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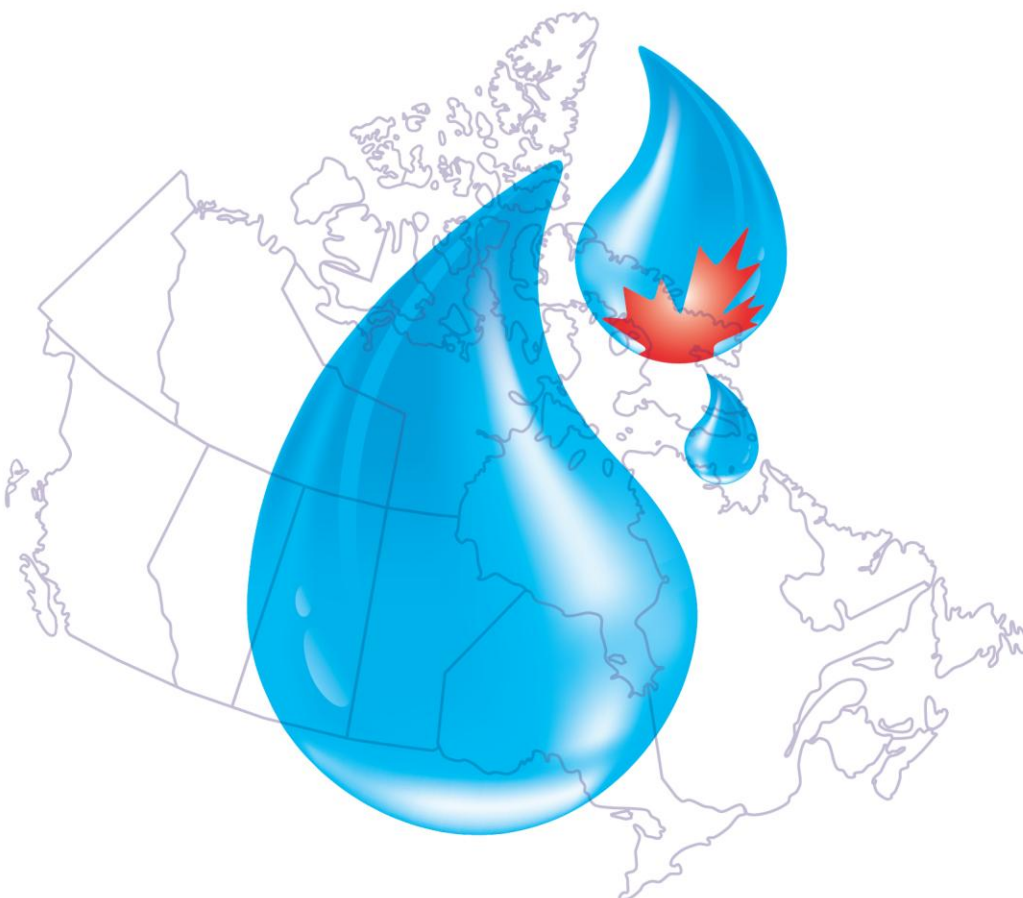
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# Guidelines for Canadian Drinking Water Quality

Guideline Technical Document

**Total Coliforms**



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# **Guidelines for Canadian Drinking Water Quality**

**Guideline Technical Document**

**Total Coliforms**

**Prepared by the  
Federal-Provincial-Territorial Committee on  
Drinking Water  
of the  
Federal-Provincial-Territorial Committee on  
Health and the Environment**

**Health Canada  
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Other Guideline Technical Documents for the Guidelines for Canadian Drinking Water Quality can be found on the following web page: [www.healthcanada.gc.ca/waterquality](http://www.healthcanada.gc.ca/waterquality)

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# Total Coliforms in Drinking Water

## Table of Contents

<b>Part I. Overview and Application.....</b>	<b>1</b>
1.0 Guideline.....	1
2.0 Executive summary.....	1
2.1 Significance of total coliforms in drinking water systems.....	1
2.2 Sampling for total coliforms .....	2
2.3 Treatment technology .....	2
3.0 Application of the guideline.....	2
3.1 Municipal scale drinking water supply systems .....	3
3.1.1 Monitoring total coliforms in water leaving the treatment plant .....	3
3.1.2 Monitoring total coliforms within water distribution and storage systems	3
3.1.3 Notification .....	3
3.1.4 Corrective actions .....	4
3.2 Residential scale.....	5
3.2.1 Testing requirements.....	5
3.2.2 Notification .....	5
3.2.3 Corrective actions for disinfected supplies .....	6
3.2.4 Corrective actions for non-disinfected wells .....	6
<b>Part II. Science and Technical Considerations .....</b>	<b>8</b>
4.0 Significance of total coliforms in drinking water .....	8
4.1 Description.....	8
4.2 Sources.....	9
4.3 Role of total coliforms in maintaining drinking water quality .....	10
4.3.1 Role in source water monitoring.....	10
4.3.2 Role in treatment and distribution system monitoring.....	10
4.3.3 Considerations for residential-scale systems .....	11
5.0 Analytical methods .....	11
5.1 Presence–absence procedure.....	13
5.2 Membrane filter procedure .....	13
5.3 Multiple tube fermentation procedure .....	14
6.0 Sampling for total coliforms .....	15
6.1 Sample collection.....	15
6.2 Sampling frequency considerations .....	16
6.3 Location of sampling points.....	18
7.0 Treatment technology .....	18
7.1 Municipal scale .....	18
7.1.1 Level of treatment necessary .....	19
7.1.2 Physical removal.....	19
7.1.3 Disinfection.....	19
7.1.3.1 Chemical disinfection .....	19

7.1.3.2	UV light disinfection.....	21
7.2	Residential scale.....	21
8.0	Risk assessment .....	23
8.1	International considerations .....	23
9.0	Rationale .....	24
10.0	References.....	24
Appendix A: Decision tree for routine microbiological testing of municipal scale systems.....		32
Appendix B: Decision tree for routine microbiological testing of residential scale systems .....		32
Appendix C: List of Acronyms.....		34

## Total Coliforms

### **Part I. Overview and Application**

#### **1.0 Guideline**

*The maximum acceptable concentration (MAC) of total coliforms in water leaving a treatment plant and in non-disinfected groundwater leaving the well is none detectable per 100 mL.*

*Total coliforms should be monitored in the distribution system because they are used to indicate changes in water quality. Detection of total coliforms from consecutive samples from the same site or from more than 10% of the samples collected in a given sampling period should be investigated.*

#### **2.0 Executive summary**

Total coliforms are a group of bacteria that are naturally found on plants and in soils, water, and in the intestines of humans and warm-blooded animals. Because total coliforms are widespread in the environment, they can be used as one of the many operational tools to determine the efficacy of a drinking water treatment system.

Health Canada recently completed its review on the usefulness of total coliforms as part of a multi-barrier approach to producing microbiologically acceptable drinking water. This guideline technical document reviews and assesses available literature on the uses of total coliforms in drinking water quality management, including as indicators of groundwater vulnerability, the adequacy of disinfection, and changes in distribution system water quality. From this review, the guideline for total coliforms in water leaving a treatment plant and in non-disinfected groundwater leaving the well is established as a maximum acceptable concentration of none detectable in 100 mL of water. This MAC does not apply to distribution systems, where total coliforms are used to indicate changes in water quality.

#### **2.1 Significance of total coliforms in drinking water systems**

Monitoring for total coliforms should be used, in conjunction with other indicators, as part of a multi-barrier approach to producing drinking water of an acceptable quality. The number, frequency, and location of samples for total coliform testing will vary according to the type and size of the system and jurisdictional requirements.

Total coliforms are naturally found in both faecal and non-faecal environments, so they are commonly present in both surface water and groundwater under the direct influence of surface water (GUDI) sources. Consequently, monitoring total coliforms in these sources does not provide information on the quality of the source water from the perspective of health risk. Protected groundwater systems, on the other hand, should not contain total coliforms. As their presence indicates that the groundwater may be vulnerable to contamination from the surrounding environment, detection of total coliforms in the water leaving the well should trigger further actions.

Monitoring for total coliforms at the treatment plant and in the distribution and storage system is carried out to provide information on the adequacy of drinking water treatment and on the microbial condition of the distribution system. The presence of total coliforms in water leaving any treatment plant signifies that inadequate treatment has taken place and therefore additional actions need to be taken. These should include actions such as notifying the responsible authorities, investigating the cause of the contamination, and implementing corrective actions; which could include issuing a boil water advisory.

The presence of total coliforms in the distribution and storage system, when water tested immediately post-treatment is free of total coliforms, indicates water quality degradation, possibly via bacterial regrowth or post-treatment contamination. In municipal-scale systems, the detection of more than 10% of samples in a given sampling period, or of consecutive samples from the same site, that are positive for total coliforms indicates changes in the quality of the water and a need for follow-up actions to be initiated. In residential-scale systems where there is little or no distribution system, the presence of any total coliforms should trigger follow-up actions to investigate the cause of the positive results.

## **2.2 Sampling for total coliforms**

As a minimum, water leaving a municipal scale treatment plant should be sampled and tested at least weekly for total coliforms as part of the verification process in a source-to-tap multi-barrier approach. In many systems, the water leaving the treatment plant will be tested well in excess of the minimum requirements. In a distribution system, the number of samples for this bacteriological testing should be increased in accordance with the size of the population served, and the samples should be taken at regular intervals throughout the month.

Sampling frequencies in residential-scale and small private systems may vary from jurisdiction to jurisdiction but should include times when the risk of contamination is greatest, for example, after spring thaw, heavy rains, or dry periods. New or rehabilitated wells should also be sampled initially to confirm acceptable bacteriological quality.

Proper procedures for collecting samples must be observed to ensure that the samples are representative of the water being examined. A minimum volume of 100 mL of water should be collected for testing, and testing should be started as soon as possible after collection.

## **2.3 Treatment technology**

Generally, minimum treatment of supplies derived from surface water or GUDI sources should include filtration (or technologies providing an equivalent log reduction credit) and disinfection. Protected groundwaters should receive adequate treatment for the removal/inactivation of enteric viruses, unless exempted by the responsible authority based on site-specific considerations, such as historical and on-going monitoring data. In systems with a distribution system, a disinfectant residual should be maintained at all times.

## **3.0 Application of the guideline**

*Note: Specific guidance related to the implementation of drinking water guidelines should be obtained from the appropriate drinking water authority in the affected jurisdiction.*

Monitoring for total coliforms should be used, in conjunction with other indicators, as part of a multi-barrier approach to producing drinking water of an acceptable quality. The number, frequency, and location of samples for total coliform testing will vary according to the



type and size of the system and jurisdictional requirements. For decision-making, the focus is the positive detection of total coliforms, regardless of quantity. However, although quantitative results are not precise, they can be used to provide an indication of the magnitude of a problem and thus inform the public health response.

### **3.1 Municipal scale drinking water supply systems**

#### *3.1.1 Monitoring total coliforms in water leaving the treatment plant*

Total coliforms should be monitored at least weekly in water leaving a treatment plant. If total coliforms are detected, this indicates a serious breach in treatment and is therefore unacceptable. These tests should be used in conjunction with other indicators, such as residual disinfectant and turbidity monitoring as part of a multi-barrier approach to producing drinking water of acceptable quality. While the required frequency for all testing at the treatment plant is prescribed by the responsible authority, best practice commonly involves a testing frequency beyond these minimum recommendations based upon the size of system, the number of consumers served, the history of the system, and other site-specific considerations.

#### *3.1.2 Monitoring total coliforms within water distribution and storage systems*

In municipal scale distribution and storage systems, the number of samples collected for total coliform testing should reflect the size of the population being served, with a minimum of four samples per month. The sampling points and testing frequencies for total coliforms, residual disinfectant, and turbidity in treated water within distribution and storage systems will be prescribed by the responsible authority. As an important part of a multi-barrier approach to ensuring safe drinking water, incorporating total coliforms into a distribution and storage system monitoring strategy can, over time, provide an enhanced knowledge of water quality throughout the system as well as overall system condition. The approach should take into account the particular characteristics of the distribution and storage system and historic knowledge of the overall system such as age, layout, or materials. This strategy allows for the detection of changing conditions, intrusion of contaminants, or areas of declining water quality, which can then be investigated further.

#### *3.1.3 Notification*

The presence of any total coliform bacteria in water leaving a treatment plant indicates a serious breach in treatment and is therefore unacceptable. This situation should be corrected immediately. The system owner should notify all responsible authorities and immediately reanalyze the coliform-positive sample(s) for *Escherichia coli* and resample and test the positive site(s) to confirm the presence or absence of both *E. coli* and total coliforms (see Appendix A). Guidance on analytical methods for *E. coli* and the actions that are required if the presence of *E. coli* is confirmed are outlined in sections 5.0 and 3.0, respectively, of the guideline technical document for *E. coli*.

In a distribution system, coliform bacteria are operational indicators. Their presence indicates water quality degradation, possibly via bacterial regrowth or post-treatment contamination. Detection of total coliforms (in the absence of *E. coli*) in more than 10% of samples in a given sampling period, or from consecutive samples from the same site, should be investigated and appropriate corrective actions taken. Some or all of the corrective actions listed in the following section may be necessary.

### 3.1.4 Corrective actions

The degree of response to the presence of total coliforms (in the absence of *E. coli*) should be discussed with the appropriate agencies and will depend on

- a risk-based assessment of the significance and extent of the problem, taking the history of the entire system into account
- the history and variability of the quality of the raw water supply
- the documented historical effectiveness of the treatment process
- the integrity of the distribution system, including the existence and effectiveness of a cross-connection control program.

Knowledge of the history of the system, including the past frequency and locations of total coliform-positive samples, enables qualified personnel to consider appropriate actions when total coliforms are detected in the absence of *E. coli*.

If corrective actions are deemed necessary, the owner of the waterworks system, in consultation with the responsible authorities, should carry out appropriate corrective actions, which could include the following measures:

- Verify the integrity and the optimal operation of the treatment process.
- Verify the integrity of the distribution system.
- Verify that the required disinfectant residual is present throughout the distribution system.
- Increase the disinfectant dosage, flush the water mains, clean treated-water storage tanks (municipal reservoirs and domestic cisterns), and check for the presence of cross-connections and pressure losses. Water should be dechlorinated before being discharged into fish-bearing waters. The responsible authority should be consulted regarding the methods available, as well as the correct procedure, for carrying out dechlorination.
- Sample and test sites adjacent to the site(s) of the positive sample(s). Tests performed should include total coliforms, *E. coli*, disinfectant residual, and turbidity. At a minimum, one sample upstream and one downstream from the original sample site(s) plus the finished water from the treatment plant as it enters the distribution system should be tested. Other samples should be collected and tested following a sampling plan appropriate for the distribution system.
- Conduct an investigation to identify the problem and prevent its recurrence, including a measure of raw water quality (e.g., bacteriology, colour, assimilable organic carbon [AOC], turbidity, conductivity) and variability.
- Continue selected sampling and testing (e.g., bacteriology, disinfectant residual, turbidity) of all identified sites during the investigative phase to confirm the extent of the problem and to verify the success of the corrective actions.

If enhanced health surveillance indicates that a waterborne outbreak may be occurring or if conditions exist that could result in a waterborne outbreak, then the necessity of issuing a boil water advisory<sup>1</sup> should be discussed immediately with senior operations personnel at the water utility and with the responsible authority. In the event that an incident that may have

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<sup>1</sup> For the purpose of this document, the use of the term “boil water advisory” is taken to mean advice given to the public by the responsible authority to boil their water, regardless of whether this advice is precautionary or in response to an outbreak. Depending on the jurisdiction, the use of this term may vary. As well, the term “boil water order” may be used in place of, or in conjunction with, a “boil water advisory.”

contaminated the distribution system or interfered with treatment is known to the owner, consumers should be notified immediately to boil the drinking water. A boil water advisory should be rescinded only after a minimum of two consecutive sets of samples, collected 24 hours apart, show negative results that demonstrate full system-wide integrity (including acceptable bacteriological quality, disinfection residuals, and/or turbidity). Additional negative results may be required by the local responsible authority. Further information on boil water advisories can be found in *Guidance for Issuing and Rescinding Boil Water Advisories*.

Minimum treatment of supplies derived from surface water sources or groundwater under the direct influence of surface waters should include adequate filtration (or technologies providing an equivalent log removal/inactivation) and disinfection. For protected groundwater sources (i.e., those not under the direct influence of surface waters), adequate treatment is recommended to ensure the removal /inactivation of enteric viruses (as outlined in the guideline technical document on enteric viruses), unless exempted by the responsible authority based on site-specific considerations including historical and on-going monitoring data. In all systems with a distribution system, a disinfectant residual should be maintained at all times. The appropriate type and level of treatment should take into account the potential fluctuations in water quality, including short-term water quality degradation, and variability in treatment performance.

## **3.2 Residential scale**

### *3.2.1 Testing requirements*

Sampling frequencies for residential-scale<sup>2</sup> systems will be determined by the authority having jurisdiction for the system and should include times when the risk of contamination is greatest, for example, early spring after the thaw, after an extended dry spell, or following heavy rains. Owners of private supplies should be encouraged to have their water tested for total coliforms during these same periods. New or rehabilitated wells should also be tested before use to confirm the microbiological quality.

### *3.2.2 Notification*

No samples from residential scale water supplies should contain coliforms. If a sample contains total coliform bacteria, it should be immediately reanalyzed and the positive site resampled and tested to confirm the presence or absence of both *E. coli* and total coliforms. If resampling confirms that the system is contaminated with *E. coli*, the actions required are outlined in the guideline technical document on *E. coli*.

Responses to total coliform-positive samples in the absence of *E. coli* can vary from jurisdiction to jurisdiction. As a precautionary measure, some jurisdictions will always advise the owner to boil the drinking water or use an alternative safe source as an interim measure until corrective actions are taken. In other jurisdictions, advice on interim measures is site-specific and depends on such factors as the historical water quality data, the health status of the users, and delays in investigation. Regardless of whether a boil water advisory is issued, the source of the

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<sup>2</sup> For the purposes of this document, a residential-scale water system is defined as a system with a minimal or no distribution system that provides water from a facility not connected to a municipal supply. Examples of such facilities include private drinking water supplies, schools, personal care homes, day care centres, hospitals, community wells, hotels, and restaurants. The definition of a residential-scale system may vary between jurisdictions.

coliforms needs to be investigated, and appropriate actions need to be taken (see Appendix B). These may include some or all of the corrective actions outlined in the following sections.

### 3.2.3 *Corrective actions for disinfected supplies*

The first step is to conduct a sanitary survey to verify the physical condition of the drinking water system as applicable, including water intake, well, well head, pump, treatment system (including chemical feed equipment, if present), plumbing, and surrounding area. Any identified faults should be corrected before proceeding. If all the physical conditions are acceptable, some or all of the following corrective actions may be necessary:

- In a chlorinated system, verify that a disinfectant residual is present throughout the system. Increase the disinfectant dosage, flush the system thoroughly, and clean treated-water storage tanks and domestic cisterns. Water should be dechlorinated before being discharged to fish-bearing waters. The responsible authority should be consulted regarding the methods available, as well as the correct procedure, for carrying out dechlorination.
- For systems where the disinfection technology does not leave a disinfectant residual, such as UV or ozone, it may be necessary to shock chlorinate the well and plumbing system; further information on shock chlorination is available in the factsheet on wells, available at [www.healthcanada.gc.ca/waterquality](http://www.healthcanada.gc.ca/waterquality).
- Ensure that the disinfection system is working properly and maintained according to manufacturer's instructions.

After the necessary corrective actions have been taken, samples should be collected and tested for both total coliforms and *E. coli* to confirm that the problem has been corrected. If total coliforms are detected after implementing these corrective actions, a boil water advisory should be issued, if one is not already in place. Alternatively, a source of water known to be safe should be used until the situation is corrected. The presence of total coliforms after corrective actions suggests that the system remains vulnerable to contamination. If the problem cannot be corrected, additional treatment or a new source of drinking water may need to be considered. In some instances, in residential-scale systems, the presence of coliform bacteria may be the result of bacterial regrowth within the distribution system biofilm as opposed to the intrusion of contaminants, and therefore a boil water advisory may not be necessary. This determination would need to be made by qualified personnel using knowledge of the history of the system and other site-specific considerations.

Minimum treatment of supplies derived from surface water sources or groundwater under the direct influence of surface waters should include adequate filtration (or technologies providing an equivalent log reduction credit) and disinfection. For groundwater sources less vulnerable to faecal contamination, adequate treatment is recommended to ensure the removal/inactivation of enteric viruses (as outlined in the guideline technical document on enteric viruses), unless exempted by the responsible authority based on site-specific considerations including historical and on-going monitoring data.

### 3.2.4 *Corrective actions for non-disinfected wells*

The first step, if it has not already been taken, is to conduct a sanitary survey to verify the physical condition of the well, well head, pump, plumbing, and surrounding area. Any identified faults should be corrected before proceeding. If all the physical conditions are acceptable, then the following corrective actions should be carried out:

- Shock-chlorinate the well and plumbing system. Further information on this topic is available in the factsheet on wells (available at [www.healthcanada.gc.ca/waterquality](http://www.healthcanada.gc.ca/waterquality)).
- Flush the system thoroughly and retest to confirm that the water is free of total coliform contamination. Confirmatory tests should be done no sooner than either 48 hours after tests indicate the absence of a chlorine residual or 5 days after the well has been treated. Local conditions may determine acceptable practice. Water should be dechlorinated before being discharged to fish-bearing waters. The responsible authority should be consulted regarding the methods available, and the correct procedure, for carrying out dechlorination.

If total coliforms are detected after implementing these corrective actions, a boil water advisory should be issued, if one is not already in place. Alternatively, a source of water known to be safe should be used until the situation is corrected. The presence of total coliforms after shock-chlorination and flushing suggests that the well remains vulnerable to contamination. If the problem cannot be reasonably identified or corrected, an appropriate disinfection device or well reconstruction or replacement should be considered. In some instances, in residential-scale systems, the presence of coliform bacteria may be the result of bacterial regrowth within the distribution system biofilm as opposed to on-going contamination, and therefore a boil water advisory may not be necessary. This determination would need to be made by qualified personnel using knowledge of the history of the system and other site-specific considerations.

A single negative total coliform test result does not necessarily indicate that the problem has been corrected. A minimum of two consecutive total coliform negative samples should be obtained. An additional test should be taken after 3–4 months to ensure that the contamination has not recurred. Only a history of data can be used to confirm the long-term integrity of a supply when applied jointly with sanitary surveys. Further information on routine monitoring can be found in section 8.0.

## **Part II. Science and Technical Considerations**

### **4.0 Significance of total coliforms in drinking water**

#### **4.1 Description**

Total coliforms belong within the family Enterobacteriaceae and have been defined in the 21st edition of *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005) as follows:

- all facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35°C
- many facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that develop red colonies with a metallic (golden) sheen within 24 hours at 35°C on an Endo-type medium containing lactose
- all bacteria possessing the enzyme  $\beta$ -galactosidase, which cleaves a chromogenic substrate (e.g., *ortho*-nitrophenyl- $\beta$ -D-galactopyranoside), resulting in release of a chromogen (e.g. *ortho*-nitrophenol).

These definitions are not to be regarded as identical; rather, they refer to three groups of coliforms that are roughly equivalent. All three groups contain various species of the genera *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, and many others (Leclerc et al., 2001). Some members of these groups are naturally occurring in the environment and may be of faecal origin, while others are found exclusively in the environment (Table 1).

Although not included in the coliform group, members of the genus *Aeromonas* can ferment lactose and possess  $\beta$ -galactosidase; therefore, they can yield false-positive total coliform reactions. *Aeromonas* species are ubiquitous in the environment, and have been found in lakes, rivers, marine waters, sewage effluents, and drinking waters, among other places (Allen et al., 1983; Nakano et al., 1990; Poffe and Op de Beeck, 1991; Payment et al., 1993; Ashbolt et al., 1995; Bernagozzi et al., 1995; Chauret et al., 2001; El-Taweel and Shaban, 2001). Information on excluding false-positives resulting from the presence of *Aeromonas* is included in Section 5.0.

**Table 1:** Selected coliform bacteria in the family Enterobacteriaceae<sup>a</sup>

	ONPG <sup>b</sup>	Faecal origin	Non-faecal origin
<i>Budvicia</i>	+	–	+
<i>Citrobacter</i>	+	+	+
<i>Enterobacter</i>	+	+	+
<i>Erwinia</i>	+	–	+
<i>Escherichia</i>	+	+	–
<i>Klebsiella</i>	+	+	+
<i>Leclercia</i>	+	–	+
<i>Pantoea</i>	+	–	+
<i>Serratia</i>	+	–	+

<sup>a</sup> Adapted from Leclerc et al. (2001)

<sup>b</sup> *ortho*-Nitrophenyl- $\beta$ -D-galactopyranoside



A subset of the total coliform group, known as the thermotolerant coliforms—coliforms that have the ability to ferment lactose at 44–45°C previously referred to as faecal coliforms—, were routinely used as faecal indicators since they were considered more faecal specific than total coliforms. By definition, thermotolerant coliforms include the portion of the total coliform group capable of forming gas within 24 hours at 44.5°C or that produce a blue colony on m-FC broth within 24 hours at 44.5°C (APHA et al., 2005). This group includes members of the genera *Escherichia*, which are faecal specific, as well as organisms that are found in both faecal and non-faecal environments such as *Klebsiella*, *Enterobacter*, and *Citrobacter*. Advances in *E. coli* detection methods have made thermotolerant coliform testing in drinking water quality management redundant.

## 4.2 Sources

The total coliform group is composed of various genera with similar characteristics. The natural niches for members of this group range from being faecal specific, such as *E. coli*, to being widely distributed in the water, soil, and vegetation (Leclerc et al., 2001; Rompré et al., 2002). Many coliform bacteria are not specific to any one source and are present in both faecal and non-faecal environments. A comparison of total coliforms within a specific environment has shown that some members of the coliform group can consistently be found in higher concentrations in that source. For example, analysis of the coliform complement of faecal matter found *Klebsiella*, *Citrobacter*, and *Enterobacter* present in small numbers compared with *E. coli* (Edberg et al., 2000). In contrast, the majority of coliforms isolated from a distribution system were *Klebsiella*, and to a lesser extent *Enterobacter*, *Pantoea*, *Escherichia*, *Citrobacter*, *Leclercia*, and *Serratia* (Geldreich, 1987; Edberg et al., 2000; Blanch, 2007).

The presence of coliforms in a distribution system, as opposed to the natural environment, can result from inadequately treated source water, which allows total coliforms to pass through the treatment system into the distribution system, or through intrusion of the organisms into the distribution system post-treatment (Besner, 2002; Blanch, 2007). A study by Kirmeyer et al. (1999) showed that coliforms could be detected surrounding distribution system pipelines; therefore, post-treatment contamination could result from numerous problems, such as pipe leaks with negative pressure events, pipe breaks, inadequate cleaning and disinfection after repairs, and cross-connections, including backflow, with non-potable water.

Once total coliforms are present in the distribution system, they can colonize and grow in the biofilm on pipe surfaces or in deposits. Their survival and possible growth depend on many factors, including water temperature, retention time of the water, type and concentration of disinfectant (if present), presence of organic nutrients, specifically the assimilable organic carbon (AOC) and the biodegradable dissolved organic carbon (BDOC) concentrations, inorganic nutrient availability, pipe material characteristics, and the presence of sediments (LeChevallier et al., 1991, 1996; Besner, 2001, 2002; Escobar and Randall, 2001; Blanch et al., 2007). Members of the coliform group differ in persistence and potential growth in water systems. *E. coli*, for example, is generally the most sensitive to environmental stressors and does not usually grow outside the human or animal gut (Geldreich, 1996). *Klebsiella*, on the other hand, is able to survive and grow in drinking water biofilms, on the interior surface of water mains, and in storage tanks (Ptak et al., 1973; LeChevallier et al., 1987; LeChevallier and McFeters, 1990; Blanch et al., 2007).

It is difficult to eliminate total coliforms once they have colonized biofilm matrices in distribution systems as the biofilm can shield the coliforms from disinfection and other eradication measures (Martin et al., 1982; Geldreich and Rice, 1987). Detachment of colonized

biofilm into the bulk water can result in total coliform detections in the distribution system (McMath, 1999). For example, activities such as hydrant tests and fire-fighting can cause surges in water mains resulting in the sloughing of biofilm and a subsequent rise in total coliform bacterial counts (Kirmeyer et al., 1999).

### **4.3 Role of total coliforms in maintaining drinking water quality**

The best means of safeguarding against the presence of waterborne pathogens in drinking water is the application of the multi-barrier approach. This approach should include assessment of the entire drinking water system, from the watershed or aquifer and intake through the treatment and distribution chain to the consumer, to assess the potential effects on drinking water quality and public health. Total coliforms are one of several indicators that are used as part of this approach. Their role in maintaining drinking water quality varies depending on where they are being measured in the drinking water system.

#### *4.3.1 Role in source water monitoring*

Because total coliforms are present in both faecal and non-faecal environments, they are not good indicators of faecal contamination. Consequently, monitoring total coliforms in raw surface water or GUDI sources does not provide information on the quality of the source water from the perspective of health risk. Other means of assessing surface waters and GUDI sources should be used to identify potential sources of faecal contamination in the watershed or aquifer that may affect the quality of the water.

Total coliform presence in groundwater sources, on the other hand, can be used to indicate that the groundwater source may be vulnerable to contamination. There is some research supporting a link between the presence of pathogens and total coliforms in groundwaters (Abbaszadegan et al., 2003; Locas et al., 2007), although because total coliforms only indicate a vulnerability to contamination, they may be present without pathogens being detected (Borchardt, 2003, Marrero-Ortiz 2009). However, the absence of total coliforms in a single sample does not necessarily indicate that the groundwater is less vulnerable to faecal contamination. There is some research that suggests groundwater sources should be sampled multiple times (i.e., 10 or more) to determine their sanitary status (Atherholt, 2003). This supports the recommendation that a history of bacteriological sampling, together with a sanitary survey and other contaminant monitoring, is needed to understand the quality of the groundwater. Collection and analysis of larger water samples may also be beneficial for determining whether a groundwater is vulnerable to contamination (Fujioka and Yoneyama, 2001; Atherholt 2003). In an investigation in Finland of three outbreaks caused by *Campylobacter* from groundwater sources, Hanninen (2003) showed that large volumes (1000–2000 mL) of water needed to be collected to detect indicator bacteria (*E. coli*) in the well water. Although collecting larger samples can provide additional information, there can be difficulties associated with analyzing large volumes of water using the current standard methods.

#### *4.3.2 Role in treatment and distribution system monitoring*

As operational indicators, total coliforms provide information on the adequacy of drinking water treatment and on the microbial condition of the distribution system. In a treated drinking water system, where each barrier in the drinking water system has been controlled to ensure that it is operating adequately based on the quality of the source water, total coliforms can be used as part of the verification process to show that the water has been adequately treated and is of an acceptable microbiological quality as it leaves the treatment plant. The presence of any



total coliform bacteria in water leaving a treatment plant shows inadequate treatment, is unacceptable, and should be corrected immediately.

If total coliforms are absent from water leaving the treatment plant but are detected in the distribution system, bacterial regrowth or post-treatment contamination may have occurred. Several studies (LeChevallier et al., 1987; LeChevallier and McFeters, 1990; Edberg et al., 1994) have documented that *Enterobacter* and *Klebsiella* frequently colonize the interior surfaces of water mains and storage tanks when conditions are favourable. Post-treatment contamination (e.g., through cross-connections, back siphonage, low pressure events), contamination of storage reservoirs, and contamination of mains from repairs have been identified as causes of distribution-system contamination linked to illness (Craun and Calderon, 2001; Hunter, 2005). A U.S. study comparing water systems for the presence of outbreaks and violations of the Total Coliform Rule found no significant difference in total coliform violations between areas with and without outbreaks of waterborne illness (Nwachuku et al., 2002). Therefore, although the presence of total coliforms in the absence of *E. coli* is of no immediate public health significance, total coliform detection should trigger an investigation and corrective actions in order to maintain the overall bacteriological quality of the water. Corrective actions, such as routine distribution system flushing, have been reported to help limit microbial regrowth in the distribution system (Lehtola, 2004). Other indicators can be used in conjunction with total coliform testing to assess the conditions that favour microbiological growth or intrusion of untreated water into the distribution system. These other indicators include turbidity, disinfectant residual, *E. coli*, aerobic endospores, and coliphage monitoring (LeChevallier et al., 2006; Cartier, 2009; Health Canada, 2012a, 2013).

#### 4.3.3 Considerations for residential-scale systems

In disinfected residential-scale systems, total coliforms are considered operational indicators. Their presence provides evidence of the inadequacy of disinfection or deterioration of water quality in the system. The presence of total coliforms in non-disinfected wells indicates that the well is either prone to surface water infiltration and therefore at risk of faecal contamination, or that bacterial regrowth is occurring within the well or plumbing system (if the sample is not taken directly from the well). Implementation of corrective actions, such as shock-chlorination and flushing, provides valuable information on the source of the total coliform bacteria. Microbial regrowth problems should be solved after these actions have been taken. The continued presence of total coliforms after shock-chlorination is probably the result of infiltration, indicating that the system is vulnerable to contamination with pathogenic microorganisms. The extent of the contamination (e.g., how many samples tested positive and the locations where they were collected) can also be used to aid in determining the cause of the contamination, interim protective measures, and the necessary corrective actions. Examples of corrective actions are outlined in section 3.2.

## 5.0 Analytical methods

In Canada, three methods are currently used for routine monitoring of total coliforms in water: presence–absence (P-A), membrane filter (MF), and multiple tube fermentation (MTF) procedures. A detailed description of these methods can be found in APHA et al. (2005).

All three detection methods use cultivation to detect or confirm the presence of total coliforms. Cultivation media can be broadly categorized into two types: (1) enzyme-based media containing a chromogenic substrate for the specific detection and confirmation of the total

coliforms in a single step (Feng and Hartman, 1982; Ley et al., 1988) and (2) presumptive coliform detection media that require additional steps to confirm the presence of total coliforms.

Methods that detect and confirm the presence of total coliforms in a single step are based on the presence of the enzyme  $\beta$ -galactosidase. By definition, all bacteria possessing the enzyme  $\beta$ -galactosidase belong to the total coliform group (APHA et al., 2005). The  $\beta$ -galactosidase activity of total coliforms is used to hydrolyze a chromogenic substrate—for example, *ortho*-nitrophenyl- $\beta$ -D-galactopyranoside—in the media to release a coloured compound (e.g., *ortho*-nitrophenol [yellow]), which changes the colour of the media. The change in medium colour indicates a positive total coliform result. There are some non-coliform bacteria, such as *Aeromonas* and *Pseudomonas* species, that can produce small amounts of  $\beta$ -galactosidase. However, they do not usually produce a positive response when they are present at low concentrations (APHA et al., 2005). However, if false-positive reactions caused by these other organisms are suspected, they can generally be ruled out by using the cytochrome oxidase test.

A distinct advantage of enzyme-based methods is that no confirmation step is required, so results are available in 24 hours or less. Both presence-absence and quantitative results are possible, depending on the enzyme-based method being used. Some enzyme-based methods are designed in such a way that they also inhibit non-coliform bacteria growth; thus, non-coliform bacteria do not interfere with the recovery of coliforms. This design is based on the principle that only the target microbe, in this case total coliforms, can utilize vital nutrients from the media (Rompré et al., 2002). Commercially available media and test kits are available that use this design. Enzyme-based methods are also capable of detecting total coliforms and simultaneously differentiating *E. coli* (Edberg et al., 1988). For these reasons, the use of enzyme-based methods is recommended. Presumptive coliform media, such as lauryl tryptose broth, m-Endo media, or LES Endo media (APHA et al., 2005), can be used for total coliform detection. When using lactose-based media for the P-A test or the MTF procedure, the formation of acid and/or gas after incubation for up to 24 hours at 35°C constitutes a positive presumptive test for total coliforms. Additional tests are required to confirm the presence of total coliforms. Using the MF procedure, colony characteristics, such as colour and surface sheen, are used for presumptive identification (APHA et al., 2005). Additional methods for the verification of total coliforms recovered by the MF technique have been described (Evans et al., 1981b; Standridge and Delfino, 1982; LeChevallier et al., 1983b).

Although multiple types of tests can be used, variability exists among these tests in their detection ability and quantification of total coliforms. Depending on the method used, non-coliform bacteria present in the sample can also affect the results (Olstadt, 2007). It is also important to use validated or standardized methods to make correct and timely public health decisions.

All analyses for total coliforms should be carried out as directed by the responsible authority. In many cases, the responsible authority will recommend or require the use of accredited laboratories. In some cases, it may be necessary to use other means to analyze samples in a timely manner, such as non-accredited laboratories or on-site testing using commercial test kits by trained operators. To ensure reliable results, a quality assurance (QA) program, which incorporates quality control (QC) practices, should be in place. In addition to the QA/QC program, any test kits used should meet minimum requirements for accuracy, detection (sensitivity), and reproducibility, and be used according to the manufacturer's instructions.

### 5.1 Presence–absence procedure

The P-A test is a qualitative procedure that was developed as a sensitive, economical, and efficient means of analyzing drinking water samples (Clark and Vlassoff, 1973). Essentially, it is a modification of the MTF procedure (see section 5.3) in which only one analysis bottle per sample is used. This test is therefore recommended only for the examination of a water supply for which a sequential or consecutive series of samples has been collected. Based on a typical 100-mL water sample, the detection limit of the procedure is one organism per 100 mL. This sensitivity is equal to that of the classical MTF and MF methods. This method can detect injured coliforms within the 24-hour response time (Rompré et al., 2002) and can be used with either enzyme-based media, or presumptive coliform media (e.g., using lauryl tryptose broth), with follow-up confirmation. Commercial test kits using enzyme-based media have been developed for P-A testing.

In comparative tests, the P-A method was at least as sensitive as the MF technique for the recovery of coliforms in drinking water samples (Clark, 1980; Jacobs et al., 1986; Pipes et al., 1986). In addition, a nationwide evaluation in the United States demonstrated no statistical difference in the number of coliform-positive samples obtained by the standard MTF method compared with the P-A procedure using enzyme-based methods (Edberg et al., 1989). Technically, the P-A test is simpler than the MF and MTF procedures and has a quicker initial processing time (less than 1 minute per sample). For lactose broth, there is a need to confirm positive results.

P-A testing does not provide any information on the actual concentration of organisms in the sample. The quantitation of organisms is sometimes used to assess the extent of the contamination, and as such is considered a benefit of the more quantitative methods such as the MF and MTF methods. For decision-making, the focus is the positive detection of total coliforms, regardless of quantity; as the guideline is for total coliform in drinking water is none per 100 mL, qualitative results are sufficient for protecting public health.

### 5.2 Membrane filter procedure

The MF procedure was introduced to bacteriological water analysis in 1951 after its capacity to produce results equivalent to those obtained by the MTF procedure was demonstrated (Clark et al., 1951; Goetz and Tsuneishi, 1951). It is a quantitative procedure that uses membrane filters with pore sizes sufficiently small to retain the target organisms. The water sample is filtered through the membrane, which is then transferred to an appropriate growth medium for identification and quantitation. Both enzyme-based methods and presumptive coliform media can be used. This procedure is able to examine larger volumes of water than can be examined with MTF, is more sensitive and reliable, and requires significantly reduced time, labour, equipment, space, and materials. These qualities have made the MF technique the method of choice in some jurisdictions for the routine enumeration of coliforms in drinking water. However, this method may underestimate the number of viable coliform bacteria in a sample. *Standard Methods for the Examination of Water and Wastewater* does provide 95% confidence limits for MF results (APHA et al., 2005). One disadvantage of the MF procedure is that it cannot be used on highly turbid water samples. The particulate matter concentrated by the filter can interfere with colony development and with the production of surface sheens used for visual detection of coliforms.

A major concern, for this and other methods that use stressful selective media (i.e., media that contain inhibitory chemicals for non-target organisms), is an inability to enumerate coliform bacteria that have been subjected to sublethal injury (e.g., caused by chlorination) in the

treatment plant or distribution system. Stressed organisms are often not able to grow on the selective coliform media but can recover through a resuscitation process. One significant improvement in the MF technique has been the development of a new medium (m-T7) for the enhanced recovery of stressed coliforms in drinking water (LeChevallier et al., 1983a). Evaluation of media using routine drinking water samples (LeChevallier et al., 1983b; McFeters et al., 1986) and surface water samples (McFeters et al., 1986; Freier and Hartman, 1987) showed a higher coliform recovery on the m-T7 medium compared with the m-Endo medium. In all of the above cases, chlorine was used as the stressing agent. Work using monochloraminated samples (Rice et al., 1987) and ozonated samples (Adams et al., 1989) showed that m-T7 performed no better than m-Endo agar in enumerating *E. coli* and *Citrobacter freundii*.

As noted previously, non-coliform heterotrophic bacteria may interfere with the recovery of coliforms when using a lactose-based medium. Data from the U.S. National Community Supply Survey (Geldreich et al., 1972) showed that the recovery of total coliforms using the MF technique decreased as the concentration of heterotrophic bacteria increased. The greatest reduction occurred when the heterotrophic plate count (HPC) exceeded 500 colony-forming units (CFU)/mL. Some researchers have shown that the composition of the heterotrophic flora may also be important. Burlingame et al. (1984) demonstrated that *Pseudomonas aeruginosa* (30 CFU/mL) and *A. hydrophila* (2 CFU/mL) caused significant reductions in sheen production by coliforms on m-Endo LES agar. *Flavobacterium* sp. and *Bacillus* sp., in contrast, were not inhibitory, even at concentrations above 1000 CFU/mL. Standridge and Sonzogni (1988) evaluated two modifications of the MF technique for total coliforms in drinking water containing high background counts. In both cases, roughly 8% of the plates originally classified as coliform negative but overgrown—i.e., confluent growth or background of more than 100 CFU/100 mL—yielded coliforms. However, most water supplies maintaining a total chlorine residual of 0.2 mg/L have an HPC below 500 CFU/mL (LeChevallier, 1990). Further information on HPC and their significance in drinking water, can be found in Health Canada (2012b).

### 5.3 Multiple tube fermentation procedure

The MTF procedure, in comparison with the MF procedure, lacks precision, is more difficult to perform, and takes longer to produce results. However, the MTF procedure is still of value when conditions render the MF technique unusable—for example, with turbid, coloured, or grossly contaminated water—and as a comparative procedure.

In the MTF procedure, 10-fold dilutions of water to be tested are added to tubes containing the appropriate media (5 or 10 tubes per dilution) and incubated. Both enzyme-based methods and presumptive coliform media can be used. For drinking water, dilution should be unnecessary because of the expected low counts. With enzyme-based methods, a confirmation step is not necessary. As mentioned previously, media containing a substrate designed for the detection of the coliform-specific enzyme  $\beta$ -galactosidase undergo a specified colour change to signify a positive confirmed total coliform result. Using presumptive media, additional tests to confirm the presence of total coliforms are required. For example, the presence of total coliforms can be confirmed with a brilliant green lactose bile broth. The formation of gas in this fermentation tube at any time within 48 hours at 35°C constitutes a positive confirmation test (Rompré et al., 2002). Regardless of media type, results are reported as a most probable number (MPN). The MPN is only a *statistical* estimate of the number of bacteria that, more than any other number, would probably give the observed result; it is not an actual count of the bacteria present. *Standard Methods for the Examination of Water and Wastewater* provides 95% confidence limits for MPN results (APHA et al., 2005). Commercial kits are available for MPN

determinations. The most widely publicized kits use a multi-well plate containing specific media and the enzyme  $\beta$ -galactosidase. A water sample is added to the plate. The wells that contain total coliforms undergo a specified colour change. The number of positive wells is then used to calculate the MPN.

High densities of non-coliform bacteria and the inhibitory nature of some MTF media may have an adverse influence on routine coliform monitoring procedures. Seidler et al. (1981) showed that the recovery of total coliforms by MTF decreased as the concentration of HPC bacteria increased, with the greatest reduction occurring when the HPC densities exceeded 250 CFU/mL. LeChevallier and McFeters (1985) hypothesized that competition for limiting organic carbon was responsible for the interference with total coliform recovery by HPC bacteria. The recovery of coliforms from gas-negative but turbid MTF tubes has demonstrated the presence of inhibitory compounds in the MTF media. When lauryl tryptose broth was the primary medium, coliform isolations from turbid gas-negative tubes increased the numbers of positive tubes in an MTF analysis by as much as 28% (McFeters et al., 1982). Comparative studies using brilliant green lactose bile broth and m-Endo LES agar as confirmatory media also demonstrated that brilliant green lactose bile broth can inhibit the growth of some coliforms. Evans et al. (1981a) developed a procedure to detect false-negative reactions. Using a modified MTF technique, the incidence of coliform detection was twice that of the standard MTF technique for drinking water. In response to these findings, the most recent edition of *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005) recommends treating all tubes with bacterial growth, regardless of gas production, as presumptive coliform-positive tubes that should be submitted to confirmation tests.

## **6.0 Sampling for total coliforms**

### **6.1 Sample collection**

Proper procedures for collecting samples must be observed to ensure that the samples are representative of the water being examined. Detailed instructions on the collection of samples for bacteriological analysis are given in *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005). To avoid unpredictable changes in the bacterial flora of the sample, examination should be started as soon as possible after collection. The sample should be transported to the laboratory in a cooler containing ice or cooling packs (at  $5 \pm 3^\circ\text{C}$ ), to minimize changes in populations and concentrations (Dutka and El-Shaarawi, 1980; McDaniels et al., 1985; ISO, 2006). As well, samples should be protected from direct contact with the ice or cooling packs to prevent freezing during transport. Ideally, the interval between collection of the sample and the beginning of its examination should not exceed 24 hours (Bartram and Rees, 2000), and analysis within 8 hours is recognized as the preferred time interval (Bartram and Rees, 2000; APHA et al., 2005). In remote areas, up to 48 hours may be an acceptable time interval; however, the implications of the extended holding time should be discussed with the responsible authorities. When delays are anticipated, a delayed incubation procedure should be employed or consideration given to on-site testing. The delayed incubation procedure is described in APHA et al. (2005). Alternatively, if normal transportation time exceeds the above recommendations, the sample should be processed and arrangements made to have another sample collected as soon as the first sample is received. Thus, if the late sample contains coliforms, a repeat sample will already have been received or will be in transit. Samples should be labelled with the time, date, location, type of sample (e.g., raw water, distribution system),



sampler's name, and identification number (if used), along with the disinfectant residual measurements and any special conditions. In most cases, much of this information, along with the identification number linked to the sample bottle, is recorded on accompanying submission forms and, in cases where samples are collected for legal purposes, chain-of-custody paperwork. When examination will be delayed, it is particularly important to record the duration and temperature of storage, as this information should be taken into consideration when interpreting the results.

A minimum volume of 100 mL of water should be examined to obtain a reliable estimate of the number of organisms (using MTF or MF) or to obtain an accurate P-A result at the expected low levels in treated drinking water. For the MTF method, a test series consisting of one 50-mL volume and five 10-mL volumes is suggested by the World Health Organization (WHO 1971) for water expected to be of good quality. Examination of larger volumes, such as in groundwaters with very low levels of contamination, can increase both the test sensitivity and the test reliability. Smaller volumes, dilutions, or other MTF combinations may be more appropriate for waters of poor quality.

## 6.2 Sampling frequency considerations

The World Health Organization lists the following factors that should be taken into account when determining sampling frequency for municipal-scale systems (WHO, 1971, 1976, 2004):

- past frequency of unsatisfactory samples
- source water quality
- the number of raw water sources
- the adequacy of treatment and capacity of the treatment plant
- the size and complexity of the distribution system
- the practice of disinfection.

These variables preclude application of a universal sampling frequency formula. Instead, the sampling frequency and location of sampling points should be decided upon by the responsible authority after due consideration of local conditions, for example, variations in raw water quality and a history of treated water quality. The sampling frequency should meet all jurisdictional requirements.

As a minimum, water leaving a treatment plant should be tested daily for disinfectant residual and turbidity and at least weekly for total coliforms as part of the verification process in a source-to-tap multi-barrier approach. Recommended sampling frequencies are presented in Table 2. In many systems, the water leaving the treatment plant will be tested for these indicators well in excess of the minimum requirements. For supplies where weekly total coliform testing is impractical (e.g., in small supplies), total coliform sampling may be reduced and other means of verifying the microbiological quality may be used, such as residual disinfectant determinations and good process control. Small supplies should also periodically carry out sanitary surveys as an additional action to verify the safety of the system.

In a distribution system, the number of samples for bacteriological testing should be increased in accordance with the size of the population served. The general practice of basing sampling requirements on the population served recognizes that smaller water supply systems may have limited resources available for monitoring. However, because small water supplies have more facility deficiencies (Schuster et al., 2005) and are responsible for more disease

outbreaks than large ones (Schuster et al., 2005), emphasis should also be placed on identified problems based on source-to-tap assessments, including sanitary surveys.

**Table 2:** Recommended sampling frequency

<b>Population served</b>	<b>Minimum number of samples per month*</b>
Up to 5000	4
5000–90 000	1 per 1000 persons
90 000+	90 + (1 per 10 000 persons)

\*The water samples should be taken at regular intervals throughout the month. For example, if four samples are required per month, samples should be taken on a weekly basis.

Disinfectant residual and turbidity analyses tests should be conducted when bacteriological samples are taken in the distribution system. Further information on monitoring for turbidity can be found in the guideline technical document for turbidity (Health Canada, 2013). Routine verification of the concentration of the disinfectant residual and the bacteriological quality of the water ensures that immediate remedial action can be taken if water of doubtful quality enters the distribution system. The preceding frequencies (Table 2) are only general guides. For small systems, additional guidance may need to be considered by the responsible authority. In supplies with a history of high-quality water, it may be possible to reduce the number of samples taken for bacteriological analysis. Alternatively, supplies with variable water quality may be required to sample on a more frequent basis. Sampling frequencies in residential scale and private systems may vary from jurisdiction to jurisdiction but should include times when the risk of contamination is greatest, for example, spring thaw, heavy rains, or dry periods. New or rehabilitated wells should also be sampled initially to confirm acceptable bacteriological quality.

Even at the recommended sampling frequencies for total coliforms, there are limitations that need to be considered when interpreting the sampling results. Simulation studies have shown that it is very difficult to detect a contamination event in a distribution system unless the contamination occurs in a main, in a reservoir, at the treatment plant, or occurs for a long duration at a high concentration (Speight et al., 2004; van Lieverloo, 2007). Therefore, even if the analytical result indicates the absence of coliforms, intrusion may be occurring in the distribution system. Some improvement in detection capabilities were found when sampling programs were designed with the lowest standard deviation in time between sampling events (van Lieverloo, 2007), such as samples collected every 5 days, regardless of weekends and holidays. There are also some limitations that are inherent when analyzing for parameters that are considered rare events, such as total coliforms. Hrudey and Rizak (2004) have reported that because the rate of total coliform positives in most distribution systems is usually lower than the false-positive rate for the method, it is difficult to determine whether the results obtained are true positives. In addition, the low rate of positive samples means that it is difficult to see statistically significant differences in total coliform positive rates, for example, before and after a corrective action, unless a very large number of samples are evaluated (Rosen, 2009). These limitations highlight the importance of implementing a source-to-tap multi-barrier approach, as opposed to relying on a single parameter for determining the microbiological quality of the drinking water.

### **6.3 Location of sampling points**

In municipal-scale systems, the location of sampling points must be decided upon by the responsible authority. The sampling locations selected may differ depending on the monitoring objectives. For example, fixed sampling points may be used to help establish a history of water quality within the distribution system, whereas sampling at different locations throughout the distribution system may provide more coverage of the system. A combination of both types of monitoring is common (Narasimhan et al., 2004). Some information is available on how to select statistically based random sampling sites (Speight et al., 2004).

In general, samples should be taken at the point where the water enters the system and from representative points throughout the distribution system. If the water supply is obtained from more than one source, the location of sampling points in the system should ensure that water from each source is periodically sampled. Distribution system drawings can provide an understanding of water flows and directions and can aid in the selection of appropriate sampling locations. The majority of samples should be taken from potential problem areas: low-pressure zones, reservoirs, dead ends, areas at the periphery of the system farthest from the treatment plant, and areas with a poor previous record. In residential-scale systems, samples are generally collected from the locations recommended by the responsible authority. More extensive sampling may be necessary depending on the system and results from previous samples.

## **7.0 Treatment technology**

The application of a multi-barrier approach, including watershed or well-head protection, optimized treatment barriers, and a well-maintained distribution system, is the best approach to reduce the presence and associated health risks of waterborne pathogens to an acceptable level. Total coliforms are one of several indicators that are used as part of the multi-barrier approach.

An array of options is available for treating source waters to provide high-quality drinking water in municipal and residential scale systems. The quality of the source water will dictate the degree of treatment necessary. Generally, minimum treatment of supplies derived from surface water sources or groundwater under the direct influence of surface waters should include adequate filtration (or technologies providing an equivalent log reduction credit) and disinfection. All groundwaters should receive adequate treatment for the removal and/or inactivation of enteric viruses, unless exempted by the responsible authority based on site-specific considerations including historical and on-going monitoring data. In systems with a distribution system, a disinfectant residual should be maintained at all times.

### **7.1 Municipal scale**

In general, all drinking water supplies should be disinfected, and a disinfectant residual should be maintained throughout the distribution system at all times. In addition, surface water sources and groundwater under the direct influence of surface water should include physical removal methods, such as chemically assisted filtration (coagulation, flocculation, clarification, and filtration) or technologies that provide an equivalent log reduction credit for microorganisms. It is essential that the removal and disinfection targets are achieved before drinking water reaches the first consumer in the distribution system. Adequate process control measures and operator training are also required to ensure the effective operation of treatment barriers at all times (U.S. EPA, 1991; Health and Welfare Canada, 1993; AWWA, 1999).



### 7.1.1 *Level of treatment necessary*

Most source waters are subject to faecal contamination, as such, treatment technologies should be in place to achieve a minimum 4 log (99.99%) removal and/or inactivation of enteric viruses and a minimum 3 log (99.9%) removal and/or inactivation of enteric protozoa in accordance with the guideline technical documents on enteric viruses and protozoa (Health Canada, 2011, 2012c). Depending on the source water quality, a higher log reduction may be necessary to produce safe drinking water. Groundwater classified as less vulnerable to faecal contamination, using procedures determined by the responsible authority, should not have protozoa present. Therefore the minimum treatment requirements for protozoa would not apply. However, even these groundwater sources will have a degree of vulnerability and should be periodically reassessed. In general, protozoa and enteric viruses are more difficult to inactivate or remove than bacterial pathogens. Therefore, water that is treated to meet the guidelines for enteric viruses and enteric protozoa should have an acceptable bacteriological quality, including meeting the MAC for total coliforms of none detectable in 100 mL of water leaving the treatment plant.

### 7.1.2 *Physical removal*

The physical removal of coliform bacteria can be achieved using various types of filtration. A review of pilot- and full-scale study data concluded that coagulation, flocculation, and sedimentation processes were associated with 1.7 log bacteria (*E. coli*, coliforms, faecal streptococci) removal credit (range, 0.5 to 3.9 log) (Hijnen et al., 2004). In studies that included pre- and post-disinfection along with coagulation, flocculation, sedimentation, and filtration, total coliform concentrations were reduced to non-detectable levels in the finished water (5 to 6 log reduction) (Payment et al., 1985; El-Taweel et al., 2001). A review of slow sand filtration studies reported a 2.4 log removal credit for bacteria (range, 1.3 to 3.2 log) (Hijnen et al., 2004). Membrane filtration technologies are also capable of removing 4.0 log to greater than 6.0 log of *E. coli* (NSF, 2002). More detailed information on filtration techniques can be found in the guideline technical document on turbidity (Health Canada, 2013).

### 7.1.3 *Disinfection*

The commonly used drinking water disinfectants are chlorine, chloramine, ultraviolet (UV) light, ozone, and chlorine dioxide. Disinfection is typically applied after treatment processes that remove particles and organic matter. This strategy helps to ensure efficient inactivation of pathogens and minimizes the formation of disinfection byproducts (DBPs). Also, when describing microbial disinfection of drinking water, the term “inactivation” is used to indicate that the pathogen is no longer able to multiply within its host and is therefore non-infectious, although it may still be present.

#### 7.1.3.1 *Chemical disinfection*

Currently, chlorine is the most widely used disinfectant in the drinking water industry. It is a strong oxidant capable of inactivating bacteria and viruses present in bulk water, although, as with most chlorine-based disinfectants, it is not as effective for the control of protozoans. Chlorine is also less effective for inactivating organisms present in biofilms. In comparison with chlorine, chloramine is a weaker oxidant. This property is advantageous in that the disinfectant resides longer in a distribution system. It is therefore easier to maintain a disinfectant residual, and the disinfectant is better able to penetrate into the biofilm found in the pipes and reservoirs, leading to superior coliform control (LeChevallier et al., 1990; LeChevallier, 2003). However,

chloramine is less efficient at controlling a sudden pulse of contamination (Snead et al., 1980), and it can lead to nitrification. Chlorine dioxide is as effective as, and in some instances more effective than, chlorine. However, this compound is difficult to work with and therefore is not widely used. Ozone, compared with chlorine-based disinfectants, is more efficient for the inactivation of bacteria, viruses, and protozoa, although ozone treatment can result in an increase in biodegradable organic compounds that can promote bacterial regrowth in the distribution system. Ozone is highly effective at the point of treatment, but an additional disinfectant (usually chlorine or chloramine) needs to be added to supply a residual. Maintaining a disinfectant residual will limit the growth of organisms within the distribution system and, depending on the residual concentration, contact time, and the pathogens present, a disinfectant residual also may afford some protection against contamination from intrusion (Besner et al., 2008). The disappearance of the residual may also provide an immediate indication of the entry of oxidizable matter into the system or a malfunction of the treatment process.

The efficacy of chemical disinfectants can be predicted based on knowledge of the residual concentration of disinfectant, temperature, pH, and contact time (AWWA, 1999b). This relationship is commonly referred to as the CT concept, where CT is the product of “C” (the residual concentration of disinfectant, measured in mg/L) and “T” (the disinfectant contact time, measured in minutes). To account for disinfectant decay, the residual concentration is usually determined at the exit of the contact chamber rather than using the applied dose or initial concentration. Also, the contact time, “T”, is often calculated using a  $T_{10}$  value, such that 90% of the water meets or exceeds the required contact time. The  $T_{10}$  values can be estimated based on the geometry and flow conditions of the disinfection chamber or basin. Hydraulic tracer tests, however, are the most accurate method to determine the contact time under actual plant flow conditions.

CT values for 99% inactivation of *E. coli* using chlorine, chlorine dioxide, chloramine, and ozone are provided in Table 3. For comparison, CT values for *Giardia lamblia* and for viruses have also been included. In a well-operated treatment system, the CT provided will result in a much greater inactivation than 99%. From Table 3, it is apparent that, in comparison with most protozoans and viruses, coliform bacteria are easier to inactivate using the common chemical disinfectants. Also, chloramines have a much higher CT value than any of the other disinfectants listed. This means that to achieve the same level of inactivation with chloramine, a higher disinfectant concentration or a longer contact time, or a combination of both, is necessary. This is consistent with the properties of chloramine as a disinfectant, as previously described.

**Table 3:** CT values for 99% inactivation at 5°C

Disinfectant agent	pH	<i>E. coli</i> <sup>a</sup> (mg·min/L)	<i>Giardia lamblia</i> <sup>b</sup> (mg·min/L)	Viruses <sup>b</sup> (mg·min/L)
Free chlorine	6–7	0.034–0.05	65–93	4.0 <sup>c</sup>
Chloramines	8–9	95–180	1470	857
Chlorine dioxide	6–7	0.4–0.75	17 <sup>c</sup>	5.6 <sup>c</sup>
Ozone	6–7	0.02	1.3	0.6

<sup>a</sup> From Hoff (1986).

<sup>b</sup> From U.S. EPA (1999).

<sup>c</sup> Value for pH 6.0-9.0

### 7.1.3.2 UV light disinfection

UV light disinfection is highly effective for inactivating many types of pathogens. Further information on inactivation of specific protozoan and viral pathogens can be found in the guideline technical documents on protozoa and enteric viruses (Health Canada, 2011, 2012c). Similar to ozone, UV light is highly effective at the point of treatment, but an additional disinfectant (usually chlorine or chloramine) needs to be added to supply a residual. When using UV light for the inactivation of *E. coli* (and other bacteria), the bacteria can undergo photo repair (Harris et al., 1987; Schoenen and Kolch, 1992; Zimmer and Slawson, 2002) and, to a lesser extent, dark repair. However, the amount of repair is not considered significant in drinking water treatment and distribution.

Log inactivations using UV light disinfection are listed in Table 4. *E. coli*, because of its importance as a public health indicator, has been used as a representative bacterial species. For comparison, UV light doses for representative protozoa and viruses have also been included. Review of the data on inactivation using UV light (Table 4) shows that, of the representative organisms, bacteria (in this instance, *E. coli*) and protozoa require comparable doses of UV light to achieve the same level of inactivation, whereas certain viruses are much more resistant.

**Table 4:** UV light dose (mJ/cm<sup>2</sup>) required for inactivation

Log inactivation	<i>E. coli</i> <sup>a,d</sup>	<i>Cryptosporidium</i> <sup>a</sup>	Adenovirus <sup>a,c,d</sup>	Rotavirus <sup>a,c,d</sup>	<i>Giardia</i> <sup>a</sup>
1	1.5–5	2.5	42–58	7.1–10	2.1
2	2.8–9	5.8	83–111	15–20	5.2
3	4.1–14	12	129–167	23–29	11
4	5.0–18	22	167–186	36–40	22

<sup>a</sup> Based on U.S. EPA (2003).

<sup>b</sup> Adenoviruses are highly UV resistant in comparison with other enteric viruses; see Health Canada (2011).

<sup>c</sup> Lechevallier and Au (2004).

<sup>d</sup> Hijnen et al., (2006).

## 7.2 Residential scale

Residential-scale treatment is also applicable to small drinking water systems. This could include both privately owned systems and systems with minimal or no distribution system that provide water to the public from a facility not connected to a municipal supply (previously referred to as semi-public systems).

Various options are available for treating source waters to provide high-quality pathogen-free drinking water. These include filtration and disinfection with chlorine-based compounds or alternative technologies, such as UV light. These technologies are similar to the municipal treatment barriers, but on a smaller scale. In addition, there are other treatment processes, such

as distillation, that can be practically applied only to small or individual water supplies. Most of these technologies have been incorporated into point-of-entry devices, which treat all water entering the system, or point-of-use devices, which treat water at only a single location—for example, at the kitchen tap. It is important to note that if point-of-use devices are used instead of a point-of-entry system, all points of water used for drinking, food and beverage preparation, hygiene or washing dishes should be equipped with a point-of-use treatment device, to minimize the potential public health risks when use of microbiologically-contaminated drinking water.

The use of UV light has increased owing to its availability, relative ease of operation, and its ability to inactivate a range of pathogenic organisms. However, scaling or fouling of the UV lamp surface is a common problem when applying UV light to raw water with moderate or high levels of hardness, such as groundwater. UV light systems are often preceded by a pretreatment filter to reduce scaling or fouling. A pretreatment filter may also be needed to achieve the water quality that is required for the UV system to operate properly. In addition, regular cleaning and replacement of the lamp, according to manufacturer's instructions, are critical in ensuring the proper functioning of the unit. Alternatively, special UV lamp-cleaning mechanisms or water softeners can be used to overcome this scaling problem.

Health Canada does not recommend specific brands of drinking water treatment devices, but it strongly recommends that consumers look for a mark or label indicating that the device has been certified by an accredited certification body as meeting the appropriate NSF International (NSF)/American National Standards Institute (ANSI) standard. These standards have been designed to safeguard drinking water by helping to ensure the safety of material and performance of products that come into contact with drinking water.

For example, NSF/ANSI Standard 55 (Ultraviolet Disinfection Systems) provides performance criteria for two categories of certified systems, Class A and Class B. UV systems certified to NSF/ANSI Standard 55 Class A are designed to deliver a UV dose at least equivalent to 40 mJ/cm<sup>2</sup> in order to inactivate microorganisms, including bacteria, viruses, *Cryptosporidium* oocysts, and *Giardia* cysts, from contaminated water. However, they are not designed to treat wastewater or water contaminated with raw sewage and should be installed in visually clear water. Also, systems certified to NSF Standard 55 Class B systems are intended for a drinking water supply that is already disinfected, tested, and deemed acceptable for human consumption. The NSF standard 62 for Drinking Water Distillation Systems also includes reduction claims for bacteria. To meet this standard, a distillation system must provide a minimum 6 log reduction of bacteria and bacterial spores. Distillation systems should only be installed at the point of use as the water they have treated may be corrosive to internal plumbing components.

Certification organizations provide assurance that a product or service conforms to applicable standards. In Canada, the following organizations have been accredited by the Standards Council of Canada ([www.scc.ca](http://www.scc.ca)) to certify drinking water devices and materials as meeting the appropriate NSF/ANSI standards:

- Canadian Standards Association International ([www.csa-international.org](http://www.csa-international.org))
- NSF International ([www.nsf.org](http://www.nsf.org))
- Underwriters Laboratories, Inc. ([www.ul.com](http://www.ul.com))
- Quality Auditing Institute Ltd. ([www.qai.org](http://www.qai.org))
- Water Quality Association ([www.wqa.org](http://www.wqa.org))
- International Association of Plumbing and Mechanical Officials ([www.iapmo.org](http://www.iapmo.org))

## 8.0 Risk assessment

The adoption of a risk-based approach, such as a multi-barrier approach, is essential to the effective management of drinking water systems (CCME, 2004). This approach should include assessment of the entire drinking water system, from the watershed or aquifer and intake through the treatment and distribution chain to the consumer, to assess potential effects on drinking water quality and public health.

A health-based risk assessment is not relevant for total coliforms since they are only used as an indicator and their presence in drinking water is not considered a risk to human health. Risk assessments have been done for specific microbiological organisms that have health implications, such as enteric viruses and the enteric protozoa *Cryptosporidium* and *Giardia* (Health Canada, 2011, 2012c)

The detection of total coliform bacteria in drinking water provides important information about a drinking water system and therefore, they should be routinely used as part of a multi-barrier approach. In groundwaters that are less vulnerable to faecal contamination, the presence of total coliforms signals that intrusion into the groundwater has occurred from surface water sources, that contamination occurred after construction of a new well or after repair or replacement of any part of the well or pump, or that growth of total coliform bacteria is occurring in the system. Total coliform bacteria are susceptible to the processes commonly used in drinking water treatment. Therefore, the presence of total coliforms in water leaving a municipal- or residential-scale treatment plant indicates a problem with the treatment. In a municipal-scale distribution system, total coliforms may result from bacterial regrowth within distribution system biofilms, so occasional detections may occur. Occasional detections (e.g., up to 10% of samples) in municipal-scale systems do not necessarily indicate a degradation in water quality and are considered acceptable. In residential-scale distribution systems, fewer samples are taken; therefore, the presence of total coliform bacteria should be investigated to determine their source and the implications for the water quality. Monitoring for total coliforms, when used in conjunction with a source-to-tap multi-barrier approach, is used as part of the verification that the drinking water system is supplying water that is of acceptable microbiological quality.

## 8.1 International considerations

Other countries use total coliforms for similar purposes. The Drinking Water Inspectorate of England and Wales has included in its regulations a mandatory value of zero coliforms per 100 mL in water leaving treatment works, a mandatory value of zero coliforms per 100 mL in 95% of samples for water in service reservoirs, and a non-mandatory value of zero coliforms per 100 mL at the consumer's tap. In these regulations, non-mandatory values do not need to be met, but exceedances need to be investigated and actions taken only if they represent a health risk (DWI, 2000). These regulations are based on the European Union's Council Directive on the quality of water intended for human consumption (Council of the European Union, 1998). The Australian Drinking Water Guidelines (NHMRC, 2004) include total coliforms as an indicator for operational monitoring. They do not set a guideline value for the parameter. Instead, if total coliforms are used for monitoring by a drinking water system, a value should be established on a system-specific basis taking into consideration historical information and system characteristics. The proposed revision to the Total Coliform Rule in the United States establishes a health goal (Maximum Contaminant Level Goal, or MCLG) and an MCL for *E. coli* and eliminates the MCLG and MCL for total coliform, replacing it with a treatment technique for coliforms that

requires assessment and corrective action (U.S. EPA, 2010). Under this treatment technique, a water system that exceeds a specified frequency of total coliforms' occurrence must conduct an assessment to determine if any sanitary defect exists and, if found, correct them. This proposed change means that, instead of triggering a non-acute MCL violation, the presence of total coliforms at a level that exceeds the treatment-technique trigger indicates potential pathways of contamination exist in the distribution system. An assessment of the system must be conducted, and any sanitary defect identified has to be corrected.

## 9.0 Rationale

Total coliform bacteria are widely distributed in surface waters and groundwaters under the direct influence of surface water, soil, and vegetation, and they are also susceptible to drinking water treatment. They are not usually found in protected groundwater sources. Sampling and analysis for total coliforms is an easy, relatively quick, inexpensive way of monitoring water. Because total coliforms are widespread in the environment, they can be used as one of the many operational tools to determine the efficacy of a drinking water treatment system. Total coliforms can also be used to indicate issues with groundwaters that are less vulnerable to faecal contamination since total coliforms should not be found in these sources. Total coliforms can colonize and grow within the biofilm that builds up on surfaces in drinking water systems.

The maximum acceptable concentration (MAC) for total coliforms in water leaving a treatment plant and in non-disinfected groundwater leaving the well is proposed as none detectable per 100 mL. In a distribution system, total coliforms should be monitored because they are used to indicate changes in water quality. Detection of total coliforms from consecutive samples from the same site or from more than 10% of the samples collected in a given sampling period should be investigated.

The sampling scheme for the MAC to be applied in distribution systems takes into account that occasional detection of total coliforms is expected to occur in a municipal-scale distribution system and does not necessarily indicate degradation of water quality. The sampling requirements are based on practical considerations and established practice.

The absence of total coliforms, when used in conjunction with a source-to-tap multi-barrier approach, is used as part of the verification that the drinking water system is producing water that is microbiologically acceptable.

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## Total coliforms (March 2012)

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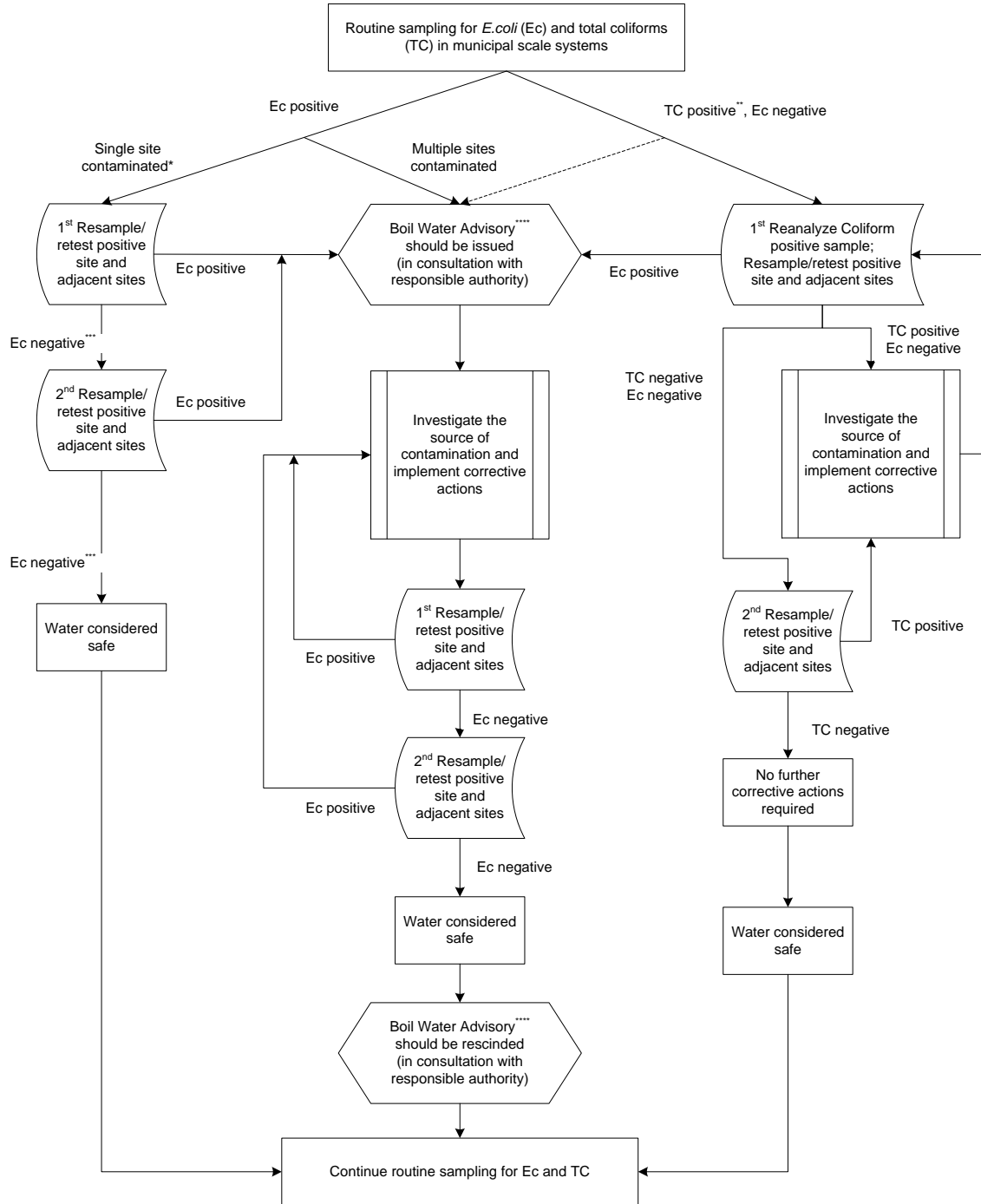
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## Appendix A: Decision tree for routine microbiological testing of municipal scale systems



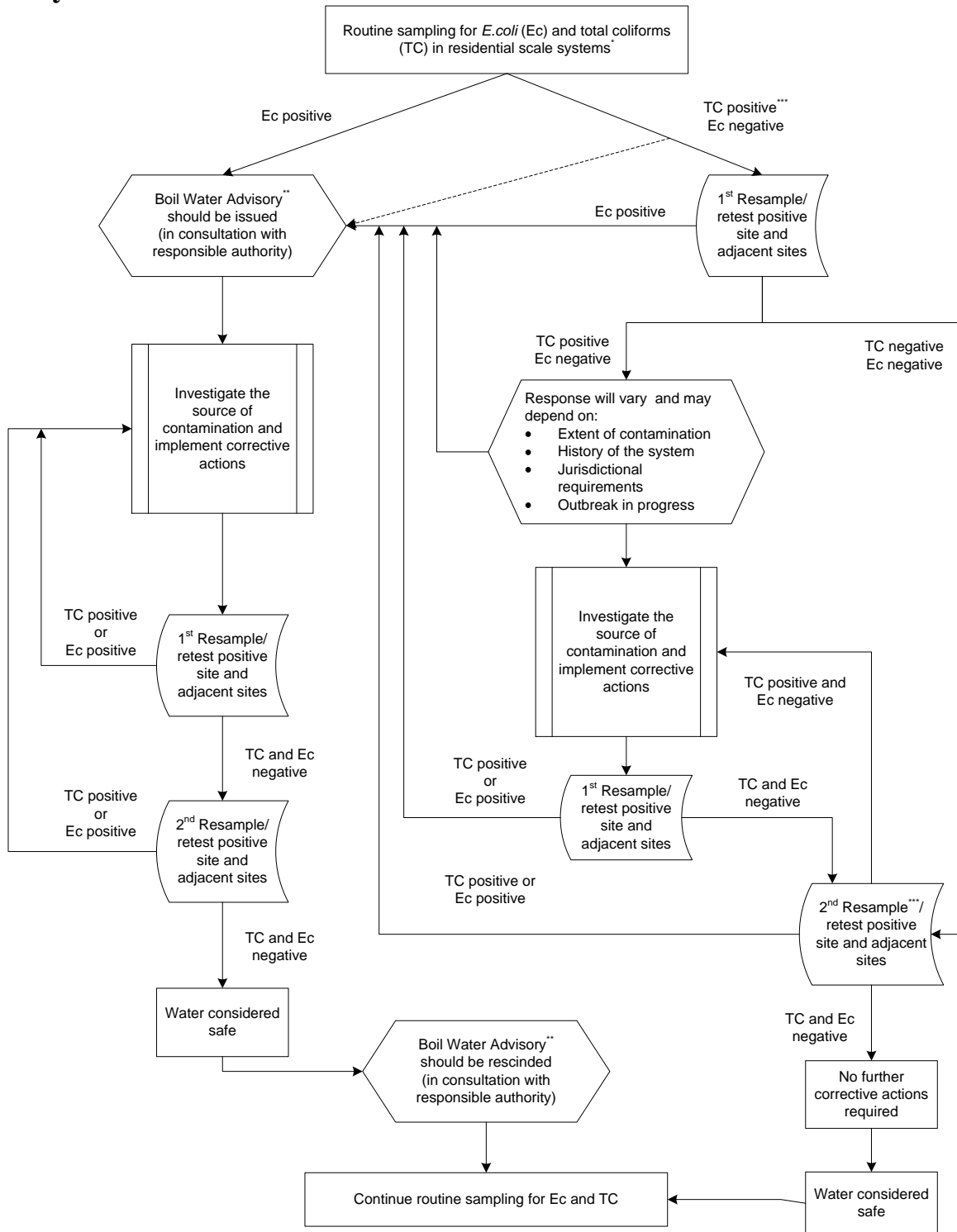
\*A boil water advisory may be issued on a single site contamination if deemed necessary by the responsible authority

\*\*A boil water advisory may be issued based on a positive total coliform, in the absence of *E. coli*, if deemed necessary by the responsible authority.

\*\*\*If a total coliform positive sample is detected during resampling for *E. coli*, the decision route for detection of a total coliform positive sample, in the absence of *E. coli*, should be followed (right-hand side of the decision tree).

\*\*\*\*Depending on the jurisdiction, "boil water order" may be used in place of, or in conjunction with, "boil water advisory."

## Appendix B: Decision tree for routine microbiological testing of residential scale systems



\* Private systems (eg. an individual well serving a rural home) are responsible for the microbiological quality of the water serving the system. Nevertheless, health authorities should be willing to provide advice on remedial actions, when necessary.

\*\* Depending on the jurisdiction, "boil water order" may be used in place of, or in conjunction with, "boil water advisory."

\*\*\* A boil water advisory may be issued based on a single positive TC result, if deemed necessary by the responsible authority.

## Appendix C: List of acronyms

ANSI	American National Standards Institute
AOC	assimilable organic carbon
CFU	colony-forming unit
EPA	Environmental Protection Agency (U.S.)
GUDI	groundwater under the direct influence of surface water
HPC	heterotrophic plate count
MAC	maximum acceptable concentration
MCL	maximum contaminant level (U.S.)
MCLG	maximum contaminant level goal (U.S.)
MF	membrane filter
MPN	most probable number
MTF	multiple tube fermentation
NSF	NSF International
P-A	presence–absence
QA	quality assurance
QC	quality control
UV	ultraviolet