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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

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Part I. Overview and Application

1.0 Guidelines

The maximum acceptable concentration (MAC) for 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 completed its review on the usefulness of total coliforms as part of a source-to-tap 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 guidelines for total coliforms in water leaving a treatment plant and in non-disinfected groundwater leaving the well is reaffirmed 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 and their sources

Monitoring of total coliforms should be used, in conjunction with other indicators, as part of a source-to-tap approach to producing drinking water of an acceptable quality. Total coliforms are naturally found in both fecal and non-fecal 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 provides 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 treatment has been inadequate 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 Treatment technology

Surface water or GUDI systems that meet the guidelines for enteric protozoa (minimum 3 log or 99.99% removal and/or inactivation) and enteric viruses (minimum 4 log or 99.99% removal and/or inactivation) and groundwater systems that meet the guidelines for enteric viruses (minimum 4 log or 99.99% removal and/or inactivation), will be capable of achieving the proposed MAC of none detectable per 100 mL for total coliforms.

For municipal-scale systems, it is important to apply a monitoring approach which includes the use of multiple operational and water quality verification parameters (e.g., turbidity, disinfection measurements, *Escherichia coli* [*E. coli*], total coliforms), in order to verify that the water has been adequately treated and is therefore of an acceptable microbiological quality. For residential-scale systems, regular monitoring of bacterial indicators (e.g., total coliforms and *E. coli*) combined with monitoring of critical processes, regular physical inspections and a source water assessment can be used to confirm the quality of the drinking water supply.

2.3 International considerations

The MAC and distribution system guidance for total coliforms are consistent with drinking water guidelines established by other countries and international organizations. The World Health Organization, the European Union, the United States Environmental Protection Agency (U.S. EPA) and the Australian National Health and Medical Research Council have all established provisions that state that total coliforms should be absent immediately after disinfection, and that their presence is indicative of inadequate treatment. They also include specific recommendations for total coliforms designed to minimize microbial risks from the drinking water distribution system.

3.0 Application of the guidelines

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 source-to-tap 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 presence 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, it 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 source-to-tap 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 the system, the number of consumers served, the history of the system, and other site-specific considerations.

3.1.2 Monitoring total coliforms in 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 specified and/or approved by the responsible authority. As an important part of a source-to-tap 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* (*E. coli*), 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 Guidelines for Canadian Drinking Water Quality: Guideline Technical Document for *E. coli* (Health Canada, 2020a).

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 section 3.1.4 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 authorities 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; and
- 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 drinking water treatment 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 the environment. 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 sample 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; and
- 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 advisory¹ should be discussed immediately with qualified operations personnel at the water utility and with the responsible authority. In the event that an incident that may have 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 in Canadian Drinking Water Supplies* (Health Canada, 2015).

Minimum treatment of supplies derived from surface water or GUDI sources should include adequate filtration (or technologies providing an equivalent log removal/inactivation) and disinfection to ensure the removal/inactivation of enteric protozoa and enteric viruses. For groundwater sources not under the direct influence of surface waters, adequate treatment is recommended to ensure the removal/inactivation of 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-scale² 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 (such as private wells) 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 for *E. coli*.

Responses to total coliform-positive samples in the absence of *E. coli* can vary from

¹ 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.”

² 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.

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 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.

Corrective actions for disinfected supplies

The first step is 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 into the environment. 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 ultraviolet (UV), 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 <https://www.canada.ca/en/health-canada/services/publications/healthy-living/water-talk-protect-clean-well.html>.
- 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 or GUDI sources should include adequate filtration (or technologies providing an equivalent log reduction credit) and disinfection to ensure the removal/inactivation of enteric protozoa and enteric viruses. For groundwater sources not under the direct influence of surface water, adequate treatment is recommended to ensure the removal/inactivation of 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 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 <https://www.canada.ca/en/health-canada/services/publications/healthy-living/water-talk-protect-clean-well.html>).
- 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 five days after the well has been treated. Local conditions may determine acceptable practice. Water should be dechlorinated before being discharged into the environment. 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 coliforms 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 three to four months to ensure that the contamination has not reoccurred. Over the long term, only a history of bacteriological and operational monitoring in conjunction with regular physical inspections and a source water assessment can be used to confirm the quality of a drinking water supply.

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 traditionally been defined based on their phenotypic characteristics, for example, their ability to ferment lactose, producing gas and acid (Leclerc et al., 2001; Rompré et al., 2002). *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2017) characterizes them 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; and
- All bacteria possessing the enzyme β -galactosidase, which cleaves a chromogenic substrate (e.g., *ortho*-nitrophenyl- β -D-galactopyranoside), resulting in the 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). It is important to note that non-fecal origins and/or adaptations to the environment have been found for organisms from all genera (Table 1) (Leclerc et al., 2001; Brennan et al. 2010; Byappanahalli and Ishii, 2011). Since phenotypic traits can be influenced by stress conditions, they cannot be consistently relied upon to detect all total coliforms all the time (e.g., injured organisms). Molecular characterization methods (see Section 5.2), including typing and sequencing, address this problem, and have led to a revised definition of total coliforms based on genetic relatedness (Fricker and Fricker, 1994; Maheux et al., 2014; Zhang et al., 2015). The total coliform group consists of 20 genera and over 80 species, including anaerogenic (producing little or no gas) lactose-fermenting microorganisms (Stevens et al., 2003; Brenner et al., 2005; Carrero-Colón et al., 2011; Maheux et al., 2014; APHA et al., 2017). Although not included in the coliform group, members of the genus *Aeromonas* can ferment lactose and possess β -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, drinking waters, and 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.

A subset of the total coliform group, known as the thermotolerant coliforms, (coliforms that have the ability to ferment lactose at 44 to 45°C previously referred to as fecal coliforms) were routinely used as fecal indicators since they were considered more fecal-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., 2017). This group includes members of the genera *Escherichia*, which are fecal specific, as well as organisms that are found in both fecal and non-

fecal environments such as *Klebsiella*, *Enterobacter*, and *Citrobacter*. Advances in *E. coli* detection methods have made thermotolerant coliform testing in drinking water quality management redundant.

Table 1: Selected coliform bacteria in the family *Enterobacteriaceae*^a

	ONPG ^b	Fecal origin	Non-fecal origin
<i>Budvicia</i>	+	—	+
<i>Citrobacter</i>	+	+	+
<i>Enterobacter</i>	+	+	+
<i>Erwinia</i>	+	—	+
<i>Escherichia</i>	+	+	+ ^c
<i>Klebsiella</i>	+	+	+
<i>Leclercia</i>	+	—	+
<i>Pantoea</i>	+	—	+
<i>Serratia</i>	+	—	+

^a Adapted from Leclerc et al. (2001)

^b *ortho*-Nitrophenyl-β-D-galactopyranoside

^c Health Canada, 2020a

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 predominantly fecal 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 fecal and non-fecal 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 fecal 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).

4.2.1 Groundwater

Total coliforms have been detected in various groundwater sources in Canada (Simpson, 2004; Payment and Locas, 2005; Locas et al., 2007, 2008; Hynds et al., 2012, 2014; Maier et al., 2014) and elsewhere (Amundson et al., 1988; Francy et al. 2000; Borchardt et al., 2003; Embrey and Runkle, 2006; Allevi et al., 2013; Atherholt et al. 2013, 2015, 2017), indicating that the groundwater is likely being impacted by surface or subsurface contamination, which may include fecal sources (DeSimone et al., 2009; Kozuskanich et al. 2011). Microbial contamination of groundwater sources can occur through various means, including infiltrating surface water, storm water, or point sources (e.g., septic tank effluents). The presence and concentration of total coliforms in groundwater can vary significantly, and is dependent on how efficiently these organisms can be transported from surface and subsurface sources. Transport of

total coliforms is affected by a number of factors, including:

- Aquifer type and geology (Embrey and Runkle, 2006; DeSimone et al., 2009; Toccalino and Hopple, 2010; Atherholt et al., 2013; Somaratne and Hallas, 2015);
- Well depth (Glanville et al., 1997; Gonzales, 2008; Allevi et al., 2013);
- Well integrity (Bacci and Chapman, 2011; Swistock et al., 2013; Hynds et al. 2012, 2014; Somaratne and Hallas, 2015); and
- Precipitation (Reid et al., 2003; Bacci and Chapman, 2011; Hynds et al., 2012; O'Dwyer et al., 2014; Procopio et al., 2017).

A number of studies have highlighted the impact of aquifer type on microorganism transport (Scandura and Sobsey, 1997; Powell et al., 2003; Embrey and Runkle, 2006; Borchardt et al., 2007; DeSimone et al., 2009; Toccalino and Hopple, 2010; Borchardt et al., 2011; Levison and Novakowski, 2012; Atherholt et al., 2013; Kozuskanich et al., 2014; Somaratne and Hallas, 2015). In general, groundwater supplies in karst and fractured bedrock aquifers are considered highly vulnerable to contamination because groundwater velocity and microorganism transport can be rapid. This was observed in a South Australian study, where higher coliform detections and levels were reported for karst and fractured rock aquifers; subtyping results showed that a variety of coliform species, including some of potentially fecal origin (e.g., *Hafnia*), were present in these groundwater supplies (Somaratne and Hallas, 2015).

Well characteristics, such as depth and integrity, can affect coliform presence. Shallow wells have been associated with higher total coliform detections (Sworobuk et al., 1987; Glanville et al., 1997; Gonzales, 2008; Allevi et al., 2013). Wells with integrity issues, such as cracked casings, have been correlated with higher total coliform concentrations (Lamka et al., 1980; Sworobuk et al., 1987; Bacci and Chapman, 2011; Swistock et al., 2013; Hynds et al., 2012, 2014; Somaratne and Hallas, 2015).

Rainfall events can have a significant impact on groundwater quality, by facilitating transportation of coliforms (and other microorganisms) from the surface (Goss et al., 1998; Fenlon et al., 2000; Samarajeewa et al., 2012; Méric et al., 2013) and subsurface (Krapac et al., 2002; Karathanasis et al., 2006; Bradbury et al., 2013; Hynds et al., 2014). Several studies have noted the impact of precipitation on coliform detection in groundwater (Reid et al., 2003; Bacci and Chapman, 2011; Hynds et al., 2012; Page et al., 2012; O'Dwyer et al., 2014). In a study by Procopio et al. (2017), the authors assessed the impact of antecedent precipitation on coliform detection rates in domestic wells. Total coliforms were detected more frequently when the cumulative precipitation in the 10 days prior to sampling exceeded 34.5 mm. An impact of seasonality was also noted by the authors, with increased coliform detection rates in the summer and autumn. This is consistent with the findings of others, who also noted increased coliform detections during specific times of the year (Raina et al., 1999; Rutter et al., 2000; Reid et al., 2003; Richardson et al., 2009; Atherholt et al., 2017).

4.2.2 *Drinking water distribution systems*

The drinking water distribution system is a complex environment, consisting of both resident and transient populations of microorganisms: the combined genetic material of these microorganisms is known as the microbiome. The microbiome is impacted by numerous factors, including nutrient availability, predation, and disinfectants (Zhang et al., 2017). Researchers have shown that microbiomes are very heterogeneous, as well as time and site-specific (within

and between distribution systems) (Gomez-Alvarez et al., 2012; Chao et al., 2013, 2015; Delafont et al., 2013; Wang et al., 2014; Zhang et al., 2017).

Coliforms have been isolated from a number of drinking water distribution systems in Canada (Coulibaly and Rodriguez, 2003, 2004; Besner et al., 2007; Gagnon et al., 2007; Locas et al., 2007; Payne et al., 2010) and elsewhere (LeChevallier, 1990; LeChevallier et al., 1996; Karim et al., 2003; Kilb et al., 2003; Batté et al., 2006; Mosher, 2011). They can be found in bulk water samples, and are associated with biofilm (Morin et al., 1996; Camper et al., 1999; Lee and Kim, 2003; Lee et al., 2005; Batté et al., 2006; Sakyi et al., 2012; Lee, 2013). Several studies have demonstrated the potential for coliforms, and other microbial contaminants, to enter the distribution system (Karim et al., 2003; LeChevallier et al., 2003; Besner et al., 2010, 2011; Yang et al., 2011; Ebacher et al., 2012; Fontanazza et al., 2015). Their presence in the distribution system is indicative of bacterial regrowth, system integrity issues (e.g., watermain break, system leaks, negative pressure events) and/or post-treatment contamination (e.g., cross-connections, non-sanitary construction/repairs) (Kirmeyer et al. 1999).

Once total coliforms are present in the distribution system, they can colonize and grow in the biofilm on pipe surfaces or in deposits. A number of factors can influence bacterial regrowth in the distribution system, including: type and concentration of organic and inorganic nutrients; type and concentration of residual disinfectant; biofilms and sediments; and distribution system conditions (e.g., disinfectant residual decay, water temperature, residence time, hydraulic conditions, pipe material and diameter, pH, corrosion rate) (LeChevallier et al., 1991b, 1996, 2015a; Besner et al., 2001, 2002, 2010, 2011; Escobar and Randall, 2001; Blanch et al., 2007; Lee, 2013; Prest et al., 2016a,b). For a comprehensive review of the impact of these factors on microbial growth in the drinking water distribution system, please refer to van der Kooij and van der Wielen, 2014; WHO, 2014; LeChevallier et al., 2015b; Prest et al., 2016a.

Biodegradable organic matter (BOM) impacts distribution system water quality by providing a source of nutrients that contributes to bacterial regrowth and biofilm development. Levels of BOM (e.g., assimilable and biodegradable organic carbon) are only one component influencing changes in water quality in the distribution system (Prest et al., 2016a,b). Other compounds have been identified as having roles in controlling microbial growth in the distribution system, including phosphorus, ammonia, manganese, iron and humic substances (Camper, 2004; Prest et al., 2016a,b; Health Canada, 2020b).

Pipe material can also impact bacterial regrowth and biofilm formation in the drinking water distribution system, although there does not appear to be a consensus regarding which materials most favour bacterial adhesion and growth (Haas et al., 1983; Schwartz et al., 1998; Niquette et al., 2000; Donlan, 2002; U.S. EPA, 2002; van der Kooij et al., 2005; White et al., 2011; Camper, 2014). In contrast, some have not observed any significant difference in bacterial concentrations or diversity among different pipe materials (i.e., stainless steel, Polyvinyl chloride [PVC] and polyethylene [PE]) (Pedersen, 1990; Zacheus et al., 2000; Wingender and Flemming, 2004).

Biofilms provide a habitat for the survival of microbial indicators, including total coliforms, and fecal pathogens that may have passed through drinking water treatment barriers or entered the distribution system directly via an integrity breach (Leclerc, 2003). Enteric pathogens have been detected in biofilms (Park et al., 2001; Howe et al., 2002; LeChevallier et al., 2003; Chang and Jung, 2004; Berry et al., 2006; September et al., 2007; Gomez-Alvarez et al., 2015; Revetta et al., 2016); although these organisms cannot grow in this environment, they can accumulate and be released over an extended period of time (Howe et al., 2002; Warnecke,

2006; Wingender and Flemming, 2011). Additionally, opportunistic premise plumbing pathogens (OPPPs), such as *Legionella pneumophila* and non-tuberculous mycobacteria (e.g., *M. avium*, *M. intracellulare*), have adapted to grow and persist in distribution and plumbing system biofilms. The potential for the multiplication of OPPPs in distribution system and plumbing system biofilms is of concern to the water utilities, especially in light of the fact that their presence does not correlate with that of microbial indicators (Falkinham et al., 2015; Wang et al., 2017).

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).

Overall, in the complex environment of the distribution system, total coliforms remain a useful indicator of potential bacterial regrowth and/or post-treatment contamination. Additional information on the drinking water distribution system can be found in *Guidance on monitoring the biological stability of drinking water in distribution systems* (Health Canada, 2020b).

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 source-to-tap approach. This approach should include an 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 fecal and non-fecal environments, they are not good indicators of fecal 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 fecal contamination in the watershed or aquifer that may affect the quality of the water.

4.3.1.1 Groundwater considerations

Total coliform presence in groundwater sources can be used to indicate that the groundwater source may be vulnerable to contamination. Groundwater is an important source of drinking water throughout the world (Chilton and Seiler, 2006), and it is often consumed with little or no treatment (Pedley et al., 2006). In Canada, 10% (3.3 million) of the population relies on a groundwater source (Statistics Canada, 2013a,b). Consuming fecally contaminated groundwater that is untreated or inadequately treated has been linked to illness, disease outbreaks (Yoder et al., 2008; Borchardt et al. 2011, 2012; Brunkard et al., 2011; Zhou et al. 2012; Gunnarsdóttir et al., 2013; Hilborn et al. 2013; Jack et al. 2013; Cho et al. 2014; Wallender et al. 2014; Beer et al., 2015; Guzman-Herrador et al. 2015). Uhlmann et al. (2009) estimated that individuals drinking water from (mostly untreated groundwater) private wells, in

a region of British Columbia, were 5.2 times more likely to develop acute gastrointestinal illness (AGI) than those drinking water from community (treated surface and groundwater) supplies. Borchardt et al. (2012) determined that between 6 and 22% of self-reported AGI cases in 13 Wisconsin communities were attributable to viruses in tap water from non-disinfected community groundwater supplies. Murphy et al. (2016a) estimated the burden of endemic AGI annually in Canada from tap water. The authors estimated that 103,230 AGI cases per year, or 0.003 cases/person-year, are due to the presence of pathogens in drinking water from private and small community water systems (defined by the authors as those serving less than 1000 people). Small community water systems relying on groundwater are estimated to account for 13,034 AGI cases per year, with the highest incidence, 0.027 cases/person-year, amongst systems without treatment (Murphy et al., 2016a).

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). Abbaszadegan et al. (2003) assessed the occurrence of viruses and microbial indicators in groundwater samples from 35 states in the United States (U.S.). Groundwater sources were located in a variety of hydrogeological settings and some were known to be under the influence of surface water. The authors reported that, overall, there was no significant correlation between microbial indicators, including total coliforms, and the presence of viruses. However, for sites where repeated sampling was conducted, there was an increased likelihood that samples testing positive for viruses would also test positive for microbial indicators, including total coliforms (i.e., positive predictive value increased) (Abbaszadegan et al., 2003). Borchardt et al. (2003) tested water samples from wells located in various geological strata, including highly vulnerable strata (e.g., fractured dolomite), for the presence of enteric viruses and water quality indicators. Twenty-five percent of the virus-impacted well samples were positive for total coliforms (Borchardt et al., 2003). Borchardt et al., (2004) did not detect any microbial indicators, including total coliforms, in municipal groundwater wells of varying susceptibility to surface water infiltration, in which enteric viruses were detected (using reverse transcription polymerase chain reaction [RT-PCR]). During an investigation of a large groundwater outbreak of gastrointestinal illness on South Bass Island, Ohio, no virus-indicator relationships were observed (Fong et al., 2007; O'Reilly et al., 2007). Similarly, no coliforms were detected in an untreated groundwater supply associated with a community campylobacteriosis outbreak in Finland (Kuusi et al., 2005).

A meta-analysis conducted by Hynds et al. (2014) focused on studies of groundwater systems in Canada and the U.S., between 1990 and 2013, that had reported contamination by enteric pathogens. No significant correlation was observed between enteric pathogens and total coliforms in groundwater. More recently, Fout et al. (2017) conducted a meta-analysis which included raw data from 12 international groundwater studies of 718 public drinking water systems located in different hydrogeological environments. Correlations between virus and indicator occurrence were assessed at the sample and well level. The authors concluded that total coliforms were statistically associated with both culturable virus and virus detected using RT-PCR, at the well level (Fout et al., 2017). Fout et al. (2017) also noted that the strength of the associations between microbial indicators and viruses changed depending on the hydrogeological setting, indicating the need for site-specific assessments. However, because total coliforms only indicate a vulnerability to contamination, they may be present without pathogens being detected (Borchardt, 2003, Marrero-Ortiz 2009).

The absence of total coliforms in a single sample does not necessarily indicate that the groundwater is less vulnerable to fecal 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). Fout et al. (2017) observed that repeat sampling for indicators, including total coliforms, improved their positive predictive value, and resulted in an improved understanding of the well's susceptibility to virus contamination. This supports the recommendation that a history of bacteriological sampling, together with a site-specific assessment 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 of three outbreaks in Finland caused by *Campylobacter* from groundwater sources, Hanninen (2003) showed that large volumes (1000 to 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.

Given the body of literature detailing the lack of correlation between total coliforms and enteric pathogens in groundwater sources, it is clear that they are not good indicators of pathogen presence in these sources. However, since their prevalence and concentrations appear to increase in situations of increased susceptibility to intrusion (e.g., after significant rainfall events, in vulnerable aquifer and geological strata types, in wells with compromised integrity), total coliforms are useful as indicators of the susceptibility of groundwater sources to contamination.

4.3.2 Role in treatment and distribution system monitoring

Distribution system contamination has been linked to a number of disease outbreaks (Craun and Calderon, 2001; Lee et al., 2002; Blackburn et al. 2004; Nygard et al. 2004, 2007; Hunter et al. 2005; Liang et al. 2006; NRC, 2006; Blokker et al., 2014). Although these outbreaks were associated with events that affected the integrity of the distribution system (e.g., watermain breaks), low level and/or intermittent contamination also occurs and contributes to endemic illness (Egorov et al., 2002; Hunter et al., 2005; Nygård et al., 2004, 2007; Messner et al., 2006; Córdoba et al., 2010; Lambertini et al., 2012; Murphy et al., 2016b). Messner et al. (2006) estimated the cases of highly credible gastrointestinal illness (HCGI) associated with drinking water among community water supplies in the U.S. The authors estimated that half of the HCGI cases (i.e., 0.02-0.06 cases per person-year) could be attributed to the distribution system. Lambertini et al. (2012) estimated the risk of AGI due to virus contamination of the distribution system in 14 municipal groundwater systems where UV disinfection had been implemented, without a chlorine residual. The AGI risk from distribution system contamination was calculated, and ranged from 0.0180 to 0.0611 episodes/person-year. These findings highlight the importance of distribution system integrity. Murphy et al. (2016b) estimated the burden of endemic AGI annually in Canada from tap water. The authors estimated that 334,966 AGI cases per year were associated with the consumption of tap water from municipal systems that serve over 1000 people in Canada; and that over 35% of these cases were attributable to the distribution system. It is clear that controlling water quality within the distribution system has a significant impact on public health.

The use of total coliforms for predicting the presence or absence of enteric pathogens in treated drinking water is hindered by the fact that enteric pathogens have different removal rates through physical processes, and dissimilar resistance to disinfectants (Hijnen and Medema, 2010). As evidence of this, enteric pathogens have been detected in filtered, treated drinking water meeting existing regulatory standards and have been linked to waterborne disease

outbreaks (LeChevallier et al., 1991a; Craun et al., 1997; Marshall et al., 1997; Nwachuku et al., 2002; Aboytes et al., 2004). Despite this, 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 watermains 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 U.S. EPA's revised 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).

Flushing (e.g., conventional flushing, unidirectional flushing) and chlorination are important corrective actions in response to fecal contamination or microbiological water quality deterioration issues (Szabo and Minamyer, 2014). However, if not properly implemented, flushing techniques can mobilize and spread deposits and contamination within the distribution system instead of facilitating their controlled removal (Hill et al., 2018). It is therefore important that water utilities identify and implement the most appropriate flushing technique for correcting or providing help with the particular microbiological water quality issue. Guidance for water utilities on managing water quality in the drinking water distribution system can be found in *Guidance on monitoring the biological stability of drinking water in distribution systems* (Health Canada, 2020b).

Despite their limitations, the presence of total coliforms in the drinking water distribution system is an indication of deterioration in microbiological quality. 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, enterococci, and coliphage monitoring (LeChevallier et al., 2006; Cartier, 2009; Health Canada, 2020a, 2013).

4.3.3 Considerations for residential-scale systems

Murphy et al. (2016a) estimated that private wells accounted for over 75% (78,073) of the estimated 103,230 AGI cases per year due to the presence of pathogens in drinking water from private and small community water systems in Canada. In North Carolina, DeFelice et al. (2016) estimated the total number of emergency department visits for AGI per year attributable

to microbiological contamination of unregulated private wells. The authors estimated that over 7% of emergency department visits from AGI, between 2007 and 2013, were related to microbial contamination of drinking water; and that of those cases related to drinking water, almost 99% were associated with the consumption of water from contaminated private wells. Their analyses also revealed a linkage between the prevalence of total coliforms and emergency room visits for AGI in one county. A 3% increase in visits was noted for every 10% increase in total coliform prevalence in private wells, providing support for the usefulness of this bacterial group as a meaningful indicator in untreated well water (DeFelice et al., 2016).

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 fecal 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

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 Culture-based methods

The *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2017) outlines the methods used for routine monitoring of total coliforms in Canadian drinking water: culture-based methods for the detection of total coliforms, including the presence-absence technique, membrane filtration (MF) technique, the multiple tube filtration (MTF) technique and an enzymatic substrate test (APHA et al., 2017). Formulations of the enzyme substrate are available commercially for use in a MTF, multi-well or presence-absence format (APHA et al., 2017). ISO Methods 9308-1, 9308-2 and 9308-3 are the specified methods of water analysis for total coliforms under the European Union (EU) Council Directive (EU, 1998; ISO, 1998, 2012, 2014). The U.S. EPA has approved several methods for the detection of total coliforms in water (U.S. EPA, 2017). Table 2 summarizes the features of the above methods.

5.1.1 Performance of culture based detection methods

A number of comparison studies have been conducted to assess culture-based methods, and have reported varying detection rates (Covert et al., 1989; Brenner et al., 1993; Hallas et al., 2003; Bernasconi et al., 2006; Chao, 2006; Olstadt et al., 2007; Maheux et al., 2008). A wide range of false positive rates have also been reported, ranging from 10% to 37.5%; and false negative rates as high as 48.6% have been reported (Covert et al., 1989; Brenner et al., 1993; Hallas et al., 2003; Bernasconi et al., 2006; Chao, 2006; Olstadt et al., 2007; Maheux et al., 2008, 2014). Maheux et al. (2015) compared four commercially-available culture-based methods (chromogenic test kits), and observed that their ability to detect total coliforms can vary significantly. The authors reported a detection difference of 47.2% between the best and worst performing methods when assessing total coliform strains of known origin. When these methods were used to assess well water samples (from Quebec), only two showed concordance – these two methods detected significantly more total coliforms than the others. Zhang et al. (2015) compared isolates detected using 12 coliform culture-based methods, approved by the U.S. EPA, by 16S rRNA gene sequencing. The authors reported a wide range of variability among the culture-based methods in detecting total coliforms, with false positive rates between 9.1% and 65.7%; and false negative rates ranged from 0.9% and 28.2%. These studies highlight the importance of analytical quality control measures, such as the use of multiple detection methods, when appropriate, performing repeat counts or duplicate analyses, and regular verification using known positive and negative controls.

There is a need to further evaluate the efficacy of these testing methods, and to improve their sensitivity and specificity. Water utilities should establish performance measures regarding method sensitivity and specificity when selecting analytical methods for internal analysis or when purchasing laboratory services.

5.2 Molecular methods

Given the issues with culture-based methods, molecular detection methods have been explored with limited success (Bej et al., 1990; Fricker and Friker, 1994; Tantawiwat et al., 2005; Liu and Stahl, 2007; Shaban et al., 2008; Worakhunpiset and Thampoophasiam, 2009; Fatemeh et al., 2014). The most significant challenge associated with drinking water analysis is the stricter limit for indicator organism presence, and therefore, the need for method sensitivity at very low concentrations. No molecular methods have been approved for drinking water compliance monitoring, and researchers continue to optimize approaches to detection (Figueras and Borrego, 2010; Maheux et al., 2014, 2017; Molina et al., 2015). Maheux et al. (2014) developed a polymerase chain reaction (PCR)-based assay, paired with a bacterial concentration and recovery procedure, aimed at detecting total coliforms in potable (well) water samples. The authors examined three gene targets: *lacZ*, *wecG* and 16S rRNA, along with two culture-based methods. The 16S rRNA molecular assay proved to be as sensitive as the culture-based methods, and only required five hours for analysis (Maheux et al., 2014). More work is needed in this area to develop standardized methods that can be used accurately, reliably and affordably.

5.3 Online methods

A number of biological sensor technologies are available for real-time detection of microorganisms or microbial activity, including: adenosine triphosphate (ATP), flow cytometry and multi-angle light scattering (Miles et al., 2011; Storey et al., 2011; Samendra et al., 2014). ATP measurements have been applied at full-scale to address increased biological activity in the distribution system and associated low chlorine residuals (Shurtz et al., 2017). The other

technologies can inform operators that water quality is degrading and a situation requires action (see Section 7.3), however, further improvements are needed before widespread use materializes (Miles et al., 2011; Samendra et al., 2014). Online monitoring tools are promising for future applications and development is progressing rapidly (Samendra et al., 2014; Ikonen et al., 2017). These and other monitoring methods and tools are further discussed in *Guidance on monitoring the biological stability of drinking water in distribution systems* (Health Canada, 2020b).

Table 2: Culture-based methods for the detection of total coliforms in water

Method	Organization/ Manufacturer	Media	Basis for detection	Detection criteria	Time to obtain results
Presence-absence (P-A)					
9221 D.2 (Presumptive Phase)	Standard Methods	P-A culture broth	Lactose fermentation	Yellow color (with or without gas production)	24-48 h
9221 D.3 (Confirmed Phase)	Standard Methods	Brilliant green lactose bile broth (BGLB)	Lactose fermentation	Gas and/or acid production	Up to 48 h
Multiple tube fermentation (MTF)					
9221 B.2 (Presumptive Phase)	Standard Methods	Lauryl tryptose broth	Lactose fermentation	Gas and/or acid production	24-48 h
9221 B.3 (Confirmed Phase)	Standard Methods	BGLB	Lactose fermentation	Gas and/or acid production	Up to 48 h
Membrane filtration (MF)					
9222 B, C	Standard Methods	Endo-type agar medium	β -galactosidase enzyme	Red colonies with golden-green metallic sheen	24 h
9308-1, 2, 3	ISO Methods	Chromocult® Coliform agar	β -galactosidase enzyme	Red colonies	24 h
9222 H	Standard Methods	m-ColiBlue24® broth	β -galactosidase enzyme	Red colonies	24 h
9222 I 1604	Standard Methods U.S. EPA	MI medium or broth	β -galactosidase enzyme	Blue-white colonies that fluorescence under longwave ultraviolet (UV) light	24 h
Enzyme substrate [MTF, multi-well (e.g., Quanti-Tray®), presence-absence]					
9223 B	Standard Methods	Various substrate media available commercially: Colilert® Colilert-18® Colisure®	β -galactosidase enzyme	Yellow or red/magenta colonies depending on substrate used	18-24 h
N/A	EMD Millipore	Readycult® Fluorocult ®	β -galactosidase enzyme	Blue-green color	24 h
N/A	CPI International, Inc.	Modified Colitag®	β -galactosidase enzyme	Yellow color	16 h
N/A	Veolia Water Solutions and Technologies	TECTA™ EC/TC	β -galactosidase enzyme	Fluorescence detected by TECTA™ instrument	2-18 h

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., 2017).

Storage temperature can have a significant impact on microbial populations within samples and thus, affect recovery of coliforms (Ahammed, 2003; Pope et al., 2003). This is an especially important consideration when collecting samples in remote locations; and for Canadian utilities in the winter months, when there is an increased likelihood of sample freezing. Commercial devices are available for verifying that the proper transport temperatures are being achieved. 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. Water utilities should record any collection or storage related issues and report them to the laboratory, so that results can be properly interpreted. Any on-going problems with sampling and/or transportation should be discussed with the responsible authority.

Studies of the effects of holding time on the detection of coliforms in water samples have been limited and contradictory. Ahammed et al. (2003) observed a sharp decline in coliform counts in various water samples between 12 and 48 hours of storage, at both ambient and refrigerator temperatures. A similar effect was noted by Toranzos and McFeters (1997) after 24-30 hours versus six hours of storage. McDaniels et al., (1985) and Ferguson (1994) indicated that holding times can be critical for members of the coliform group when their concentrations are low. In contrast, Bushon et al. (2015) reported no significant difference in total coliform detections in groundwater samples tested after eight hours versus those tested within 18-30 hours. These different findings may be related to a number of factors, including: variations in survival rates among coliform isolates; sampling location; detection methods used; and how polluted water samples are (i.e., presence and density of competitor organisms) (Ahammed et al., 2003; Pope et al., 2003; Maier et al., 2014). To avoid unpredictable changes in the bacterial flora of the sample, examination should be started as soon as possible after collection. Where on-site facilities are available or when an accredited laboratory is within an acceptable travel distance, analysis of samples within six to eight hours is suggested (Payment et al., 2003; APHA et al., 2017). Ideally, for total coliform analysis of drinking water samples, the holding time between the collection of the sample and the beginning of its examination should not exceed 30 hours (APHA et al., 2017). 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. (2017). 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 presence-absence 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, 1972) for water expected to be of good quality. Examination of larger volumes 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. Hargy et al. (2010) demonstrated that large sample volumes were useful in improving the detection of total coliforms. The authors examined 100 mL and 20 L drinking water distribution system samples ($n = 252$), obtained from three utilities, using a commercially available capsule filter in conjunction with a substrate-based indicator reagent. Total coliforms were detected in 18 of the 20 L samples, but in only two of the corresponding 100 mL samples. The authors concluded that the microbiological quality of distribution systems may be better characterized by collecting larger volume samples (Hargy et al., 2010). An increased concentration of microorganisms is generally detected after periods of stagnation related to disinfectant decay and bacterial regrowth in the distribution system, and is reflective of the conditions under which OPPPs might be present (NRC, 2006; Lautenschlager et al., 2010; Wang et al., 2017). Thus, improved detection of total coliforms in the drinking water distribution system may be aided by not flushing and disinfecting the tap prior to sampling (NRC, 2006; Lautenschlager et al., 2010; Wang et al., 2017).

6.2 Sampling frequency considerations

The WHO lists the following factors that should be taken into account when determining sampling frequency for municipal-scale systems (WHO, 1972, 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; and
- 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 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 approach. 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.

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. Minimum recommended sampling frequencies for total coliform testing in the distribution system are provided in Table 3. However, systems should conduct a site-specific assessment to ensure that their sampling frequency meets the requirements of the responsible authority. The WHO also provides guidance on the minimum number of samples for indicator testing in drinking water distribution systems (WHO, 2014).

Table 3: Recommended sampling frequency for total coliform testing in the drinking water distribution system.

Population served	Minimum number of samples per month ^a
Up to 5000	4
5000–90,000	1 per 1000 persons
90 000+	1 per 10,000 persons

^a 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 *Guidelines for Canadian drinking water quality: guideline technical document — turbidity* (Health Canada, 2012). 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 3) 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 watermain, in a reservoir, at the treatment plant, or occurs for a long duration at a high concentration (Speight et al., 2004; van Lieverloo et al., 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 et al., 2007), such as samples collected every five 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 et al., 2009). These limitations highlight the importance of implementing a source-to-tap 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 selected or approved 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.

Given the presence of biofilm in drinking water distribution systems, consideration should be given to including biofilm sampling as part of total coliform detection, as it would likely provide a more accurate assessment of potential risk (WHO, 2011, 2014; Botsaris et al., 2015; Wang et al., 2017). Careful consideration should also be given to identifying locations within the distribution system where the risk of microbial contamination (either because of regrowth, intrusion or biofilm dislodgement) is likely highest (e.g., areas where disinfectant residual decay is anticipated to occur), and to sample a variety of these locations. The composition of the sampling tap also needs to be considered when determining sampling locations, as it can affect microbial test results (Geldreich et al, 1985; Cox and Giron, 1993; Goatcher et al, 1992; Geldreich and LeChevallier, 1999). The U.S. EPA's Revised Total Coliform Rule addresses this issue by allowing the collection of samples from dedicated (distribution system) sampling stations – this also ensures spatially representative sampling (U.S. EPA, 2013; LeChevallier, 2014). Detection may be improved by rotating among sampling sites throughout the distribution system (WHO, 2014). Additional guidance on selecting sampling points within the drinking water distribution system can be found in *Guidance on monitoring the biological stability of drinking water in distribution systems* (Health Canada, 2020b).

7.0 Treatment technology

The application of a source-to-tap 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 source-to-tap 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 or GUDI sources should include adequate filtration (or technologies providing an equivalent log reduction credit) and disinfection to ensure removal/inactivation of enteric protozoa and enteric viruses. 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 or GUDI sources 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 fecal 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, 2019a, 2019b). Depending on the source water quality, a higher log reduction may be necessary to produce safe drinking water. Groundwater, classified as less vulnerable to fecal contamination, should not have protozoa present when using procedures determined by the responsible authority. Therefore, the minimum treatment requirements for protozoa would not apply. However, even 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. Although physical removal of bacteria is achievable, Smeets et al. (2006) found that most filtration technologies achieved less than 6 log reduction in bacteria on their own; thus,

disinfection is a critically important additional treatment barrier (see Section 7.1.3).

Physical log removal credits for several filtration technologies are outlined in Table 4. More detailed information on filtration techniques can be found in the guideline technical document on turbidity (Health Canada, 2012).

Table 4: Range of log removals from filtration studies^a

Technology	Bacterial log removals			
	Minimum	Mean	Median	Maximum
Conventional filtration	1.0	2.1	2.1	3.4
Direct filtration	0.8	1.4	1.5	3.3
Slow sand filtration	1.2	2.7	2.4	4.8
Microfiltration (pore size – 0.1-1 µm)	0	Not given	Not given	4.3
Ultrafiltration (pore size – 0.01-0.1 µm)	Not given	>7	Not given	Not given

^a Adapted from Smeets et al., 2006

7.1.3 Disinfection

The commonly used drinking water disinfectants are chlorine, chloramine, UV light, ozone, and chlorine dioxide. Disinfection is typically applied after treatment processes that remove particles and organic matter (Health Canada, 2019c). This strategy helps to ensure efficient inactivation of pathogens and minimizes the formation of disinfection by-products (DBP). 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. Primary disinfection is a critically important barrier to reduce bacteria in drinking water. Thus operators should understand and maintain the required CT/IT values (where CT = concentration of disinfectant x contact time, and IT = intensity of UV light x contact time, see sections 7.1.3.1 and 7.3.1.2 for descriptions of the CT and IT concepts, respectively) for primary disinfection, in addition to disinfectant residual requirements for secondary disinfection. Typically, bacterial reduction goals will be achieved if primary disinfection for viruses is achieved.

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, 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., 2007). 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, 1999). 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. 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 5. 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 5, 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. Thus, chloramine is not recommended as a primary disinfectant because of the high CT values required for inactivation of bacteria.

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

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

^a From Hoff (1986)^b From U.S. EPA (1999)^c 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 enteric viruses and enteric protozoa (Health Canada, 2019a, 2019b).

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 (i.e., photoreactivation) (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.

For UV disinfection, the product of light intensity “I” (measured in mW/cm² or W/m²) and time “T” (measured in seconds) results in a computed dose (fluence) in mJ/cm² for a specific microorganism. This relationship is referred to as the IT concept. Log inactivations using UV light disinfection are listed in Table 6, and show 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. *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.

Table 6: UV light dose (mJ/cm²) required for inactivation

Log inactivation	<i>E. coli</i> ^{a,d}	<i>Cryptosporidium</i> ^a	Adenovirus ^{a,b,c,d}	Rotavirus ^{a,c,d}	<i>Giardia</i> ^a
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

^a Based on U.S. EPA (2003)

^b Adenoviruses are highly UV resistant in comparison with other enteric viruses, however drinking water is not a typical exposure route; see Health Canada (2019a)

^c LeChevallier and Au (2004)

^d Hijnen et al. (2006)

With regards to UV disinfection, Sommer et al. (2000) found the dose (fluence) required to inactivate eight pathogenic *E. coli* strains differed significantly. The authors reported a dose of 1.2 mJ/cm² was required to achieve 6 log reduction for the most susceptible strain, whereas 12.5 mJ/cm² was required for the most resistant strain. Up to 30 mJ/cm² was necessary when photoreactivation for 2 hours was considered. Typically, photoreactivation is not a concern for drinking water applications, however, it may be a consideration for potable water use in food production facilities that have their own private water supply (i.e., a semi-public system). One strain was also found to repair under dark conditions; a dose of approximately 10 mJ/cm² was required to achieve 6 log reduction for this strain.

Many jurisdictions require a minimum UV dose of 40 mJ/cm² on the basis that 4 log inactivation can be achieved for most pathogens, including bacteria, viruses and protozoan (oo)cysts (ÖNORM, 2001, 2003; DVGW, 2006a,b,c; Ontario Ministry of Environment, 2006; Nova Scotia Environment, 2012). The responsible jurisdiction should be contacted to confirm the UV dose requirements.

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).

Groundwater is a common source of drinking water for small or individual water supplies, and the consumption of fecally contaminated groundwater has been associated with illness (see Section 4.3.3). As a result, small groundwater supplies providing drinking water to the public (i.e., semi-public systems) that are vulnerable to fecal contamination should be treated.

In cases where an individual household obtains its drinking water from a private well, the vulnerability of the source to fecal contamination should be assessed. Although it is difficult for homeowners to conduct a detailed assessment of the vulnerability of their well to fecal contamination, steps can be taken to minimize the likelihood of a well becoming contaminated. General guidance on well construction, maintenance, protection and testing is typically available from provincial/territorial jurisdictions. When considering the potential for fecal contamination, well owners should have an understanding of the well construction, type of aquifer material

surrounding the well and location of the well in relation to sources of fecal contamination (e.g., septic systems, sanitary sewers, animal waste, etc.). This information can be obtained from records provided to the homeowner during well and septic system construction, and well log databases, aquifer vulnerability maps, and regional groundwater assessments that are generally available from the responsible drinking water authority. If insufficient information is available to determine if a well is vulnerable to fecal contamination, treatment of the well is a way to reduce risk. In general, surface water is not recommended as a private or semi-public water supply unless it is properly filtered, disinfected and monitored for water quality.

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. Many 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. To minimize the potential health risks from the use of microbiologically-contaminated drinking water, it is important to note that in the absence 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.

Specific guidance on technologies that can be used in small systems should be obtained from the responsible drinking water authority in the affected jurisdiction. 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.

Certification organizations provide assurance that a product conforms to applicable standards and must be accredited by the Standards Council of Canada (SCC). Accredited organizations in Canada (SCC, 2019) include:

- CSA Group;
- NSF International;
- Water Quality Association;
- UL LLC;
- Truesdail Laboratories Inc.;
- Bureau de Normalisation du Québec (available in French only); and
- International Association of Plumbing and Mechanical Officials.

An up-to-date list of accredited certification organizations can be obtained from the SCC web site.

Private and semi-public supplies that use liquid chlorine should use hypochlorite solutions that are certified as meeting NSF/ANSI/CAN Standard 60 (NSF/ANSI/CAN, 2018) and follow the handling and storage recommendations for hypochlorite outlined in (Health Canada, 2016).

NSF/ANSI Standard 55 (Ultraviolet Disinfection Systems) (NSF/ANSI, 2019) 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/cm² 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.

Reverse osmosis (RO) membranes have a pore size smaller than bacteria and could provide a physical barrier to remove them. However, NSF/ANSI Standard 58 (NSF/ANSI, 2018a) does not include a claim for bacterial reduction. It is important to note that RO systems are intended for point-of-use installation only. This is because water treated by a RO system may be corrosive to internal plumbing components. These systems also require larger quantities of influent water to obtain the required volume of drinking water and are generally not practical for point-of-entry installation.

Ultrafiltration membranes also have a pore size smaller than bacteria and could also provide a physical barrier to bacteria, although there is currently no NSF/ANSI standard for residential-scale ultrafiltration systems. Semi-public systems that require higher capacity may refer to ultrafiltration membranes certified under NSF/ANSI Standard 419 (NSF/ANSI, 2018b). Although units are not certified for bacterial reduction, those with a pore size of <0.1 µm should be effective.

The NSF/ANSI Standard 62 (NSF/ANSI, 2018c) for Drinking Water Distillation Systems 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.

Periodic testing for *E. coli* and total coliforms by an accredited laboratory should be conducted on both the water entering the treatment device and the treated water to verify that the treatment device is effective. Treatment devices lose their removal capacity through usage and time and need to be maintained and/or replaced. Consumers should verify the expected longevity of the components in their treatment device according to the manufacturer's recommendations and establish a clearly defined maintenance schedule. Treatment devices should be inspected and serviced in accordance with the maintenance schedule and manufacturer's recommendations.

7.3 Distribution system

A well-maintained distribution system is a critical component of the multi-barrier approach for management of drinking water safety (Fisher et al., 2000). Distribution system optimization is a complex process involving numerous concomitant goals (e.g., microbial, DBPs, corrosion, physical integrity).

Distribution system water quality is known to deteriorate due to a variety of issues, including long-term biofilm regrowth and short-term transients/intrusions that may result due to normal day-to-day operations (e.g., start/stop pump; open/close valve), as well as accidental cross-contamination or intentional contamination. Contamination events are difficult to detect due to the low frequency of microbial indicator sampling (Hall et al., 2007; Storey et al., 2011; Ikonen et al., 2013). Distribution system water quality should be regularly monitored. Several instruments and water quality probes, including those for chlorine, conductivity, flow, pH, pressure, temperature, total/dissolved organic carbon and turbidity, provide reliable means by which changes in the distribution system can be measured in real-time (Hall et al., 2007; Miles

et al., 2011; Rosen and Bartrand, 2013; Durand et al., 2016; Ikonen et al., 2017). The most sensitive sensors are reported to be total organic carbon and free/total chlorine, while the five most commonly used by water utilities in the U.S., Netherlands, Belgium and Australia include: flow, turbidity, pH, pressure and chlorine (van der Gaag and Volt, 2008). Online monitoring shows promise to better understand distribution system dynamics such that problems are rapidly detected, quick response is taken and drinking water is transported to the consumer with minimum loss of quality (van der Gaag and Volt, 2008; U.S. EPA, 2010; Storey et al., 2011; Rosen and Bartrand, 2013; Samendra et al., 2014; WHO, 2014; Durand et al., 2016; Ikonen et al., 2017).

In addition to regularly monitoring distribution system water quality, source water protection measures should be implemented, along with treatment optimization, maintenance of physical/hydraulic integrity of the distribution system, and minimization of negative- or low-pressure events (Karim et al., 2003; Fleming et al., 2006; Friedman et al., 2009; WHO, 2014). Treatment optimization should include biofilm reduction strategies, including: optimized removal of natural organic matter; maintenance of an effective disinfectant residual; maintenance of low levels of biostability indicators in treated water (e.g., AOC, biodegradable dissolved organic carbon [BDOC], biofilm formation rate); controlling corrosion; and managing water temperatures (Prévost et al., 2005; van der Kooij and van der Wielen, 2014; LeChevallier et al., 2015a,b; Prest et al., 2016a,b; Health Canada, 2019c). Operations and maintenance programs should be in place (e.g., watermain cleaning, cross-connection control, asset management) and strict hygiene should be practiced during watermain repairs to ensure drinking water is transported to the consumer with minimum loss of quality (Kirmeyer et al., 2001, 2014). Further details on monitoring and managing biostability in the drinking water distribution system can be found in *Guidance on monitoring the biological stability of drinking water in distribution systems* (Health Canada, 2020b).

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, 2019a, 2019b).

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 source-to-tap approach. In groundwaters that are less vulnerable to fecal 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 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 and international organisations use total coliforms in drinking water quality management. 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 plant, 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 EU's Council Directive on the quality of water intended for human consumption (Council of the European Union, 1998).

The WHO Guidelines for Drinking Water Quality (2017) specify that total coliforms should be absent immediately after disinfection, and that their presence is indicative of inadequate treatment. These Guidelines also mention that the presence of total coliforms in distribution systems may relate to regrowth and possible biofilm formation. However, no guideline values are specified for total coliforms; instead, guideline values for thermotolerant coliforms and *E. coli* are established (WHO, 2017).

The Australian Drinking Water Guidelines do not establish a guideline value for total coliforms; instead, guideline values for thermotolerant coliforms and *E. coli* are established (NHMRC, 2016).

The U.S. EPA (2013) revised the Total Coliform Rule to include specific provisions designed to minimize microbial risks from the drinking water distribution system, including the establishment of a total coliform treatment technique requirement, and monitoring requirements for total coliforms according to site-specific plans. Under this treatment technique, a water system that exceeds a specified frequency of total coliform occurrence must conduct an assessment to determine if any sanitary defect exists and, if found, correct them. This change means that, instead of triggering a non-acute Maximum Contaminant Level 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.

The U.S. EPA Groundwater Rule establishes a risk-based approach to identify subsurface sources that are susceptible to fecal contamination, and requires corrective action to address deficiencies. Systems not providing 4 log virus reduction must monitor for *E. coli*, enterococci or somatic coliphage within 24 hours of receiving notice of a total coliform positive result (U.S. EPA, 2006).

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 fecal 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 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 approach, is used as part of the verification that the drinking water system is producing water that is microbiologically acceptable.

10.0 References

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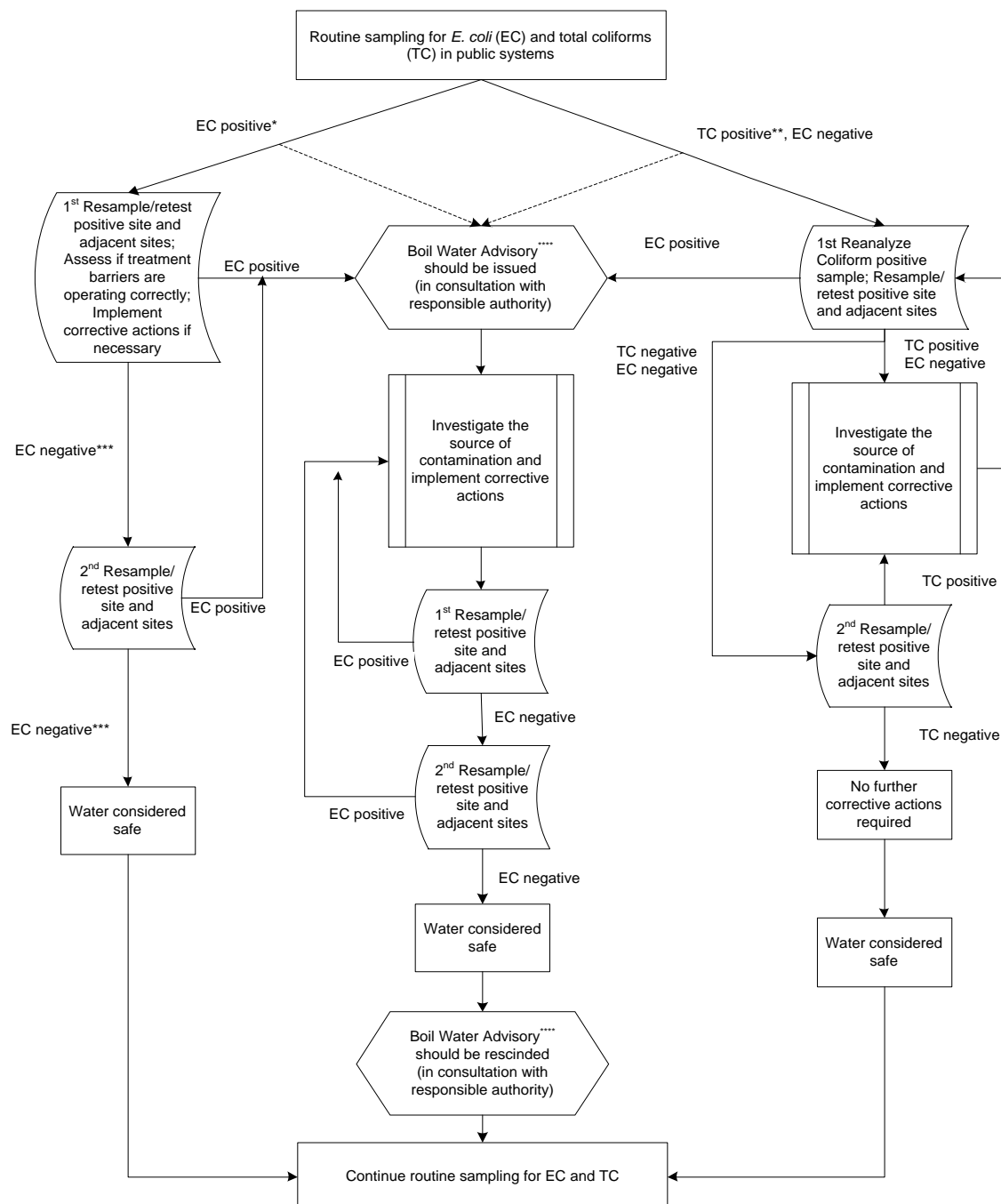
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Appendix A: Decision tree for routine microbiological testing of municipal-scale systems



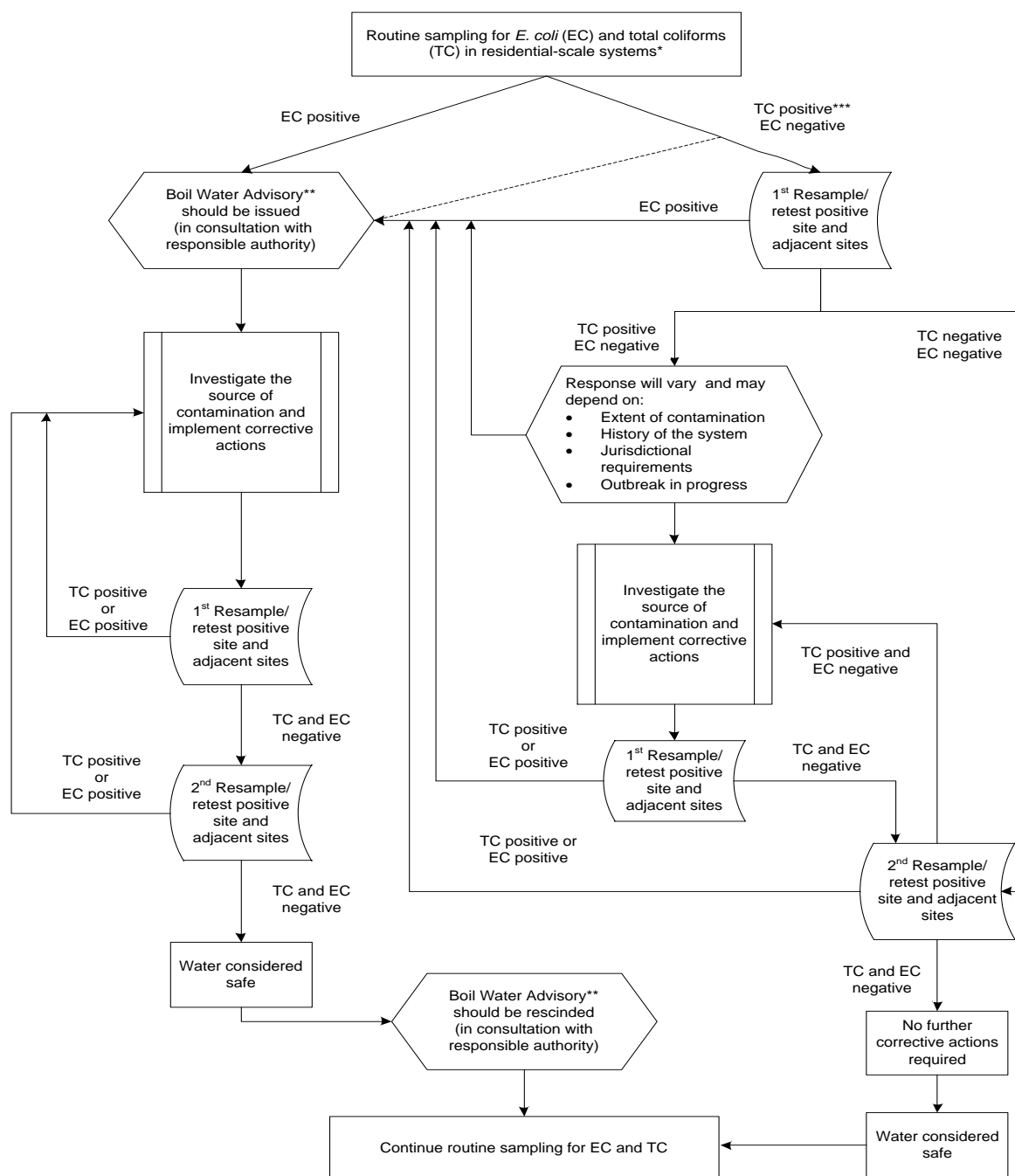
* (dashed line) A boil water advisory may be issued on an initial positive detection of *E. coli* (e.g. from single or multiple sites) if deemed necessary by the responsible authority. A boil water advisory should be issued if there is also strong evidence of a significant treatment failure or illness associated with the drinking water.

** (dashed line) A boil water advisory may be issued based on a positive total coliform sample, 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"

*** (dashed line) 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

AGI	acute gastrointestinal illness
ANSI	American National Standards Institute
AOC	assimilable organic carbon
ATP	adenosine triphosphate
BDOC	biodegradable organic carbon
BGLB	brilliant green lactose bile broth
BOM	biodegradable organic matter
CT	concentration of disinfectant \times contact time
DBP	disinfection by-product
<i>E. coli</i>	<i>Escherichia coli</i>
EU	European Union
GUDI	groundwater under the direct influence of surface water
HCGI	highly credible gastrointestinal illness
ISO	International Organization for Standardization
IT	intensity of UV light \times contact time
MAC	maximum acceptable concentration
MF	membrane filtration
MTF	multiple tube filtration
NSF	NSF International
OPPP	opportunistic premise plumbing pathogen
P-A	presence–absence
PCR	polymerase chain reaction
PE	Polyethylene
PVC	Polyvinyl chloride
QA	quality assurance
QC	quality control
RO	reverse osmosis
RT-PCR	reverse transcription polymerase chain reaction
SCC	Standards Council of Canada
U.S.	United States
U.S. EPA	United States Environmental Protection Agency
UV	ultraviolet
WHO	World Health Organization