforms and total coliforms) should be used concurrently (reference 9). Descriptions of the tests are provided below and the complete methodologies may be found in Standard Methods (reference 3).

b. Because of certain limitations of the coliform group as a general indicator of drinking water quality, research studies have continually searched for a better indicator than the coliform group. Although no other single group of organisms has been found to be a satisfactory replacement for total coliform, mentioning briefly the importance of these other indicator groups is pertinent:

(1) Fecal coliforms (defined as those organisms that develop on media incubated at 44.5 °C) have frequently been used in stream and lake pollution work, but are not suitable as the only indicator of drinking water quality. The number of fecal coliforms is considerably lower in source waters than total coliforms, making the test less sensitive (reference 8). Testing drinking water only for fecal coliforms could miss contamination of a nonfecal nature.

(2) Fecal streptococci (defined as those organisms able to grow on medium containing sodium azide) have been used in water pollution work, but have not proven suitable for drinking water analysis because of low recovery rates, poor agreements between various methods, and uncertainty as to their significance in water.

(3) Several other organisms have been suggested as indicators, but have found even less acceptance: *Clostridium perfringens, Bifidobacterium, Pseudomonas, Staphylococcus* (reference 8). *Clostridium perfringens* may be the most promising indicator of fecal contamination, according to new research from the American Water Works Association. It is

more stable and can sustain itself longer than fecal coliforms in water (reference 10). Smaller water utilities can adopt the procedure for detecting *Clostridium* easily since the MF technique can be used. Further research may ultimately lead to increased use of *Clostridium* as an indicator.

c. Detection and estimation of the coliform group of bacteria has occasionally required specific identification of species to determine the exact nature of the pollution source. This is because not all strains taxonomically assigned to the coliform group conform to the coliform definition used (i.e., they may not ferment lactose; or if they do, they may not produce gas). Further, not all lactose-fermenting or sheen-producing, gram-negative rods found in water are coliforms (they may be *Aeromonas*), and not all strains of a given species will react uniformly in a given substrate (reference 8).

(1) The traditional IMViC (indol, methyl red, Voges-Proskauer, and citrate utilization) tests are useful for coliform differentiation, but do not provide for complete identification. Additional biochemical tests may be necessary.

(2) Commercial kits for identification are available and are viable alternatives to conventional media. Using these commercial materials reduces many required quality control activities. Prepackaged kits are simple to store and use, and give reproducible and accurate results.

2-5. Suitability of Coliform Standard

a. Ultimately, the testing for coliforms is still the most reliable indicator of the possible presence of fecal contamination and pathogens in water. The present coliform standards appear adequate to protect public health against outbreaks of bacterial diseases when raw water is obtained from a protected source, appropriately treated, and distributed in a contamination-free system.

b. Since many water systems are deficient in one or more of these areas, frequent microbiological sampling of the water system is by far the most critical aspect in water production and distribution to consumers (reference 5).

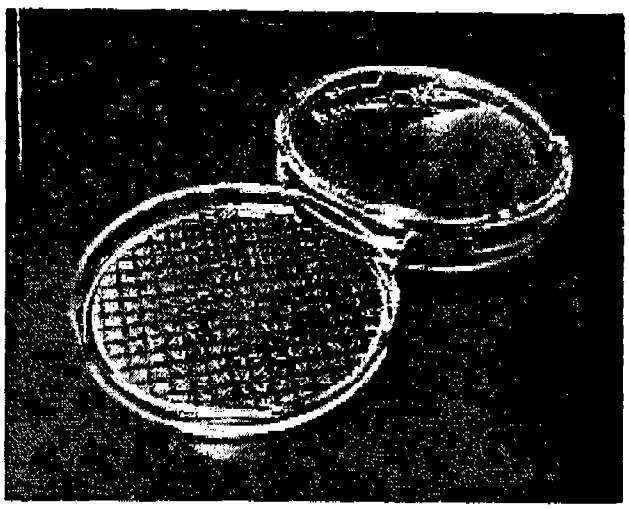
CHAPTER 3
CURRENT TOTAL COLIFORM REGULATIONS

3-1. Monitoring Regulations

Regulations for microbiological monitoring of drinking water, known as the Total Coliform Rule (TCR), are part of the National Primary Drinking Water Regulations (NPDWR) (reference 2) . Presently, regulatory compliance from a microbiological standpoint is based on the absence of total coliforms in the water. The NPDWR contains subparts specifying maximum contaminant levels (MCLs), monitoring and analytical requirements, and reporting and public notification requirements.

3-2. Total Coliform MCL Goal and MCL

a. The MCL goal (MCLG) was established at zero, the level at which there would be no anticipated adverse health impact, allowing for a margin of safety. It is not an enforceable level. The presence of total coliforms in drinking water indicates the potential presence of fecal pathogens. Therefore, the EPA feels the appropriate MCLG is zero.



Coliform colonies

b. There are several reasons why the MCL is based on the presence or absence of total coliforms in the sample, rather than an estimate of coliform density. Data in the literature does not demonstrate a quantitative relationship between coliform densities and either pathogenic density or potential for a waterborne disease outbreak. In addition, it is easier to determine the presence or absence of coliforms, sample transit time is less critical, and calculation of densities is eliminated (e.g., the multiple tube fermentation test uses probability tables to estimate coliform quantities).

c. Determination of whether the MCL is exceeded is based on the number of samples tested and the number found positive during the month.

(1) If fewer than 40 samples/month are analyzed within a system, no more than one sample/month can be coliform-positive.

(2) If at least 40 samples/month are analyzed within a system, no more than 5.0 percent of the samples can be coliform-positive. Notice that 5.0 percent is taken to one decimal place to determine the MCL.

3-3. Monitoring for Total Coliforms

a. The frequency that a public drinking water system must monitor for total coliforms is based on the population served, as shown in Tables 1 and 2.

b. Systems using unfiltered surface water, regardless of population served, must collect and analyze one coliform sample from near the first service connection each day the turbidity of the source water exceeds 1 nephelometric turbidity unit (NTU).

c. The current regulation states that "...samples will be taken at points which are representative of the conditions within the distribution system" [40 CFR 141.21(a)]. This suggests, but does not specify, sampling throughout the water distribution system. The EPA intended in its proposal that sampling sites be varied over a year in this manner, so that the use of the same sites is confined to a short period of time (e.g., 1 or 2 months). In addition, new sampling sites would be selected every sampling plan. The sampling plan should be designed so that there is no place in the distribution system where microbiological contamination could persist indefinitely with little chance of detection. Larger systems tend to maintain the same sample sites in order to build historical records on coliform incidence. These sites are often selected as key locations within the distribution systems. The intention was that all isolated sections of the distribution system would be sampled periodically. The state has the final authority to approve the system.

TABLE 1. COLIFORM MONITORING REQUIREMENTS SYSTEMS SERVING POPULATION < 4900

System size	# Samples	# Repeats	More monitoring for
NCWS†	quarterly	4	5/mo for 1 addl mo
25-1000	monthly*	4	5/mo for 1 addl mo
1001-2500	2/mo	3	5/mo for 1 addl mo
2501-3300	3/mo	3	5/mo for 1 addl mo
3301-4100	4/mo	3	5/mo for 1 addl mo
4101-4900	5/mo	3	NA
>4900	Table 2	3	NA

†non-community water system, * for exceptions see Table 2

TABLE 2. COLIFORM MONITORING FREQUENCY BY POPULATION SERVED

Population Served	Minimum number samples per month*
25 - 1,000	1**
1,001 - 2,500	2
2,501 - 3,300	3
3,301 - 4,100	4
4,101 - 4,900	5
4,901 - 5,800	6
5,801 - 6,700	7
6,701 - 7,600	8
7,601 - 8,500	9
8,501 - 12,900	10
12,901 - 17,200	15
17,201 - 21,500	20
21,501 - 25,000	25
25,001 - 33,000	30
33,001 - 41,000	40
41,001 - 50,000	50
50,001 - 59,000	60
59,001 - 70,000	70
70,001 - 83,000	80
83,001 - 96,000	90
96,001 - 130,000	100
130,001 - 220,000	120

Listing abbreviated based on average effective population of most military installations.

* A NCWS will monitor in each calendar quarter during which the system provides water to the public. If such a system uses unfiltered surface water, it must sample at the same frequency as a community water system, except that in no case will it be reduced to less than once per month.

** Based on a history of no coliform contamination and on a sanitary survey every 5 years by the state showing the water system to be supplied solely by a protected ground water source and free of sanitary defects, a community public water system serving 25 to 1,000 persons may reduce this sampling frequency with the written permission of the state, except that in no case will it be reduced to less than once per quarter.

3-4. Response to a Coliform-Positive Sample

a. The EPA will require a set of repeat samples for every positive coliform sample, to evaluate the significance of a coliform-positive occurrence. The number of repeat samples required is scaled according to how many are normally collected each month (see Table 1). The EPA believes a set of repeat samples is appropriate because it would allow the system to determine quickly whether a serious contamination problem exists, and whether an MCL has been exceeded. Negative repeat samples would give some assurance that the contamination is not extensive or has been eliminated. Moreover, negative repeat samples provide confidence to a small system that a much smaller percentage of its samples are coliform-positive. Coliform-positive repeat samples would indicate a serious continuing problem. See the enclosed flowchart (Figure 1) for the complete repeat monitoring procedure.

(1) At least one repeat sample must be from the same tap as the original positive, and the others must be within five service connections of the original to include an upstream and a downstream tap. Repeat samples must be collected within 24 hours after notification of the original positive result. If any repeat samples are total coliform-positive, additional repeat samples are required until an MCL has been violated and the state has been notified. Continued monitoring will be accomplished at the state's discretion.

(2) Water systems that normally test four or fewer routine samples have a special requirement following a coliform positive sample. When total coliforms are detected in any original or repeat samples, and the sample is not invalidated by the state, the system must collect a minimum of five routine samples the next month the system is in operation. This is in addition to the repeat sampling requirement noted in paragraph 3-4.a.(1).

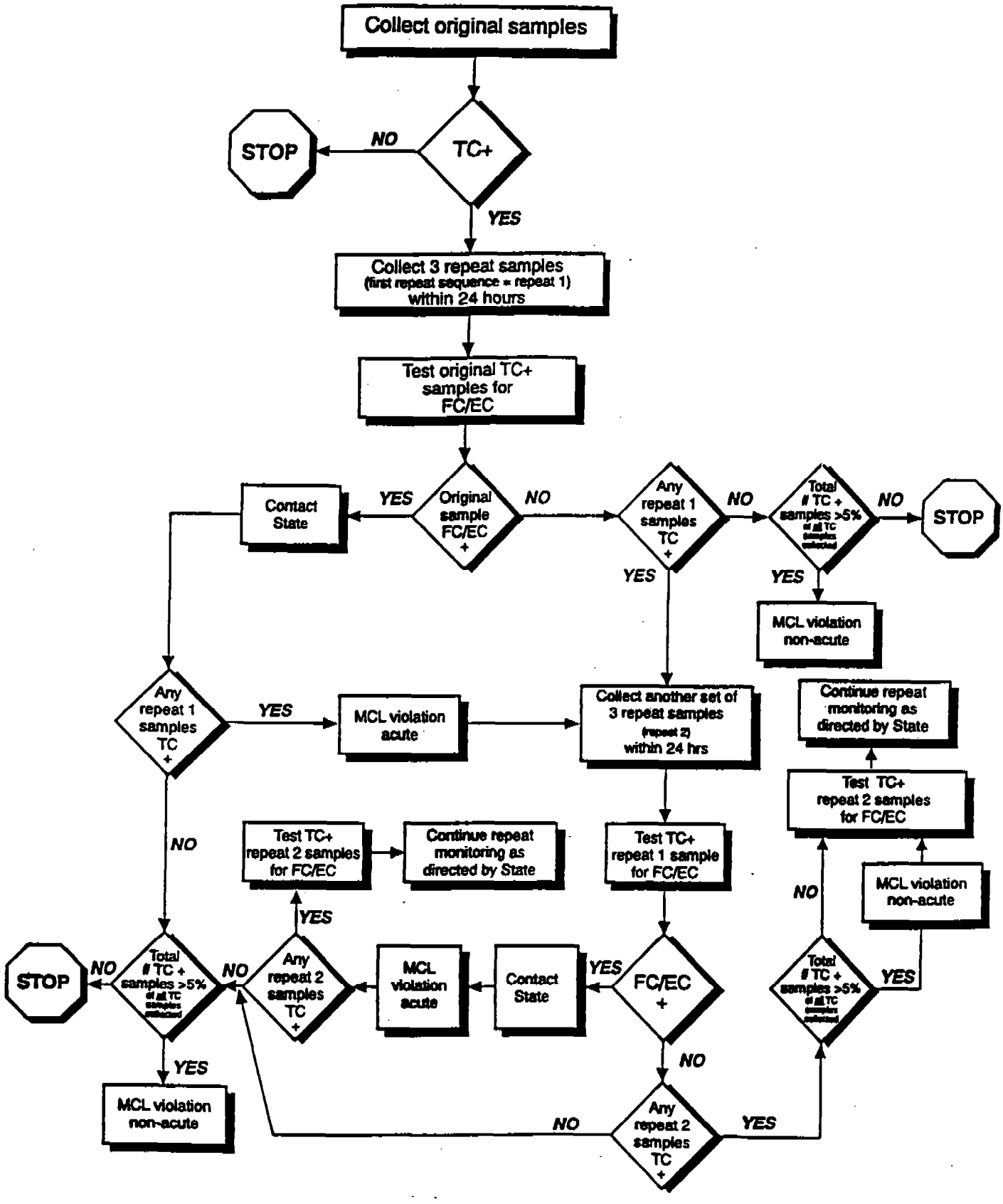
(3) Invalidation of a sample by the state may occur if the laboratory acknowledges analytical error or written documentation reflects that the coliform-positive result was due to a local circumstance, and not an indication of distribution system quality. Samples that are invalidated do not count toward the minimum monitoring frequency.

b. The flowchart in Figure 1 describes repeat monitoring, type of tests, and reporting that occurs following coliform positives. Systems must report a violation of the total coliform MCL no later than the close of the next business day after learning of the violation.

c. Total coliform-positive samples must be analyzed for either fecal coliforms or *E. coli*. If a total coliform-positive sample is also shown to be fecal coliform or *E. coli*-positive, the system must notify the state no later than the end of the next business day.

FIGURE 1.

Total Coliform Monitoring Flowchart



KEY: TC - total coliform; + - positive; FC/EC - fecal coliform or e. coli; # - number

NOTE: When an MCL is violated, the State must be contacted and public notification provided. Acute violations require public notification within 72 hours by electronic media. Non-acute violations require public notification within 14 days.

(1) A fecal coliform or *E. coli*-positive repeat sample, or a fecal coliform or *E. coli*-positive original sample followed by a total coliform-positive repeat, is considered an acute violation of the MCL for total coliforms. This occurrence would require public notification by electronic media within 72 hours.

(2) Invalidation of a total coliform-positive sample also invalidates any subsequent fecal testing on the same sample.

3-5. Heterotrophic Bacteria Interference

a. The EPA is concerned that heterotrophic bacteria, if present in the distribution system, could interfere with the analysis for total coliforms. Therefore, the analyst will visually inspect the coliform media to determine if interference has occurred. A sample is considered invalid if any of the following three conditions are present: the sample produces a turbid culture in the absence of gas production using the multiple tube fermentation method; it produces a turbid culture in the absence of an acid reaction with the P-A test; it produces either a confluent growth or a colony number that is "too numerous to count" using the MF procedure (unless total coliforms are detected). The water purveyor must, within 24 hours of receiving the result, collect another sample from the same location as the original and analyze for total coliforms. The EPA recommends using media less prone to interference by the heterotrophic bacteria in such cases. One such media is the ONPG-MUG, as discussed in chapter 5, paragraph 5-3.

b. The second sample should be counted toward total coliform compliance calculations unless it, too, demonstrates interference by the heterotrophic bacteria. The system must continue to resample within 24 hours until a valid result is obtained.

3-6. Sanitary Survey Requirements

a. States must determine the vulnerability of systems to coliform contamination as a condition for reduced monitoring. The TCR requires that the state, or an agent acceptable to the state, conduct sanitary surveys and the state determine whether the results are satisfactory. Guidance for conducting a sanitary survey is provided in the EPA's Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources (reference 11).

b. All systems collecting fewer than 5 samples per month must have periodic sanitary surveys as shown in Table 3. Community water systems should have completed an initial survey by 29 June 1994 and noncommunity systems by 29 June 1999. As stated in the Surface Water Treatment Rule (reference 12), systems using unfiltered surface water must perform an annual on-site inspection [40 CFR 141.71(b)]. However, the on-site inspection is not equivalent to the

sanitary survey. Therefore, additional criteria may need to be met to satisfy the coliform rule requirements.

TABLE 3. FREQUENCY OF SANITARY SURVEYS SYSTEMS COLLECTING FEWER THAN 5 SAMPLES PER MONTH

Type System	Sanitary Survey within (yrs) Initial	Subsequent
Community water systems		
Filtered surface water	5	5
Unfiltered surface water	*	3/5*
Undisinfected ground water	5	5
Disinfected ground water	5	5
Nontransient noncommunity system	5	5
Other noncommunity systems		
Filtered surface water	10	5
Unfiltered surface water	*	3/5*
Undisinfected ground water	10	5
Disinfected ground water	10	10

* Sanitary surveys will be performed at a frequency specified by 40 CFR 141, Subpart H.

3-7. Analytical Methodology for Total Coliforms

a. The current EPA regulations (reference 10) specify the use of either the 10-tube multiple tube fermentation (MTF) test, the membrane filter (MF) test, the presence-absence (P-A) test using a standard 100 mL (milliliter) volume of sample, or the ONPG-MUG test to test for the presence of coliforms in drinking water (see chapter 5, Standard Methods Test Procedures).

b. The protocol for each of these tests is provided in the 19th edition of Standard Methods, Parts 9221 A, B, and C (pp. 9.44-p.50); 9222 A, B, and C (pp. 9.53-9.59); 9221 D (pp. 9.50-9.51); and 9223 (pp.9.64-9.66), respectively.

3-8. Reporting, Public Notification, and Record Maintenance

a. The current requirements for reporting, public notification, and record maintenance are in 40 CFR 141.31, 141.32, and 141.33, respectively.

b. The final TCR requires that the state be notified of a violation of an MCL by the end of the next business day after the violation is discovered. Also, if a coliform monitoring requirement (e.g., a sample siting plan or collection of required repeat samples) is violated, the system must notify the state within 10 days.

c. The public notification regulations require that notices of MCL violations include a description of adverse health effects. The presence of fecal coliforms/*E. coli* in treated water is cause for grave concern and may pose an acute risk to human health. When fecal coliforms are detected, it is likely that human pathogens are present. Therefore, the EPA believes that more urgent public notice language is needed when fecal coliforms are detected, compared to that for total coliforms, even though the detection of fecal coliforms may only constitute a violation of the monthly MCL for total coliforms. The EPA has revised the mandatory language to be used by the system to notify consumers of the presence of fecal coliforms/*E. coli* (reference 2). The notice should read as follows:

"The United States Environmental Protection Agency sets drinking water standards and has determined that the presence of fecal coliforms or *E. coli* is a serious health concern. Fecal coliforms and *E. coli* are generally not harmful themselves, but their presence in drinking water is serious because they are usually associated with sewage or animal wastes. The presence of these bacteria in drinking water is generally a result of a problem with water treatment or the pipes that distribute the water, and indicates that the water may be contaminated with organisms that can cause disease. Disease symptoms may include diarrhea, cramps, nausea, and possibly jaundice, and associated headaches and fatigue. These symptoms, however, are not just associated with disease-causing organisms in drinking water, but also may by caused by a number of factors other than your drinking water. The EPA has set an enforceable drinking water standard for fecal coliforms and *E. coli* to reduce the risk of these adverse health effects. Under this standard all drinking water samples must be free of these bacteria. Drinking water which meets this standard is associated with little or none of this risk and should be considered safe. State and local health authorities recommend that consumers take the following precautions: [To be inserted by the public water systems, according to instructions from state of local authorities]."

d. The public water system must maintain records pertaining to coliform monitoring of drinking water for 5 years.

CHAPTER 4
SAMPLE COLLECTION

4-1. General

Make bacteriological examinations on samples collected at representative points throughout the distribution system. As good management practice, add a sampling point at the distribution system entry point. Select the frequency of sampling and the location of sampling points to ensure accurate determination of the bacteriological quality of the treated water supply, which may be controlled in part by the known quality of the untreated water and thus by the need for treatment. Base the minimum number of samples to be collected and examined each month on the population served by. It is important to examine repetitive samples from designated points, as well as samples from a number of widely distributed sampling points. Take samples at reasonably evenly spaced intervals.

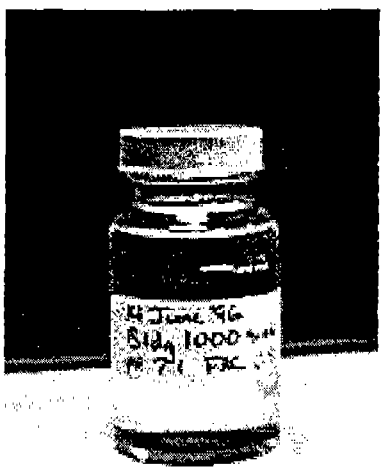
Collection of a drinking water sample

4-2. Sample Collection and Preservation

a. If the water sample is to be taken from a distribution system tap without attachments, select a tap that is supplying water from a service pipe directly connected with the main, and is not, for example, served from a cistern or storage tank. Remove faucet attachments such as screen or splashguard, open tap fully and let water run to waste for 2 or 3 minutes, or for a time sufficient to permit clearing the service line. Reduce water flow to a steady, moderate stream. In sampling from a mixing faucet (which mixes hot and cold water) run hot water for 2 minutes, cold water for 2 to 3 minutes, and collect the sample.

b. If the sample is to be taken from a well fitted with a hand-pump, pump water to waste for about 5 minutes before collecting samples. If the well is equipped with a mechanical pump, collect samples from a tap on the discharge. If there is no pumping machinery, collect a sample directly from the well by means of a sterilized bottle fitted with a weight at the base. Take care to avoid contaminating samples by any surface scum.

c. Collect samples for microbiological examination in bottles washed with soapy water and rinsed with tapwater, given a final rinse with distilled water, and sterilized. Add a reducing agent to containers intended for the collection of water having residual chlorine or other halogen. Adding 0.1 mL 10 percent solution of sodium thiosulfate to a 120 mL bottle will neutralize a sample containing about 15 mg/L residual chlorine. The examination then will indicate more accurately the true microbial content of the water at the time of sampling, since the sodium thiosulfate neutralizes residual halogen and prevents continuation of bactericidal action during sample transit.



Drinking water sample

d. Collect sufficient volume of samples to perform the required tests. Leave ample air space in the bottle [at least 2.5 centimeters (cm)] to facilitate mixing by shaking, preparatory to examination. Clearly label the sample with location and date according to EPA guidelines (reference 13).

e. A disinfectant residual (e.g. free chlorine) is generally measured at the time of sample collection.

f. Experience in the shipment of un-iced samples by mail indicates that changes in type or numbers of bacteria during such shipment, for even limited periods of time, are not negligible. Therefore, refrigeration during transportation is recommended to minimize changes, particularly when ambient air temperature exceeds 13°C.

4-3. Sample Storage Time

a. Start microbiological examination of a water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 1 hour after collection, use an iced cooler for storage during transport to the laboratory. Prompt delivery to the laboratory within 6 hours and strict chain of custody is critical if analyses are to be used for legal proceedings.

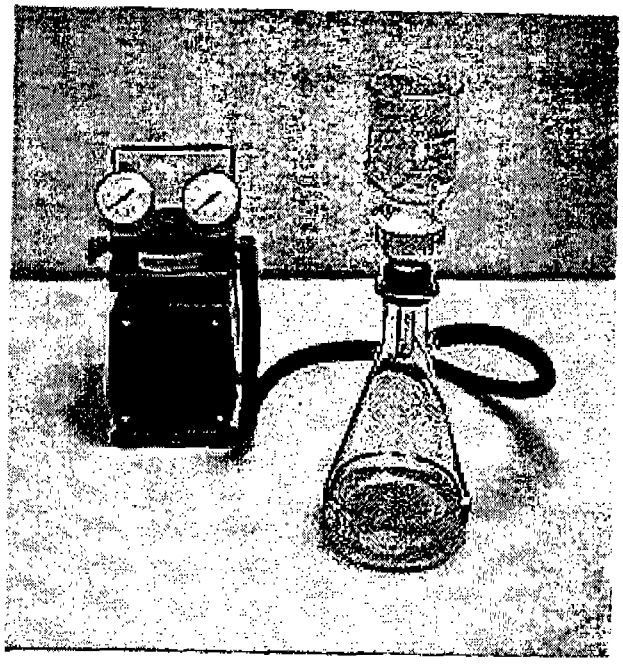
b. These requirements are seldom are realistic in the case of individual potable water samples sent to the laboratory by mail service, but the time elapsing between collection and examination must not exceed 30 hours. Where refrigeration of individual water samples sent by mail is not possible, a thermos-type insulated sample bottle that can be sterilized may be used. Record time and temperature of storage of all samples and consider this information in the interpretation of data.

CHAPTER 5
DRINKING WATER TEST PROCEDURES

5-1. General Discussion

a. The following sections discuss the general procedures for making bacteriological examination of water samples based on analytical methods outlined in Standard Methods. These analytical procedures are the best currently available; however, their limitations should be thoroughly understood.

b. Experience has established the significance of coliform group density as a criterion of the degree of pollution and thus of sanitary quality. The significance of the tests and the interpretation of results are well authenticated and have been used as a basis for standards to assess the bacteriological quality of water supplies.



MF apparatus

c. Direct plating methods, such as the MF procedure, permit a direct count of coliform colonies. By contrast, it is customary to report results of the coliform test by the MTF procedure as a Most Probable Number (MPN) index. This is an index of the coliform bacteria that, more probably than any other number, would give the results shown by the laboratory examination; it is not an actual enumeration. In both procedures, coliform density is reported conventionally as a count or MPN per 100 mL. Use of either procedure permits appraising the sanitary quality of water and the effectiveness of treatment processes. Because total coliform regulations no longer require a qualitative assessment, the Minimal Media ONPG-MUG test has been added, as well as the Presence-Absence (P-A) test.

d. The heterotrophic plate count (HPC) may be determined by pour plate, spread plate, or MF procedure. It provides an approximate enumeration of total numbers of bacteria multiplying at 35°C that may yield useful information about water quality, and may provide supporting data on the significance of coliform test results. The HPC is useful in judging the efficiency of various treatment processes and may have significant application as an in-plant control test. It

also is valuable for checking quality of finished water in a distribution system as an indicator of microbial regrowth and sediment buildup in slow-flow sections and dead ends.

5-2. MF Method

a. Although the MF procedure is limited in testing waters with high turbidity and high non-coliform bacteria numbers, the test does have a number of advantages. It is more precise than the MTF method and is highly reproducible. It can test large volumes of sample and gives definite test results faster than the MTF procedure. However, if the MF test has not been previously used, it is a good idea to test samples using the MTF procedure at the same time to provide a point of comparison for results.

b. The standard sample volume for TCR compliance is 100 mL, which can be separated among multiple membranes, if needed. As good operational practice, water treatment facilities (WTF) may monitor the bacteriological quality as the water exits the facility. Since there may be undetectable densities of total coliforms in 100 mL, the WTF operator can test 1 L samples of water (divide the sample into four 250 mL samples and total the coliform counts).

c. Numerous species of facultative anaerobic, gram-negative, rod-shaped bacteria comprise the coliform group. In the MF test, these species develop red colonies with a metallic (golden) sheen within 24 hours at 35 °C on an Endo-type medium containing lactose.

d. Standard total coliform MF procedure (see Figure 1). A detailed description may be found in Standard Methods, section 9222B.5 (reference 3).

(1) The sample is sent through a membrane filter using a vacuum. The membrane filter is placed on a medium, inverted, then incubated at 35±0.5 °C for 22-24 hours. After incubation, the colonies are counted and verified.

(2) For drinking water samples with colony growth, verify up to 10 typical colonies. It is advisable to verify all suspect colonies.

Application of MF filter

(3) Enrichment procedures can be used to improve assessment of drinking water quality. However, this step is not necessary if use of standard MF procedures has proven to be reliable.

(4) Coliform Density.

(a) Report coliform density as (total) coliforms/100 mL. Compute the count, using the formula:

$$\text{Coliform colonies/100 mL} = \frac{\text{coliform colonies counted} \times 100}{\text{mL sample filtered}}$$

(b) With water of good quality, the occurrence of coliforms generally will be minimal. Therefore, count all coliform colonies and use the formula given above to obtain coliform density.

(c) If growth covers either the entire filtration area of the membrane or a portion thereof (confluent growth), and colonies are not discrete, report results as confluent growth with (or without) coliforms and request another sample from the same sampling point.

Clockwise from top: incubator, m-Endo media, culture dish, membrane filter

(d) If the total number of bacterial colonies, coliforms and noncoliforms, exceeds 200 per membrane, or if the colonies are not distinct enough for accurate counting, report results as too numerous to count (TNTC). The presence of coliforms in such cultures showing no sheen may be verified by placing the entire membrane filter culture into a sterile tube of brilliant green lactose bile broth. As an alternative, brush the entire surface with a sterile loop or applicator stick and inoculate this growth to the tube of brilliant green lactose bile broth. If gas is produced from this culture within 48 hours at 35± 0.5°C, conclude that coliforms are present. In any case, request a new sample and select more appropriate volumes to be filtered per membrane, remembering that the standard drinking water portion is 100 mL.

(5) Although the statistical reliability of the method is greater than that of the MPN procedure, membrane counts really are not absolute numbers. Standard Methods Table 9222:II illustrates some 95 percent confidence limits. These values assume that bacteria are distributed randomly.

e. Delayed-incubation total coliform procedure.

(1) Certain circumstances may not allow the use of conventional MF procedures. Therefore, the MF method has been modified to allow for testing in the event the desired sample temperature cannot be maintained, the time span between sample collection and analysis is unacceptable, or for other reasons the test cannot be completed.

(2) The modifications enable transport of the sample after filtration to a laboratory for completion of the test. Transport media prevent growth during travel time but keep organisms viable. Once the sample is transferred to a fresh medium, normal coliform growth should commence.

(3) The delayed-incubation test follows the methods outlined for the total coliform MF procedure, except as indicated in Section 9222C 1-4 of Standard Methods.

(4) Comparisons between the delayed-incubation test and immediate standard test have shown results to be consistent between the two. However, it is recommended that both tests should be administered when first testing a specific water source to determine if the delayed-incubation test yields satisfactory results.

5-3. ONPG-MUG Test

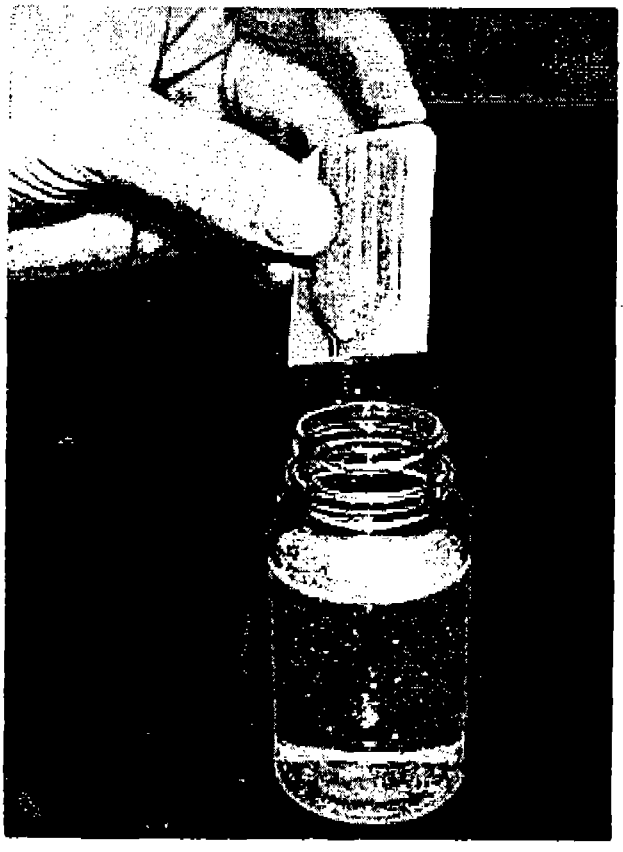
a. The Minimal Media ONPG-MUG (marketed as the Autoanalysis Colilert System®) test utilizes hydrolyzable substrates for the simultaneous detection of total coliform bacteria and *E. coli*. The enzyme β-galactosidase (present in all coliforms) hydrolyzes the substrate ortho-nitrophenyl-β-d-galactopyranside (ONPG) producing a yellow color. The enzyme 4-β-glucuronidase hydrolyzes 4 methylumbelliferyl-β-d-glucuronide (MUG), which then fluoresces under ultraviolet light, indicating the presence of *E. coli*. Noncoliform bacteria cannot metabolize the indicator nutrients and therefore do not interfere with the test.

b. The ONPG-MUG test is recommended for the analysis of drinking and fresh source water samples. The ONPG-MUG test is available in P-A and MPN formats. Samples are incubated at 35°C for 24 hours.

® Autoanalysis Colilert System is a registered trademark of IDEXX Laboratories, Inc., Westbrook, ME.

c. Many studies have provided comparisons between the MF procedure and the ONPG-MUG test and have found there are no significant differences in coliform enumeration. It is a fast, simple test, easy to interpret, sufficiently sensitive in detecting coliforms and *E. coli*, and needs no confirmatory tests. Ideally, laboratories planning to use this procedure will conduct parallel tests with one of the standard coliform tests over a period of several months to assess the effectiveness of the test for the specific water type being analyzed, and to determine the comparability of the two techniques.

d. The Army currently has a federal supply contract with the manufacturer of Colilert. A Colilert P/A 20® count pack is $80.00; a Colilert Economy P/A 200® count pack is $640.00. The ONPG-MUG media has a higher initial cost than the MF and MTF tests, but savings may be gained through decreased labor, reduced equipment to perform the test, and better quality control.



Addition of Colilert®

e. Colisure® is the fifth EPA approved method to test for total coliforms in drinking water. It is very similar to Colilert in that the media is a dry reagent powder, presence-absence or MPN formats are available, and *E. coli* can be detected. Compared to Colilert, a different total coliform substrate is used, resulting in a total coliform-positive sample turning red.

f. The substrate media has many qualities valuable to the Army. Their simplicity and capability for field use lends itself well to Army field applications. In addition, they may be used to test untreated, treated, and bottled waters.

5-4. HPC Method

a. The 16th edition of Standard Methods introduced the first major procedural change in what had been called the standard plate count (SPC), now known as the heterotrophic plate count

® Colisure is a registered trademark of Millipore Corporation, Bedford, MA.

(HPC). The HPC attempts to provide a single standardized means of determining the density of aerobic and facultative anaerobic heterotrophic bacteria in water. Because it is impossible to recover all viable bacteria present in a water sample with a single procedure, methods have been developed to improve recovery of the heterotrophic bacteria population. Three alternative methods and four media are presented. Any measurement is still empirical because bacteria occur singly or in pairs, chains, clusters, or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in the water sample. Whichever procedure is used, the HPC is the best available measure of water treatment plant efficiency, aftergrowth in transmission lines, and general bacterial composition of source water.

b. The pour plate method, formerly known as the SPC (and now the HPC), has several disadvantages that limit recovery of the maximum number of organisms irrespective of medium and incubation temperatures and the time used. Tempered medium at 44 to 46°C may cause heat shock to stressed bacteria, and the nutritionally rich medium may decrease recovery of starved bacteria. The alternative methods have attempted to overcome these liabilities.

c. The spread plate method eliminates heat shock caused by tempered agar. Additionally, all colonies will be on the agar surface where they can be seen and counted readily and can be distinguished easily from particulates or air bubbles. This procedure may require less time and space than the pour plate method but is limited by the small sample volume that can be used: 0.1 to 0.5 mL depending on absorbency of the agar. As in the pour plate method, spreaders also may be a problem.

d. The MF method permits testing large volumes of low-turbidity waters. It may require the least analytical time. Disadvantages include problems with spreaders, contamination if plates are prepared and stored for too long, and variable recovery dependent on quality of membrane filter used.

e. There are four different types of media available for use. Plate count agar (tryptose glucose yeast agar) is for use in the pour or spread plate methods. The m-HPC agar is used only in the MF method. The R2A agar and NWRI agar may be used in any of the HPC methods.

f. The HPC procedure counts both bacterial pathogens and innocuous bacteria. There is no way to know whether the bacteria counted are pathogens or innocuous, or the proportion of each (50 FR 46955). Therefore, it is impossible to specify a scientifically rational MCLG, and to set any particular HPC level (other than at zero) at which no adverse health effects occur. Drinking water with any given HPC level might contain numerous, few, or no pathogens

5-5. MTF Method

a. This method uses a fermentation technique and a series of tubes to determine bacterial density. Results of the examination of replicate tubes and dilutions are reported in terms of the MPN. This number is an estimate of the mean density of coliforms in the sample.

b. Standard Total Coliform MPN Tests.

(1) Inoculate the sample into test tubes containing lauryl tryptose broth (LTB), then incubate the tubes at 35°C±5°C.

(2) If gas forms in the inner, inverted tube or an acidic reaction occurs within 48±3 hours, this is a positive presumptive reaction. Inoculum from the positive LTB tube is transferred to confirmatory brilliant green lactose bile (BGLB) broth.

(3) Gas formation in the inverted vial of BGLB is a positive, confirmed total coliform test.

(4) The completed phase is used in at least 10 percent of positive confirmed tubes to establish definitively the presence of coliform bacteria. Conduct parallel testing of the sample in BGLB (for total coliforms) and EC broth (for fecal coliforms) or EC-MUG broth (for *E. coli*). Positive EC and EC-MUG results are positive completed test indicators. Positive BGLB broth cultures combined with negative EC or EC-MUG broth cultures show that there are nonfecal coliforms present.

c. The precision of any single test depends on the number of tubes used. Unless a large number of sample portions is examined, the precision of the MTF test is rather low. Consequently, exercise great caution when interpreting the sanitary significance of coliform results obtained from the use of a few tubes with each sample dilution, especially when the number of samples from a given sampling point is limited. Adequate sample shaking is another factor that affects accuracy of analysis. If the sample is not adequately shaken before the portions are removed or if clumping of bacterial cells occurs, the MPN value will be an underestimate of the actual bacterial density. The most satisfactory information will be obtained when the largest sample inoculum examination shows gas in some or all of the tubes and the smallest sample inoculum shows no gas in all or a majority of the tubes.

5-6. P-A Coliform Test

a. The P-A coliform test is a simplification of the MTF procedure. It uses one 100 mL sample in a single culture bottle to detect the presence of coliforms. The simplified method is

justified on the basis that no coliforms should be present in a 100 mL sample of drinking water. A positive total coliform test is indicated by gas bubbles and/or a change in the medium color from purple to yellow.

b. The intended use for the test is routine water treatment plant samples or distribution system samples. One notable advantage is the possibility of examining a larger number of samples in a given amount of time.

CHAPTER 6
QUALITY ASSURANCE

6-1. Laboratory Quality Assurance

a. The growing emphasis on water quality standards, enforcement, and monitoring has required the establishment of a quality assurance program to substantiate the validity of analytical data. A laboratory quality assurance program is the orderly application of the practices necessary to remove or reduce errors that may occur in any laboratory operation, caused by personnel, equipment, supplies, sampling procedures, and analytical methodology.

b. The program must be practical and integrated. It should require only a reasonable time or it will be bypassed. When properly administered, a balanced, conscientiously applied quality assurance program will yield uniformly high-quality data without interfering with the primary analytical functions of the laboratory. Generally, 15 percent of total analyst time should be spent on different aspects of a quality assurance program.

c. The quality assurance guidelines discussed in Part 9020A of Standard Methods (reference 3) are recommended as a minimal program for a microbiology laboratory. In addition to Standard Methods, most state regulatory agencies use the Manual for the Certification of Laboratories Analyzing Drinking Water to evaluate and certify laboratories for microbiological analyses of water (reference 13).

6-2. Intralaboratory Quality Control

a. An intralaboratory quality control program is a system of agreed-upon requirements and laboratory practices necessary to maintain minimal quality standards among a group of participant laboratories. Minimal standards are set for laboratory operations (personnel, facilities, equipment, supplies, data handling, and quality control). To safeguard drinking water and assure a level of data reliability, most states have approval, registration or certification programs for laboratories that test water. These state programs provided the base for the Federal/State program for the certification of water supply laboratories developed under the Safe Drinking Water Act (references 14 and 15).

b. Criteria have been established for laboratory operations and methodology, and for on-site inspections by the certifying state agency or its surrogate to verify these minimal standards. On-site inspections of laboratories in the present certification program have shown that the primary causes for discrepancies have been inadequate equipment, improperly prepared media, incorrect analytical procedures, and insufficiently trained personnel. References 13 and

16 provide certification requirements and guidance on equipment selection and use, sampling procedures, analytical methodologies, and quality assurance practices.

c. Analytical Quality Control Procedures.

(1) General.

(a) For MF procedures check sterility of media, membrane filters, dilution and rinse water, and glassware and equipment once during each series of samples using sterile water as the sample. For each type of test conducted, verify colonies monthly from a known positive sample. Verify suspected coliforms by testing for lactose fermentation or by use of rapid biochemical tests or multitest systems. For drinking water samples, verify all sheen colonies counted as coliforms when this number is <10/100 mL. When the number exceeds 10/100 mL, randomly pick and verify at least 10 colonies representative of all sheen types. If no positives result from testing drinking water samples, analyze at least one known positive source water quarterly.

(b) Each lot of media for the ONPG-MUG test should be quality control tested. Mix the media with distilled water and divide into three culture vessels. Label the vessels "*Escherichia coli*," "*Klebsiella pneumoniae*," and "*Pseudomonas aeruginosa*." Inoculate each bottle with their respective culture and incubate at 35±0.5°C for 24 hours. *E. coli* should be yellow and fluorescent, *K. pneumoniae* should be yellow with no fluorescence, and *Pseudomonas aeruginosa* should produce no color and no fluorescence.

(c) For MTF procedures, check sterility of media, dilution water, and glassware. Media may be checked by incubating a representative portion at the appropriate temperature and observing for growth. Sterility of glassware is confirmed by the addition of 25 mL of sterile nonselective broth to a random container, incubation, and observance for growth. For routine analyses, do the completed test on 10 percent of positive samples. If no positives result from potable water samples, complete at least one positive source water quarterly. For public water supply samples with a history of heavy growth without gas in presumptive-phase tubes, submit such tubes to the confirmed phase to check for coliform bacteria.

(d) For each lot of medium, check analytical procedures by inoculating with known positive and negative control cultures for the organism(s) under test.

(e) Perform duplicate analyses on 5 percent of samples and on at least one sample per test run.

(2) Measurement of method precision.

(a) Calculate precision of duplicate analyses for each type of sample examined (e.g., drinking water, wastewater).

(b) Make duplicate analyses on the first 15 positive samples of a specific type. Thereafter, analyze 10 percent of routine samples in duplicate.

CHAPTER 7
RECOMMENDED ACTIONS: RESPONSE TO THE PRESENCE OF COLIFORMS

7-1. Background

The Installation Medical Authority (IMA), often the Preventive Medicine Service, must ensure all measures necessary for the provision of a safe, healthful water supply and, therefore, must be actively involved in the potable water surveillance process. The IMA may provide the laboratory performing certified analyses or render oversight regarding the potability of the water and significant health issues. Regardless of whether the laboratory is certified to perform regulatory analyses, the IMA has several key functions when there is a coliform occurrence in the drinking water.

7-2. Total Coliform Rule Compliance

a. Under the Total Coliform Rule (TCR), the following actions are required by the water supplier and the certified laboratory involved in sample analysis when a water sample is total coliform-positive.

(1) Repeat samples must be collected at the coliform-positive locations within one day. The original positive tap should be included, as well as upstream and downstream faucets, both within five service connections of the original site. The purpose is to determine whether contamination is confined to a single building (or tap).

(2) If any repeat samples are also total coliform positive, additional repeat samples must be collected, using the upstream/downstream format. Repeat sampling is required until all samples are negative, the MCL has been exceeded, or the primacy authority instructs otherwise.

(3) If a sample is total coliform positive, testing for either fecal coliforms or *E. coli* must be initiated. Membrane filters or colony growth from the filter must be transferred into one of the following media: EC Medium (fecal coliform), EC Medium + MUG, or nutrient agar + MUG. Testing total coliform-positive samples for *E. coli* is generally preferable, because *E. coli* is the most specific indicator of fecal contamination. Performing one of these tests will provide critical information as to the public health significance of the coliform results.

b. Several types of notification may be required.

(1) The primacy authority must be notified if any water samples are fecal or *E. coli* positive. The notification must occur before the end of the next business day following receipt of

laboratory results. The installation commander [Directorate of Public Works (DPW) representative, Public Affairs Office] is responsible for notifying the authority, not the IMA.

(2) For acute violations (see paragraph 3-4.c.) notice must be posted on the main television and radio stations in the area served within 72 hours. If a non-acute violations occurs, notice must be posted in a daily circulating newspaper (or weekly newspaper if there is no daily newspaper) within 14 days.

7-3. Remedial Actions

a. Beyond the actions required by the TCR, the IMA should actively participate in resolution of the problem. The following four areas may serve as a starting point for the IMA involvement: source of the coliforms, sampling and laboratory techniques, adjustments to monitoring, and elimination of the coliforms. Some functions will be performed by the DPW, so coordinate closely with that office.

(1) Look at numerous aspects of the water system to determine potential coliform sources.

(a) Examine the WTF operations for any unusual conditions (e.g., high turbidity or improper backwashing of filters). The result may be passage of coliforms into the distribution system. Locate any storage tanks in the vicinity of the coliform positives. Examine the tank exterior for broken screens or vents. Check the interior conditions for signs of trapped wildlife (i.e., birds and rodents).

(b) Coliforms may be trapped in the biofilm and protected from disinfectant. Shifts in water pressure or direction can cause portions of biofilm to break away and disperse in the system. See the discussion in chapter 2, paragraph 2-2.c. in regards to biofilm.

(c) A thorough review of the distribution system should be performed to identify potential cross connections. Examples include improper direct connections of potable and non-potable water lines, insufficient air gaps at sinks, or water line breaks. Points of new main construction, flushing, or fire flow testing that may have occurred should also be examined. All installations should have a cross connection control plan.

(d) Even if water treatment facility and distribution system operations appear normal, disinfectant residuals (free chlorine, chloramine) may have dropped below effective levels in the system. All sampling locations should indicate a minimum of 0.2 mg/L of FAC or 1.0 mg/L chloramine. Ensure adequate disinfection of repaired mains and valves takes place.

(2) Review sampling techniques, whether performed by the IMA, DPW, or a contractor. Factors include the cleanliness of the sample faucet, proper flushing prior to collection, and the chlorine residual. In the laboratory, examine all procedures with particular attention to quality control checks for all phases. Check for any recent changes in laboratory methods or analysts.

(3) The repeat sampling should help define the extent of the coliform contamination. If repeat results indicate the contamination exists in several areas, adjustment to monitoring with an expanded sampling plan may be required to pinpoint the source of contamination. For instance, test water samples from the WTF output daily, for both total and fecal coliforms. Also, test water from storage tanks if not part of routine monitoring.

(4) Several actions may be required in order to eliminate coliform bacteria following a contamination episode. Perhaps a combination of steps will be effective. Laboratory identification of bacterial species may provide important information regarding the contamination. Certain species, such as *Enterobacter cloacae* and *Klebsiella oxytoca*, are characteristic of biofilm growth.

(a) If data indicates inadequate chlorine residuals within the system, raise the level leaving the WTF until FAC levels of 1.0 to 2.0 mg/L are measurable. Evaluate the effect of that FAC level on coliform results to determine if even higher residuals are necessary. The boost in chlorine residual may cause sloughing of biofilm bacteria and initially result in additional coliform positives. At least 2 days of monitoring results may be required to assess the effectiveness of the elevated FAC. If the 1.0-2.0 mg/L FAC level does not appear effective, the FAC may next be raised to 2.0-4.0 mg/L. Be aware that consumer complaints about a "chlorine taste" may follow.

(b) Flushing water through the distribution system assists in removal of sediment and corrosion products and helps eliminate pockets of low chlorine residual. The installation should maintain a flushing program so the entire system is methodically flushed at least yearly. During a coliform episode, flushing can be a useful tool if performed correctly. Start at a source, (e.g., a storage tank) and sequentially open and close valves to force water through all pipelines to the distant points of a particular section of the system. Flushing will initially change water flow patterns and can cause an increase in coliforms, as with boosting chlorine residuals. At least 1 day may be required for the system to settle following flushing.

(c) Corrosion materials in pipelines have been identified as a significant factor in biofilm development. If the water is corrosive, addition of a corrosion sequestrant/inhibitor has been demonstrated to reduce coliform levels by limiting the amount of places the bacteria can attach and grow (reference 6). The newest chemical sequestrant/inhibitors used in corrosion

attach and grow (reference 6). The newest chemical sequestrant/inhibitors used in corrosion control are polyphosphates or orthophosphates in combination with zinc (reference 17). South Central Connecticut Regional Water Authority increased their use of zinc metaphosphate from 1 to 2 mg/L after years of problems with coliform occurrences (reference 18). The concentration of total phosphorus within the distribution system was consequently raised to 0.43 mg/L and did not stimulate bacterial growth. In fact, coliform levels dropped by a substantial amount over the next 2 years. The decision to use a certain type of corrosion sequestrant/inhibitor will be dependent on the water quality and pipe material in question.

b. If the total coliform MCL is exceeded or if an acute violation occurs, the installation may be required to provide an alternate water supply (e.g. bottled water). The regulatory authority will assist with this decision. The installation should have an emergency water supply plan in place.

c. The guidance in chapter 7 is intended to outline procedures that the IMA and DPW can follow to help eliminate coliform contamination from the water system. More specific assistance (e.g., regarding elevating chlorine levels or flushing) may be obtained by contacting the Water Supply Management Program at DSN 584-3919, or commercial (410) 671-3919. Further information regarding cross connection is contained in Water Quality Information Paper No. 42, Cross Connection Control and Backflow Prevention, August 1987. Copies may be obtained from the WSMP.

APPENDIX A
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23. Title 40, CFR, 1995 rev, Part 142, National Primary Drinking Water Regulations Implementation.

24. Title 40, CFR, 1995 rev, Part 143, National Secondary Drinking Water Regulations.

25. Proposed Rule, National Primary Drinking Water Regulations; Total Coliforms; 52 Federal Register (FR) 42224, 3 November 1987.

26. Final Rule, Drinking Water; National Primary Drinking Water Regulations; Total Coliforms (Including Fecal Coliforms and E. coli); 54 FR 27544, 29 June 1989.

27. Final Rule, National Primary Drinking Water Regulations: Analytical Techniques; Coliform Bacteria; 54 FR 29998, 17 July 1989.

APPENDIX B
GLOSSARY

GLOSSARY

AOC	assimilable organic carbon
BGLB	brilliant green lactose bile
CFR	Code of Federal Regulations
cm	centimeter
DPW	Directorate of Public Works
DSA	Direct Support Activity
E. coli	*Escherichia coli*
EPA	U. S. Environmental Protection Agency
FAC	free available chlorine
FR	Federal Register
HPC	heterotrophic plate count
IMA	installation medical authority
IMViC	indol, methyl red, Voges-Proskauer, and citrate utilization
K.	Klebsiella
L	liter
LTB	lauryl tryptose broth
M-FC	membrane-fecal coliform
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	membrane filter method
mg/L	milligram per liter
mL	milliliter
MPN	Most Probable Number
MTF	multiple-tube fermentation method
NCWS	non-community water system
NPDWR	National Primary Drinking Water Regulations
NTU	nephelometric turbidity unit
ONPG-MUG	ortho-nitrophenyl-β-d-galactopyranside + 4-β-glucuronidase
P-A	presence-absence test
pH	negative logarithm of hydrogen ion
SPC	standard plate count
TCR	Total Coliform Rule
TNTC	too numerous to count
USACHPPM	U.S. Center for Health Promotion and Preventive Medicine
USPHS	United States Public Health Service
WSMP	Water Supply Management Program
WTF	water treatment facility
μg/L	micrograms per liter

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