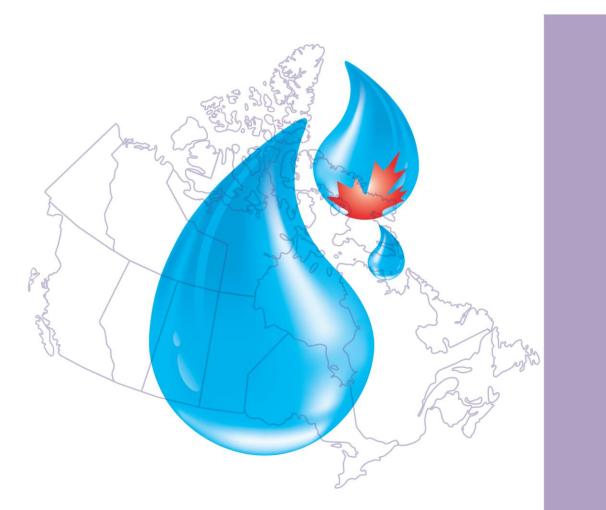
Guidelines for Canadian Drinking Water Quality

Guideline Technical Document

Escherichia coli





Health Canada is the federal department responsible for helping the people of Canada maintain and improve their health. We assess the safety of drugs and many consumer products, help improve the safety of food, and provide information to Canadians to help them make healthy decisions. We provide health services to First Nations people and to Inuit communities. We work with the provinces to ensure our health care system serves the needs of Canadians.

Published by authority of the Minister of Health.

Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Escherichia coli

is available on Internet at the following address: www.healthcanada.gc.ca

Également disponible en français sous le titre :

Recommandations pour la qualité de l'eau potable au Canada : Document technique – Escherichia coli

This publication can be made available on request in a variety of alternative formats.

© Her Majesty the Queen in Right of Canada, represented by the Minister of Health, 2013

This publication may be reproduced without permission provided the source is fully acknowledged.

Pub. Number: 130021 Cat.: H144-7/2013E-PDF ISBN: 978-1-100-21737-6

Guidelines for Canadian Drinking Water Quality

Guideline Technical Document Escherichia coli

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

Health Canada Ottawa, Ontario

March, 2012

This document may be cited as follows:

Health Canada (2012). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — *Escherichia coli*. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H144-7/2013E-PDF).

The document was prepared by the Federal-Provincial-Territorial Committee on Drinking Water of the Federal-Provincial-Territorial Committee on Health and the Environment.

Any questions or comments on this document may be directed to:

Water, Air and Climate Change Bureau Healthy Environments and Consumer Safety Branch Health Canada 269 Laurier Avenue West, Address Locator 4903D Ottawa, Ontario Canada K1A 0K9

Tel.: 613-948-2566 Fax: 613-952-2574

E-mail: water_eau@hc-sc.gc.ca

Other Guideline Technical Documents for the Guidelines for Canadian Drinking Water Quality can be found on the following web page: www.healthcanada.gc.ca/waterquality

Table of Contents

Part	I. Over	view and Application	1				
1.0	Guide	eline	1				
2.0	Execu	utive summary	1				
	2.1	Significance of <i>E. coli</i> in drinking water systems and their sources					
	2.2	Sampling and testing for <i>E. coli</i>					
	2.3	Treatment technology					
3.0	Application of the guideline						
	3.1	Municipal-scale drinking water supply systems	3				
		3.1.1 Monitoring <i>E. coli</i> in water leaving the treatment plant					
		3.1.2 Monitoring <i>E. coli</i> within water distribution and storage systems					
		3.1.3 Notification.					
		3.1.4 Corrective actions	3				
	3.2	Residential-scale and private drinking water systems	4				
		3.2.1 Testing requirements					
		3.2.2 Notification					
		3.2.3 Corrective actions for disinfected supplies	5				
		3.2.4 Corrective actions for non-disinfected wells					
Part	II. Scie	nce and Technical Considerations	7				
4 0	Signit	ficance of E. coli in drinking water	7				
4.0	4.1	Description	7				
	4.2	Sources					
4.0 S 4 4 4	4.3	Survival					
	4.4	Role of <i>E. coli</i> as an indicator of microbiological safety					
		4.4.1 Role in source water monitoring					
		4.4.2 Role in treatment and distribution system monitoring					
		4.4.3 Considerations for residential-scale systems					
5.0	Analy	ytical methods	10				
5.0	5.1	Presence–absence procedure					
	5.2	Membrane filter procedure					
	5.3	Multiple tube fermentation procedure					
6.0	Samp	oling for E. coli	13				
	6.1	Sample collection					
	6.2	Sampling frequency considerations					
	6.3						
7.0	Treatment technology						
	7.1	Municipal scale					
		7.1.1 Level of treatment necessary					
		7.1.2 Physical removal					
		7.1.3 Disinfection					

		7.1.3.1 Chemical disinfection	17
		7.1.3.2 UV light disinfection	19
	7.2	Residential scale	
8.0	Rick a	assessment	20
0.0		International considerations	
9.0	Ration	nale	21
10.0	Refere	ences	22
Appen	dix A:	Decision Tree for Routine Microbiological Testing of Municipal Scale Systems	. 30
Appen	dix B:	Decision Tree for Routine Microbiological Testing of Residential Scale System	s 31
Appen	dix C:]	List of Acronyms	32

Escherichia coli

Part I. Overview and Application

1.0 Guideline

The maximum acceptable concentration (MAC) of Escherichia coli is none detectable per 100 mL.

2.0 Executive summary

Escherichia coli is a member of the coliform group of bacteria that is naturally found in the intestines of humans and warm-blooded animals. As it is not usually found naturally in other environments such as on plants or in soils or water, the presence of E. coli in a water sample is a good indicator of recent faecal contamination. The ability to detect faecal contamination in drinking water is a necessity, as pathogenic microorganisms from human and animal faeces in drinking water pose the greatest danger to public health.

Health Canada recently completed its review on the usefulness of *E. coli* as an indicator of faecal contamination in drinking water systems. This guideline technical document reviews and assesses available literature on the uses of *E. coli* as an indicator of pathogen presence in source waters, the potential for *E. coli* to be present in the absence of recent faecal contamination, and improvements in methods for the detection of *E. coli*. From this review, the guideline for *E. coli* in drinking water is established as a maximum acceptable concentration of none detectable in 100 mL of water.

2.1 Significance of *E. coli* in drinking water systems and their sources

E. coli monitoring should be used, in conjunction with other indicators, as part of a multibarrier approach to producing drinking water of an acceptable quality. The number, frequency, and location of samples for *E. coli* testing will vary according to the type and size of the system and jurisdictional requirements.

Surface water and groundwater under the direct influence of surface water (GUDI) are commonly impacted by faecal contamination from either human or animal sources and, as a result, usually contain *E. coli*. If *E. coli* monitoring results are available for these sources, they can be used as part of the source water assessment to identify changes in its microbiological quality. The presence of *E. coli* in any groundwater sources indicates that the groundwater is contaminated by faecal material and is microbiologically unsafe for drinking without further treatment.

Monitoring for *E. coli* in treated water at the treatment plant and in the distribution system is carried out to provide information on the adequacy of drinking water treatment and on the microbial condition of the distribution system. The presence of *E. coli* in water leaving a treatment plant signifies that treatment has been inadequate, while the presence of *E. coli* in the distribution system, when water tested immediately post-treatment is free of *E. coli*, suggests that post-treatment contamination with faecal material has occurred. The presence of *E. coli* at any point in the treated water indicates there is a potential health risk from consuming the water. Additional actions to be taken in these cases include notifying the responsible authorities, issuing

a boil water advisory, investigating the cause of the contamination, and implementing corrective actions.

Although the presence of *E. coli* is a good indicator of recent faecal contamination, such contamination is often intermittent and may not be revealed by the examination of a single sample. Therefore, if a sanitary inspection shows that an untreated supply is subject to faecal contamination, or that treated water is subject to faecal contamination during storage or distribution or is inadequately treated, the water should be considered unsafe, irrespective of the results of *E. coli* analysis.

2.2 Sampling and testing for *E. coli*

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

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

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

2.3 Treatment technology

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

3.0 Application of the guideline

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

E. coli is currently the best available indicator of recent faecal contamination in drinking water systems. Consequently, detection of E. coli in any drinking water system is unacceptable. For decision-making, the focus is the positive detection of E. coli, 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. Faecal contamination is often intermittent and may not be revealed by the examination of a single sample. Therefore, if a sanitary inspection shows that an untreated supply is subject to faecal contamination, or that treated water is subject to faecal contamination during storage or distribution or is inadequately treated, the water should be considered unsafe, irrespective of the results of E. coli examination.

3.1 Municipal-scale drinking water supply systems

3.1.1 Monitoring E. coli in water leaving the treatment plant

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

3.1.2 Monitoring E. coli within water distribution and storage systems

In municipal scale distribution and storage systems, the number of samples collected for *E. coli* 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 *E. coli* in treated water within distribution and storage systems will be prescribed by the responsible authority.

3.1.3 Notification

If *E.coli* is detected in a municipal-scale drinking water system, the system owner/operator and the laboratory processing the samples should immediately notify the responsible authorities. The system owner/operator should resample and test the positive site(s) and adjacent sites. If resampling and testing confirm the presence of *E. coli* in drinking water, the system owner/operator should immediately: (1) issue a boil water advisory¹ in consultation with the responsible authorities, (2) carry out the corrective actions described below, and (3) cooperate with the local responsible authority in any surveillance for possible waterborne disease outbreaks (see Appendix A for decision tree). In addition, where *E. coli* contamination is detected in the first sampling—for example, positive sample results from a single site, or from more than one location in the distribution system—the owner or the responsible authority may decide to notify consumers immediately to boil their drinking water or use a safe alternative source and initiate corrective actions without waiting for confirmation.

3.1.4 Corrective actions

If the presence of *E. coli* in drinking water is confirmed, the owner of the waterworks system 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.

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."

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

If a boil water advisory is issued, it should be rescinded only after a minimum of two consecutive sets of samples, collected 24 hours apart, show negative results demonstrating full system-wide integrity (including acceptable microbiological quality, turbidity, and/or disinfectant residuals). 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*. Only a history of data together with the verification of the suitability of the system design and its operation and maintenance can be used to confirm the long-term integrity of a supply.

Minimum treatment of supplies derived from surface water sources or groundwater under the direct influence of surface waters should include adequate filtration (or technologies providing an equivalent log removal/inactivation) and disinfection. For groundwater sources less vulnerable to faecal contamination, adequate treatment is recommended to ensure removal/inactivation of enteric viruses, unless these sources are exempted by the responsible authority based on site-specific considerations such as historical and on-going monitoring data. In systems with a distribution system, a disinfectant residual should be maintained at all times. 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 and private drinking water systems

3.2.1 Testing requirements

_

Testing frequencies for residential-scale² systems will be determined by the responsible authority and should include times when the risk of contamination is greatest, for example, in early spring after the thaw, after an extended dry spell, or following heavy rains. Owners of

² For the purposes of this document, a residential-scale water supply system is defined as a system with a minimal or no distribution system that provides water to the public 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 supply may vary between jurisdictions.

private supplies should be encouraged to have their water tested during these same periods. New or rehabilitated wells should also be tested before use to confirm microbiological safety.

3.2.2 Notification

The presence of *E. coli* in a residential-scale or private drinking water system demonstrates that the source or the system has been impacted by recent faecal contamination; as a result, the water is unsafe to drink. The drinking water should be immediately retested to confirm the presence of *E. coli*. The responsible authority should advise the owner to boil the drinking water or to use a safe alternative source in the interim. If resampling confirms that the source is contaminated with *E. coli*, the corrective actions described in the next section should be taken immediately. As a precautionary measure, some jurisdictions may recommend immediate corrective actions without waiting for confirmatory results (see Appendix B).

3.2.3 Corrective actions for disinfected supplies

The first step, if it has not already been taken, is to conduct a sanitary survey to evaluate the physical condition of the drinking water system as applicable, including water intake, well, well head, pump, treatment system (including chemical feed equipment, if present), plumbing, and surrounding area.

Any identified faults should be corrected before proceeding. If all the physical conditions are acceptable, some or all of the following corrective actions may be necessary:

- In a chlorinated system, verify that a disinfectant residual is present throughout the system.
- Increase the disinfectant dosage, flush the system thoroughly, and clean treated water storage tanks and domestic cisterns. Water should be dechlorinated before being discharged to fish-bearing waters. The responsible authority should be consulted regarding the methods available and the correct procedure for carrying out dechlorination.
- For systems where the disinfection technology does not leave a disinfectant residual, such as UV or ozone, it may be necessary to shock chlorinate the well and plumbing system; further information on shock chlorination is available in the factsheet *What's in Your Well? A Guide to Well Water Treatment and Maintenance* (www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/well-puits-eng.php).
- 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 *E. coli* to confirm that the problem has been corrected. If the problem cannot be corrected, additional treatment or a new source of drinking water should be considered. In the interim, any initial precautionary measures should continue; for example, drinking water should continue to be boiled or an alternative safe source of water should continue to be used.

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

3.2.4 Corrective actions for non-disinfected wells

The first step, if it has not already been taken, is to conduct a sanitary survey to evaluate 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 *What's in Your Well? A Guide to Well Water Treatment and Maintenance* (www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/well-puits-eng.php).
- Flush the system thoroughly and retest to confirm the absence of *E. coli*. Confirmatory tests should be done no sooner than either 48 hours after tests indicate the absence of a chlorine residual or 5 days after the well has been treated. Local conditions may determine acceptable practice. Water should be dechlorinated before being discharged to fish-bearing waters. The responsible authority should be consulted regarding the methods available and the correct procedure for carrying out dechlorination.

If the water remains contaminated after shock-chlorination, further investigation into the source of the contamination should be carried out. If the source cannot be found or corrected, either an appropriate disinfection device or well reconstruction or replacement should be considered. Drinking water should be boiled or an alternative safe source of water should continue to be used in the interim.

A boil water advisory should be rescinded only after a minimum of two consecutive sets of samples, collected 24 hours apart, show negative results. Further information on boil water advisories can be found in *Guidance for Issuing and Rescinding Boil Water Advisories*. Additional tests should be taken after 3 to 4 months to ensure that the contamination has not recurred. Only a history of data can be used to confirm the long-term integrity of a supply when applied jointly with sanitary surveys. Further information on routine monitoring can be found in section 6.0.

Part II. Science and Technical Considerations

4.0 Significance of *E. coli* in drinking water

4.1 Description

Escherichia coli is a member of the coliform group of bacteria, part of the family Enterobacteriaceae, and described as a facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacterium. The vast majority of waterborne $E.\ coli$ isolates have been found to be capable of producing the enzyme β-glucuronidase (Martins et al., 1993; Fricker et al., 2008b, 2010), and it is this characteristic that currently facilitates their detection and identification. Further information on the coliform group of organisms can be found in the guideline technical document on total coliforms (Health Canada, 2012a).

4.2 Sources

As a member of the Enterobacteriaceae family, *E. coli* is naturally found in the intestines of humans and warm-blooded animals. Unlike other bacteria in this family, *E. coli* does not usually occur naturally on plants or in soil and water, although there is evidence that some strains may be able to survive and grow in soils (Winfield and Groisman, 2003; Byappanahalli et al., 2006; Ishii et al., 2006). Within human and animal faeces, *E. coli* is present at a concentration of approximately 10⁹ cells per gram (Edberg et al., 2000) and comprises about 1% of the total biomass in the large intestine (Leclerc et al., 2001). Although *E. coli* are part of the natural faecal flora, some strains of this bacterium can cause gastrointestinal illness along with other, more serious health problems. Faecal concentrations of the typical non-pathogenic *E. coli*, used to indicate recent faecal contamination, will always be greater than those of the pathogenic strains, even during outbreaks. Further information on illness-associated *E. coli* strains can be found in *Guidance on Waterborne Bacterial Pathogens* (Health Canada, 2013a).

4.3 Survival

The survival time of E. coli in the environment is dependent on many factors including temperature, exposure to sunlight, presence and types of other microflora, and the type of water involved (e.g., groundwater, surface water, or treated distribution water) (Foppen and Schijven, 2006). In general terms, E. coli survives for about 4–12 weeks in water containing a moderate microflora at a temperature of 15–18°C (Kudryavtseva, 1972; Filip et al., 1987; Edberg et al., 2000). A concept that is receiving increasing attention within the scientific community is that if favorable conditions (e.g., nutrients, temperature, reduced environmental stresses) are present in the environment, E. coli may be capable of prolonged survival and growth, even in the absence of recent faecal contamination. Several studies have been published demonstrating evidence of the survival and growth of E. coli populations in nutrient-rich environments such as soils, beach sand, cyanobacterial bloom material and mats of the algal species Cladophora, in both tropical and temperate environments (Carrillo et al., 1985; Hardina and Fujioka, 1991; Power et al., 2005; Byappanahalli et al., 2006; Ishii et al., 2006; Heuvel et al., 2010). Compared to soils or masses of vegetative material, most source water environments provide conditions that are less protective and less nutrient-rich. As a result, bacterial regrowth is not expected to be a concern in a water setting. E. coli is generally the most sensitive of the coliform bacteria to environmental stressors and does not survive as long in the environment as do protozoans and some viruses (Edberg et al., 2000). E. coli does have similar survival rates to many faecal bacterial pathogens (Jimenez et al., 1989; Artz and Killham, 2002; Karim et al., 2004; Cook and Bolster, 2007).

The ability of *E. coli* to survive and grow in distribution system biofilms has been the subject of several recent studies. In several research settings that used model and pilot-scale systems, *E. coli* were able to survive in the biofilm under various conditions, although in most cases colonization was transient (Fass et al., 1996; Williams and Braun-Howland, 2003; Lehtola et al., 2007). In full-scale drinking water distribution systems, when *E. coli* has been detected in the biofilm, it generally makes up a small portion of the total coliforms isolated (Lechevallier et al., 1987; Blanch et al., 2007; Juhna et al., 2007). Although in some instances *E. coli* can be detected in biofilms in distribution systems, they do not seem to be a significant component of the biofilm matrix; consequently, the detection of *E. coli* in a water distribution system is a good indication of recent faecal contamination.

4.4 Role of *E. coli* as an indicator of microbiological safety

Although modern microbiological techniques have made the detection of pathogenic bacteria, viruses, and protozoa possible, it is currently not practical to attempt to routinely isolate them from drinking water (Allen et al., 2000; Payment and Pintar, 2006). Reasons for this include the large number of different pathogens that exist, their uneven distribution in water, and the time and expense associated with routine monitoring of all pathogens. It is better to use a variety of indicators that are less difficult, less expensive, and less time consuming to monitor, which will encourage a higher number of samples to be tested, giving a better overall picture of the water quality and, therefore, better protection of public health. Of the contaminants that may be regularly found in surface and groundwater sources, pathogenic microorganisms from human and animal faeces pose the greatest danger to public health. For this reason, the ability to detect faecal contamination in drinking water is a necessity for ensuring public safety. As early as the 19th century, E. coli was recognized as a good indicator of faecal contamination, but there were no easy, simple, and low-cost methods to easily speciate E. coli until the late 1980s (Edberg et al., 2000). Of the coliform group of organisms, E. coli are considered a more specific indicator of faecal contamination and can be rapidly and easily enumerated in water. In addition to being faecal specific, E. coli do not usually multiply in the environment, are excreted in the faeces in high numbers (approximately 10^9 cells per gram) making detection possible even when greatly diluted, and have a life span on the same order of magnitude as those of other enteric bacterial pathogens. These features make E. coli the best available indicator of faecal contamination. A subset of the total coliform group, known as the thermotolerant coliforms—coliforms that have the ability to ferment lactose at 44–45°C previously referred to as faecal coliforms—has been used as a surrogate for E. coli in water quality testing. Thermotolerant coliforms were considered more faecal specific than total coliforms, and, given that E. coli testing was difficult, thermotolerant coliform detection was used routinely. Advances in E. coli detection methods have made the need for thermotolerant coliform testing in drinking water quality management redundant.

Although *E. coli* is the best available indicator of recent faecal contamination, there are waterborne illnesses that result from pathogens not transmitted by the faecal—oral route and, therefore, the detection of faecal indicators does not provide any information on their potential presence. No indicators are currently known for such pathogens. Further information can be found in *Guidance on Waterborne Bacterial Pathogens* (Health Canada, 2013a). The best means of safeguarding against the presence of waterborne pathogens in drinking water, including non-faecal pathogens, is the application of the multi-barrier approach that includes adequate treatment, a well-maintained distribution system, and source protection. This approach can reduce both faecal and non-faecal pathogens to non-detectable levels or to levels that have not

been associated with human illness. *E. coli* sampling should be used as part of this approach to verify the microbiological water quality and safety. Bacteriological analysis needs to be used in conjunction with numerous other indicators and process controls to reliably produce drinking water of an acceptable quality.

4.4.1 Role in source water monitoring

E. coli is well recognized as an indicator of recent faecal contamination; consequently numerous studies have attempted to link the presence of this indicator with the presence of specific faecal pathogens in both surface and groundwater sources. For bacterial faecal pathogens, such as *Salmonella*, pathogenic *E. coli*, and *Campylobacter*, studies have shown that, in general, *E. coli* can be used to indicate the increased potential for all these pathogens to be present in both surface and groundwaters (Mitchell and Starzyk, 1975; Schaffter and Parriaux, 2002; Jokinen et al., 2010), although this relationship is not always found (Dorner et al., 2007). On the other hand, enteric protozoan pathogens can have little (Medema et al., 1997; Atherholt et al., 1998; Payment et al., 2000) or no (Rose at al., 1988, 1991; Chauret et al., 1995; Stevens et al., 2001; Hörman et al., 2004; Dorner et al., 2007; Sunderland et al., 2007) correlation to faecal indicators in surface water sources. In the cases where a correlation has been reported, it is with *Giardia* and at high indicator levels (Wallis et al., 1998). Studies investigating the presence of protozoa in groundwater sources are lacking.

A relationship between the presence of *E. coli* and of enteric viruses in surface water sources has been reported when the water source is known to be contaminated by human faecal pollution (Payment and Franco, 1993; Payment et al., 2000; Ashbolt et al., 2001; Hörman et al., 2004). In a study of groundwater quality in three provinces in Canada, Locas et al. (2007, 2008) reported that most groundwater sites that did not contain any faecal or bacterial indicators were also free of any enteric viruses. However, the detection of enteric viruses in the absence of indicator bacteria has been reported in these and other groundwater studies (Abbaszadegan et al., 1998, 1999; Borchardt et al., 2003, 2004, Locas et al., 2007, 2008). Although the presence of *E. coli* is not necessarily associated with the presence of specific pathogens, studies have shown a link between *E. coli* presence in groundwater and the development of gastrointestinal illness (Craun et al., 1997; Raina et al., 1999).

4.4.2 Role in treatment and distribution system monitoring

Monitoring for *E. coli* 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. *E. coli* are more susceptible to many of the disinfectants commonly used in the drinking water industry than are protozoans and some viruses (Edberg et al., 2000). Nevertheless, if a multi-barrier, source-to-tap approach is in place and 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, then *E. coli* can be used as part of the verification process to show that the water has been adequately treated and is therefore of an acceptable microbiological quality. The presence of any *E. coli* in water leaving a treatment plant or in any treated water immediately post-treatment signifies inadequate treatment and is unacceptable.

The presence of *E. coli* in the distribution and storage system, when water tested immediately post-treatment is free of *E. coli*, suggests that post-treatment contamination has occurred. Post-treatment contamination, for example, through cross-connections, back siphonage, low pressure events, contamination of storage reservoirs, and contamination of mains from repairs, have been identified as causes of distribution system contamination linked to illness

(Craun, 2001; Hunter, 2005). The presence of any *E. coli* in the distribution and/or storage system is unacceptable.

4.4.3 Considerations for residential-scale systems

E. coli is an indicator of recent faecal contamination. Therefore, their presence in disinfected residential-scale systems provides evidence of inadequate disinfection. The presence of *E. coli* in non-disinfected wells indicates that the well is being affected by faecal contamination. Therefore, the water should be boiled or an alternative safe source of water should be used, and an investigation to determine the source of the contamination needs to be carried out. Further information is outlined in section 3.2. If the contamination source cannot be found or corrected, either an appropriate disinfection device or well reconstruction or replacement should be considered.

5.0 Analytical methods

Currently, three methods are routinely used to detect *Escherichia coli* organisms in water: the presence—absence (P-A) method, which is a qualitative test, as well as two quantitative methods, membrane filter (MF) and multiple tube fermentation (MTF). A detailed description of each method is given in *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005). All three detection methods use cultivation to detect or confirm the presence of *E. coli*. Newer molecular methods are available for the detection of pathogens or indicators. Although much progress has been made in this area, molecular methods still have numerous challenges and are not used for routine monitoring. The literature and/or the responsible authority should be consulted for further information regarding these methods.

Cultivation media can be broadly categorized into two types: (1) enzyme-based media that contain fluorogenic or chromogenic substrates for the specific detection and confirmation of the bacterium in a single step (Feng and Hartman, 1982; Ley et al., 1988); and (2) presumptive coliform detection media that require a second step to confirm the presence of *E. coli*.

Methods that detect and confirm the presence of E. coli in a single step are based on the presence of the enzyme β -glucuronidase. This is a unique constitutive enzyme that is found in the vast majority of E. coli isolates, as well as some Shigella spp., and Salmonella spp., but is rarely present in other coliforms (Manafi et al., 1991, Fricker et al., 2008b, 2010). The most publicized methods use the β-glucuronidase activity of E. coli to hydrolyse 4-methylumbelliferyl-β-Dglucuronide to form 4-methylumbelliferone, which fluoresces under longwave ultraviolet (UV) light (Feng and Hartman, 1982). A distinct advantage of enzyme-based methods is that no confirmation step is required. Both presence-absence and quantitative results are possible, depending on the enzyme-based method being used. Some enzyme-based methods also inhibit non-coliform bacterial growth, and thus non-coliform bacteria cannot interfere with the recovery of coliforms. This design is based on the principle that only the target microbe, in this case E. coli, can utilize vital nutrients from the media (Rompré et al., 2002). For these reasons, the use of enzyme-based methods is recommended. Various enzyme-based methods have been approved by the U.S. Environmental Protection Agency (U.S. EPA) as acceptable means for the detection of E. coli in drinking water (U.S. EPA, 1992; Allen et al., 2010). Enzyme-based methods have also been developed that are capable of detecting total coliforms and simultaneously differentiating E. coli (Edberg et al., 1988).

Presumptive coliform media, such as lauryl tryptose broth, m-Endo media or EC media, can make a presumptive determination but cannot distinguish *E. coli* colonies from other types of

coliforms. Therefore, a confirmation step is required. Several options are available for confirmation of $E.\ coli$. For example, the classical "IMViC" test uses biochemical reactions to differentiate the members of the coliform group. Various media and reagents, which are available commercially prepared, are needed to complete the test. Also, $E.\ coli$ confirmation can be done by subjecting the coliform-positive sample to media that tests for the enzyme β -glucuronidase (APHA et al., 2005). The main disadvantages to using presumptive coliform media are the necessity of a confirmation step, which requires additional time (24 hours) to complete the analyses, and the possible interference with recovery by non-coliform bacteria.

Although multiple types of tests can be used, variability exists among these tests in their sensitivity for the detection and quantification of *E. coli*. The method and media composition used, as well as the presence of non-coliform bacteria in the sample, have varying effects on the results (Olstadt, 2007; Fricker et al., 2008a, 2008b). It is also important to use validated or standardized methods to make correct and timely public health decisions.

All analyses for *E. coli* 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 the manufacturer's instructions.

5.1 Presence–absence procedure

The P-A test was developed as a more sensitive, economical, and efficient means of analyzing drinking water samples (Clark and Vlassoff, 1973). This procedure is currently the preferred method in many jurisdictions for verifying the bacteriological safety of public drinking water supplies (i.e., the absence of *E. coli*). Essentially the P-A test is a modification of the MTF procedure (see section 5.3) in which only one analysis bottle per sample is used. This method can be used with either enzyme-based media or presumptive coliform media (e.g., using lauryl tryptose broth), with follow-up *E. coli* confirmation. Commercial test kits using enzyme-based media have been developed for P-A testing. Studies performed on the effectiveness of the commercial tests compared with classical MTF and MF approaches showed that the commercial kits were usually as sensitive as the MTF approach for the detection of *E. coli*, and sometimes more sensitive for the detection of total coliforms (Rompré et al., 2002). Also, data illustrate that some media based on enzyme-based methods can detect injured coliforms within 24 hours (Edberg and Edberg, 1988).

In comparative tests using lactose-based media, the P-A method was shown to be at least as sensitive as the MF and MTF techniques for the recovery of both total coliforms and *E. coli* (Clark, 1980; Jacobs et al., 1986; Pipes et al., 1986; Clark and El-Shaarawi, 1993), and it required a similar amount of time to obtain results. Technically, P-A testing (using lactose-based and enzyme-based technologies) is simpler than the MF and MTF procedures and has a shorter processing time (less than 1 minute per sample).

P-A testing does not provide any information on the actual concentration of organisms in the sample. The quantitation of organisms is sometimes used to assess the extent of the contamination, and as such is considered a benefit of the more quantitative methods such as the MF and MTF methods. For decision-making, the focus is the positive detection of *E. coli*,

regardless of quantity; as the guideline for *E. coli* in drinking water is none per 100 mL, qualitative results are sufficient for protecting public health.

5.2 Membrane filter procedure

The MF procedure was introduced to bacteriological water analysis in 1951, after its capability to produce results equivalent to those obtained by the MTF procedure was demonstrated (Clark et al., 1951; Goetz and Tsuneishi, 1951). Both enzyme-based media and presumptive coliform media can be used. With this technique, the water sample is passed through a filter that retains bacteria. The filter is then placed on a standard presumptive coliform medium or on a medium containing substrates designed for the detection of the enzyme βglucuronidase (Dufour et al., 1981; Ciebin et al., 1995) and incubated. The advantages of the technique were quickly recognized because it made the examination of larger volumes of water practical. Sensitivity and reliability were increased, whereas time, labour, equipment, space, and material requirements were significantly reduced. The MF technique remains the method of choice in some jurisdictions for the routine enumeration of coliforms in drinking water; however, this method may underestimate the number of viable coliform bacteria in a sample. Standard Methods for the Examination of Water and Wastewater provides 95% confidence limits for MF results (APHA et al., 2005). When the MF method is used with media capable of detecting the enzyme β -glucuronidase, it is an efficient means of enumerating E. coli in water; however, it does not satisfactorily solve the problems linked to the presence of non-culturable bacteria (discussed below) (Rompré et al., 2002). Commercial agar is available for routine enumeration.

The major concern for this and other methods that use stressful selective media (i.e., media that contain inhibitory chemicals for non-target organisms) is an inability to enumerate bacteria that have been subjected to sublethal injury (e.g., caused by chlorination) in a treatment plant or distribution system. The resultant false-negative findings could lead to the acceptance of water of potentially hazardous quality. Although stressed organisms may not grow on selective media, they can recover through a resuscitation process. Detection of stressed coliforms, in general, has been improved using enhanced recovery media such as m-T7 (LeChevallier et al., 1983) or through the addition of substances, such as catalase and/or sodium pyruvate, to m-Endo or m-FC media (Calabrese and Bissonnette, 1990). Since these media are not specific for *E. coli*, additional confirmation steps are still needed. To overcome the need for a confirmation step, substrates such as 4-methylumbelliferyl- β -D-glucuronide can be added to selective and non-selective coliform media. As described above, the β -glucoronidase enzyme produced by *E. coli* will cleave the substrate in the media, resulting in a fluorescent product that can be visualized under UV light.

High turbidity can also interfere with the MF method. The retention of particulate matter by the filter can interfere with colony development and the production of surface sheens and/or fluorescence by presumptive coliforms or *E. coli*. Similarly, concentrations of heterotrophic bacteria in excess of 500 colony-forming units (CFU) per millilitre can interfere with coliform recovery when using presumptive coliform media (Geldreich et al., 1972; Clark, 1980; Burlingame et al., 1984), even with the addition of substrates for detecting the β-glucuronidase enzyme. Most water supplies that maintain a total chlorine residual of 0.2 mg/L have a heterotrophic plate count (HPC) below 500 CFU/mL (LeChevallier, 1990). Further information on HPC along with their significance in drinking water, can be found in the guidance document on heterotrophic plate count (Health Canada, 2012b). Background colony counts can also be used for determining whether there is interference with coliform recovery when using presumptive coliform media. Although this method is quantitative, clumping of *E. coli* or

masking because of growth of other microorganisms, can also lead to underestimations in concentrations. Since *E. coli* should not be present in treated drinking water, these underestimations are not as much of a concern as are false-negative findings.

5.3 Multiple tube fermentation procedure

In the MTF procedure, 10-fold dilutions of the water to be tested are added to tubes containing the appropriate medium (5 or 10 tubes per dilution) and incubated. Both enzyme-based media and presumptive coliform media can be used. For drinking water, dilution should be unnecessary because of the expected low counts. Commercial kits using enzymatic methods have been developed for enumeration by the multiple tube technique (Rompré et al., 2002). Results are reported as a most probable number (MPN). The MPN is only a *statistical* estimate of the number of bacteria that, more than any other number, would probably give the observed result; it is not an actual count of the bacteria present. *Standard Methods for the Examination of Water and Wastewater* provides 95% confidence limits for MPN results (APHA et al., 2005). Studies performed on the effectiveness of the commercial tests compared with classical MTF and MF approaches showed that the commercial kits were usually as sensitive as the MTF approach for the detection of *E. coli* and sometimes more sensitive for the detection of total coliforms (Rompré et al., 2002).

Similar to the situation with the MF procedure, high densities of non-coliform bacteria and the inhibitory nature of some presumptive coliform MTF media may have an adverse influence on *E. coli* detection. For example, many heterotrophic bacteria can inhibit the detection of *E. coli* (Waksman, 1941; Hutchison et al., 1943; Means and Olson, 1981). In addition, the recovery of coliforms from gas-negative MTF tubes has demonstrated the presence of inhibitory compounds in the MTF media (Evans et al., 1981; McFeters et al., 1982). In response to these findings, APHA et al. (2005) recommends treating all tubes with turbidity, regardless of gas production, as presumptive coliform-positive tubes. Clumping of coliforms can lead to an underestimation of their concentrations in both MF and MTF methods.

The MTF procedure has a longer turnaround time for results and the MF procedure has largely replaced it for routine examinations of drinking water when using presumptive coliform media. However, the MTF technique is used more extensively with enzyme-based tests when conditions render the MF technique unusable, for example, with turbid, coloured, or grossly contaminated water.

6.0 Sampling for E. coli

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 APHA et al. (2005). To avoid unpredictable changes in the bacterial flora of the sample, examination should be started as soon as possible after collection. The sample should be transported to the laboratory in a cooler with ice or cooling packs (at $5 \pm 3^{\circ}$ C), to minimize changes in populations and concentrations (Dutka and El-Shaarawi, 1980; McDaniels et al., 1985; ISO, 2006). As well, samples should be protected from direct contact with the ice or cooling packs to prevent freezing during transport. Ideally, the interval between collection of the sample and the beginning of its examination should not exceed 24 hours (Bartram and Rees, 2000), and analysis within 8 hours is recognized as the preferred time interval (Bartram and Rees, 2000; APHA et al., 2005). In remote areas, up to 48 hours may be an

acceptable time interval; however, the implications of the extended holding time should be discussed with the responsible authorities. When delays are anticipated, a delayed incubation procedure, described in APHA et al. (2005), should be employed or consideration given to onsite testing. Alternatively, if normal transportation time exceeds 24 or 48 hours (depending on circumstances noted above), the sample should be processed and arrangements made to have another sample collected as soon as the first sample is received. Thus, if the first sample contains *E. coli*, a repeat sample will already have been received or will be in transit. Samples should be labelled with the time, date, location, type of sample (e.g., raw water, distribution system), sampler's name, and identification number (if used), along with the disinfectant residual measurements and any special conditions. In most cases, much of this information, along with the identification number linked to the sample bottle, is recorded on accompanying submission forms and, in cases where samples are collected for legal purposes, chain-of-custody paperwork. When examination will be delayed, it is particularly important to record the duration and temperature of storage, as this information should be taken into consideration when interpreting the results.

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

6.2 Sampling frequency considerations

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

- past frequency of unsatisfactory samples;
- source water quality;
- the number of raw water sources;
- the adequacy of treatment and capacity of the treatment plant;
- the size and complexity of the distribution system; 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 upon by the responsible authority after due consideration of local conditions—for example, variations in raw water quality and a history of treated water quality. The sampling frequency should meet all jurisdictional requirements.

As a minimum, water leaving a treatment plant should be tested daily for disinfectant residual and turbidity and tested at least weekly for *E. coli* as part of the verification process in a source-to-tap multi-barrier approach. A guide for the recommended sampling frequency is provided in Table 1. 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 *E. coli* testing is impractical (e.g., in small supplies), *E. coli* sampling may be reduced and other means of verifying the microbiological quality may be used, such as residual disinfectant determinations and good process control. Small supplies should also periodically carry out

sanitary surveys as an additional action to verify the safety of the system. The daily sampling recommendations for disinfectant residual and turbidity testing may not apply to supplies served by groundwater sources of excellent quality in which disinfection is practised to increase the safety margin.

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

Table 1: Recommended sampling frequency.

- 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4	-J
Population served	Minimum number of samples per month*
Up to 5000	4
5000–90 000	1 per 1000 persons
90 000+	90 + (1 per 10 000 persons)

^{*} The 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 tests should be conducted when bacteriological samples are taken. Further information on monitoring for turbidity can be found in the guideline technical document for turbidity (Health Canada, 2013b). The majority of samples should be taken in potential problem areas. 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 a distribution system. It must be emphasized that the frequencies suggested in Table 1 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 with jurisdiction but should include times when the risk of contamination is greatest, for example, during 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 *E. coli*, 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 water main, in a reservoir, at the treatment plant, or for a long duration at a high concentration (Speight et al., 2004; van Lieverloo, 2007). Therefore, even if the analytical result indicates the absence of *E. coli*, intrusion may be occurring in the distribution system. Some improvement in detection capabilities were found when sampling programs were designed with the lowest standard deviation in time between sampling events (van Lieverloo, 2007), such as samples collected every 5 days regardless of weekends and holidays. This highlights the importance of implementing a source-to-tap multi-barrier approach, as opposed to relying on monitoring a single parameter for verifying the microbiological quality of the drinking water.

6.3 Location of sampling points

In municipal-scale systems, the location of sampling points must be chosen by the responsible authority. The sampling locations selected may vary 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 work has been published on how to select statistically based random sampling sites (Speight et al., 2004).

Samples should be taken at the point where the water enters a system and from representative points throughout a 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 in potential problem areas, such as low-pressure zones, reservoirs, dead ends, areas at the periphery of the system farthest from the treatment plant, and areas with a poor previous record.

In residential-scale systems, samples are generally collected from the locations recommended by the responsible authority. More extensive sampling may be necessary, depending on the system and results from previous samples.

7.0 Treatment technology

The application of a multi-barrier approach, including watershed or well-head protection, optimized treatment barriers, and a well-maintained distribution system, is the best approach to reduce the presence and associated health risks of waterborne pathogens to an acceptable level. *E. coli* monitoring is part of the multi-barrier approach.

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

7.1 Municipal scale

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

7.1.1 Level of treatment necessary

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

7.1.2 Physical removal

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

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. This strategy helps to ensure efficient inactivation of pathogens and minimizes the formation of disinfection byproducts (DBPs). It is important to note that when describing microbial disinfection of drinking water, the term "inactivation" is used to indicate that the pathogen is no longer able to multiply within its host and is therefore non-infectious, although it may still be present.

7.1.3.1 Chemical disinfection

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

leading to superior coliform control (LeChevallier et al., 1990; LeChevallier, 2003). However, chloramine is less efficient at controlling a sudden pulse of contamination (Snead et al., 1980) and it can lead to nitrification. Chlorine dioxide is as effective as, and in some instances more effective than, chlorine. However, this compound is difficult to work with and therefore is not widely used. Ozone is more efficient for the inactivation of bacteria, viruses, and protozoa compared with chlorine-based disinfectants, although ozone treatment can result in an increase in biodegradable organics that can promote bacterial regrowth in the distribution system. Ozone is highly effective at the point of treatment, but an additional disinfectant (usually chlorine or chloramine) needs to be added to supply a residual. Maintaining a disinfectant residual will limit the growth of organisms within the distribution system and, depending on the residual concentration, contact time, and the pathogens present, a disinfectant residual also may afford some protection against contamination from intrusion (Besner et al., 2008). The disappearance of the residual may also provide an immediate indication of the entry of oxidizable matter into the system or a malfunction of the treatment process.

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

CT values for 99% inactivation of *E. coli* using chlorine, chlorine dioxide, chloramine, and ozone are provided in Table 2. 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 2, it is apparent that, compared with most protozoans and viruses, coliform bacteria are easier to inactivate using the common chemical disinfectants. Also, chloramines have a much higher CT value than any of the other disinfectants listed. This means that to achieve the same level of inactivation with chloramine, a higher disinfectant concentration or a longer contact time, or a combination of both, is necessary. This is consistent with the properties of chloramine as a disinfectant, as previously described.

Table 2: CT values for 99% inactivation at 5°C

Disinfectant agent	pН	E. coli ^a (mg·min/L)	Giardia lamblia ^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°
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

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

Log inactivations using UV light disinfection are listed in Table 3. *E. coli*, because of its importance as a public health indicator, has been used as a representative bacterial species. For comparison, UV light doses for representative protozoa and viruses have also been included. Review of the data on inactivation using UV light (Table 3) shows that, of the representative organisms, bacteria (in this instance, *E. coli*) and protozoa require comparable doses of UV light to achieve the same level of inactivation, whereas certain viruses are much more resistant.

Table 3: UV light dose (mJ/cm²) required for inactivation

Log inactivation	E. coli ^{a,d}	Cryptosporidium ^a	Adenovirus ^{a,b,d}	Rotavirus ^{a,c,d}	Giardia ^b
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).

7.2 Residential scale

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

Various options are available for treating source waters to provide high-quality pathogen-free drinking water. These include filtration and disinfection with chlorine-based compounds or alternative technologies, such as UV light. These technologies are similar to the municipal treatment barriers, but on a smaller scale. In addition, there are other treatment processes, such as distillation, that can be practically applied only to small or individual water supplies. Most of these technologies have been incorporated into point-of-entry devices, which treat all water entering the system, or point-of-use devices, which treat water at only a single location—for example, at the kitchen tap. It is important to note that if point-of-use devices are used instead of a point-of-entry system, all points of water used for drinking, food and beverage preparation, hygiene or washing dishes should be equipped with a point-of-use treatment device, to minimize the potential public health risks when use of microbiologically-contaminated drinking water.

^b Adenoviruses are highly UV resistant in comparison with other enteric viruses. Further information is available in Health Canada's guideline technical document on enteric viruses (Health Canada, 2011).

^c LeChevallier and Au (2004)

^d Hijnen et al. (2006)

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

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

NSF/ANSI Standard 55 (Ultraviolet Disinfection Systems) provides performance criteria for two categories of certified systems, Class A and Class B. UV systems certified to NSF/ANSI Standard 55 Class A are designed to deliver a UV dose that is 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. 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. NSF Standard 62 for Drinking Water Distillation Systems also includes reduction claims for bacteria. To meet this standard, a distillation system must provide a minimum 6 log reduction of bacteria and bacterial spores. Distillation systems should only be installed at the point of use as the water they have treated may be corrosive to internal plumbing components.

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

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

8.0 Risk assessment

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

A health-based risk assessment for *E. coli* is not appropriate since *E. coli* is used only as an indicator organism. Risk assessments have been done for specific microbiological organisms that have health implications, such as enteric viruses and the enteric protozoa *Cryptosporidium* and *Giardia* (Health Canada, 2011, 2012c).

Current drinking water guidelines encourage the adoption of a multi-barrier approach to produce clean, safe, and reliable drinking water. *E. coli* is a bacteriological indicator that should be routinely used as part of this approach. *E. coli* is well recognized as an indicator of recent faecal contamination. In surface and GUDI water sources, *E. coli* monitoring results can be used as part of the source water assessment to look at the microbiological quality of the water and to indicate changes in this quality. In protected groundwater systems, the presence of *E. coli* signals that the groundwater is being impacted by faecal contamination and is microbiologically unsafe for drinking without further treatment. Monitoring for *E. coli*, when used in conjunction with a source-to-tap multi-barrier approach, is used as part of the verification that the drinking water system is producing water that is microbiologically acceptable.

8.1 International considerations

Other countries use *E. coli* for similar purposes. The Drinking Water Inspectorate of England and Wales has included in its regulations a mandatory value of zero *E. coli* per 100 mL in water leaving treatment works, in service reservoirs, and at the consumer's tap (DWI, 2000). These regulations are based on the European Union's Council Directive on the quality of water intended for human consumption, which specifies a maximum contaminant level of zero for *E. coli* in 100mL of water (Council of the European Union, 1998). The Australian Drinking Water Guidelines (NHMRC, 2011) include *E. coli* as an indicator for faecal contamination and set a guideline of no *E. coli* in any sample of drinking water (minimum of 100 mL). The proposed revision to the Total Coliform Rule in the United States includes a maximum contaminant level (MCL) and a maximum contaminant level goal (MCLG) of zero for *E. coli* because it is a more specific indicator of faecal contamination (than total coliforms) (U.S. EPA, 2010). The presence of *E. coli* in a repeat sample or the presence of *E. coli* in a routine sample followed by a positive test for either total coliforms or *E. coli* in the repeat sample exceeds the MCL. A system can also be non-compliant with the MCL for *E. coli* if they fail to perform all the necessary testing.

9.0 Rationale

Of the contaminants that may be regularly found in drinking water, those present in human and animal faeces pose the greatest danger to public health. Faecal pathogens of concern for transmission through water include bacteria (e.g., *Campylobacter*, pathogenic *E. coli*), viruses (e.g., rotaviruses, enteroviruses), and protozoa (e.g., *Cryptosporidium*, *Giardia*). Routinely detecting pathogenic bacteria, viruses, and protozoa is currently not practical. Reasons for this include the large number of different pathogens that exist, their uneven distribution in water, and the time and expense associated with routine monitoring of all pathogens. It is better to use an indicator that is less difficult, less expensive, and less time consuming to monitor. This encourages a higher number of samples to be tested, giving a better overall picture of the water quality and therefore better protection of public health.

Although *E. coli* are not necessarily related to the presence of specific faecal pathogens, the presence of *E. coli* is a definite indicator of the presence of recent human or animal faeces. In addition to being faecal specific, *E. coli* do not usually multiply in the environment, are excreted in the faeces in high numbers, making detection possible even when greatly diluted, and have a

life span on the same order of magnitude as those of other enteric bacterial pathogens. This makes *E. coli* the best available indicator of faecal contamination and the potential presence of faecal pathogens. Any level of *E. coli* in drinking water would be related to faecal contamination and is deemed unacceptable. Consequently, the guideline for *E. coli* in drinking water systems is proposed as a maximum acceptable concentration of none detectable per 100 mL.

Sampling and analysis for *E. coli* is an easy, relatively quick, inexpensive way of monitoring the quality of drinking water. The absence of *E. coli*, when used in conjunction with a source-to-tap multi-barrier approach, is used as part of the verification that the drinking water system is producing water that is microbiologically acceptable.

10.0 References

Abbaszadegan, M., Stewart, P.W., LeChevallier, M.W. and Gerba, C.P. (1998). Application of PCR technologies for virus detection in groundwater. American Water Works Association, Denver, Colorado (Report No. 90740).

Abbaszadegan, M., Stewart, P. and LeChevallier, M. (1999). A strategy for detection of viruses in groundwater by PCR. Appl. Environ. Microbiol., 65(2): 444–449.

Allen, M.J., Clancy, J.L. and Rice, E.W. (2000). The plain, hard truth about pathogen monitoring. J. Am. Water Works Assoc., 92(9): 64–76.

Allen, M.J., Payment, P. and Clancy, J.L. (2010). Rapid microbial methods can improve public health protection. J. Am. Water Works Assoc., 102(8): 44–51.

APHA, AWWA and WEF (2005). Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC.

Artz, R.R.E. and Killham, K. (2002). Survival of *Escherichia coli* O157:H7 in private drinking water wells: influences of protozoan grazing and elevated copper concentrations. FEMS Microbiol. Lett., 216: 117–122.

Ashbolt, N.J., Grabow, W.O.K. and Snozzi, M. (2001). Indicators of microbial water quality. In: Water quality: guidelines, standards and health—assessment of risk and risk management for water-related infectious disease. L. Fewtrell and J. Bartram (eds.). IWA Publishing, London, UK, on behalf of the World Health Organization, Geneva. pp. 289–315.

Atherholt, T.B., LeChevallier, M.W., Norton, W.D. and Rosen, J.S. (1998). Effect of rainfall on *Giardia* and *Cryptosporidium*. J. Am. Water Works Assoc., 90: 66–80.

AWWA (1999). Water quality and treatment: a handbook of community water supplies. American Water Works Association. McGraw-Hill, New York, New York.

Bartram, J. and Rees, G. (2000). Monitoring bathing waters. E & FN Spon, New York, New York.

Blanch A.R., Galofré, B., Lucena, F., Terradillos, A., Vilanova, X. and Ribas, F. (2007). Characterization of bacterial coliform occurrences in different zones of a drinking water distribution system. J. Appl. Microbiol., 102:711–721.

Borchardt, M.A., Bertz, P.D., Spencer, S.K. and Battigelli, D.A. (2003). Incidence of enteric viruses in groundwater from household wells in Wisconsin. Appl. Environ. Microbiol., 69: 1172–1180.

Escherichia coli (March 2012)

Borchardt, M.A., Haas, N.L. and Hunt, R.L. (2004). Vulnerability of drinking-water wells in La Crosse, Wisconsin, to enteric-virus contamination from surface water contributions. Appl. Environ. Microbiol., 70: 5937–5946.

Burlingame, G.A., McElhaney, J., Bennett, M. and Pipes, W.O. (1984). Bacterial interference with coliform colony sheen production on membrane filters. Appl. Environ. Microbiol., 47: 56–60.

Byappanahalli, M.N., Whitman, R.L., Shively, D.A., Sadowsky, M.J. and Ishii, S. (2006). Population structure, persistence, and seasonality of autochthonous *Escherichia coli* in temperate, coastal forest soil from a Great Lakes watershed. Environ. Microbiol., 8(3): 504–513.

Calabrese, J.P. and Bissonnette, G.K. (1990). Improved membrane filtration method incorporating catalase and sodium pyruvate for detection of chlorine-stressed coliform bacteria. Appl. Environ. Microbiol., 56: 3558–3564.

Carrillo, M., Estrada, E. and Hazen, T.C. (1985). Survival and enumeration of the fecal indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a tropical rain forest watershed. Appl. Environ. Microbiol. 50: 468–476.

CCME (2004). From source to tap: guidance on the multi-barrier approach to safe drinking water. Produced jointly by the Federal-Provincial-Territorial Committee on Drinking Water and the CCME Water Quality Task Group. Canadian Council of Ministers of the Environment, Winnipeg, Manitoba. Available at: www.ccme.ca/assets/pdf/mba_guidance_doc_e.pdf

Chauret, C., Armstrong, N., Fisher, J., Sharma, R., Springthorpe, S. and Sattar, S. (1995). Correlating *Cryptosporidium* and *Giardia* with microbial indicators. J. Am. Water Works Assoc., 87(11): 76–84.

Ciebin, B.W., Brodsky, M.H., Eddington, R., Horsnell, G., Choney, A., Palmateer, G., Ley, A., Joshi, R. and Shears, G. (1995). Comparative evaluation of modified m-TEC media for membrane filter enumeration of *Escherichia coli* in water. Appl. Environ. Microbiol., 61(11): 3940–3942.

Clark, H.F., Geldreich, E.E., Jeter, H.L. and Kabler, P.W. (1951). The membrane filter in sanitary microbiology. Public Health Rep., 66: 951–977.

Clark, J.A. (1980). The influence of increasing numbers of non-indicator organisms by the membrane filter and presence–absence test. Can. J. Microbiol., 26: 827–832.

Clark, J.A. and El-Shaarawi, A.H. (1993). Evaluation of commercial presence–absence test kits for detection of total coliforms, *Escherichia coli*, and other indicator bacteria. Appl. Environ. Microbiol., 59(2): 380–388.

Clark, J.A. and Vlassoff, L.T. (1973). Relationships among pollution indicator bacteria isolated from raw water and distribution systems by the presence–absence (P-A) test. Health Lab. Sci., 10: 163–172.

Cook, K.L. and Bolster, C.H. (2007). Survival of *Campylobacter jejuni* and *Escherichia coli* in groundwater during prolonged starvation at low temperatures. J. Appl. Microbiol., 103: 573–583.

Council of the European Union (1998). Council directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Off. J. Eur. Commun., L330: 32.

Craun, G.F., Berger, P.S. and Calderon, R.L. (1997). Coliform bacteria and waterborne disease outbreaks. J. Am. Water Works Assoc., 89(3): 96–104.

Craun, G.F. and Calderon, R.L. (2001). Waterborne disease outbreaks caused by distribution system deficiencies. J. Am. Water Works Assoc., 93: 64–75.

Department of National Health and Welfare (1977). Microbiological quality of drinking water. 77-EHD-2, Environmental Health Directorate, Ottawa, Ontario.

Dorner, S.M., Anderson, W.B., Gaulin, T., Candon, H.L., Slawson, R.M., Payment, P. and Huck, P.M. (2007). Pathogen and indicator variability in a heavily impacted watershed. J. Water Health, 5: 241–257.

Dufour, A., Strickland, E. and Cabelli, V. (1981). Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol., 41: 1152–1158.

Dutka, B.J. and El-Shaarawi, A. (1980). Microbiological water and effluent sample preservation. Can. J. Microbiol., 26: 921–929.

DWI (2000). Water, England and Wales: the water supply (water quality) regulations 2000 no. 3184. Drinking Water Inspectorate, London, UK. Available at: http://www.dwi.gov.uk/regs/si3184/3184.htm#sch1pA

Edberg, S.C. and Edberg, M.M. (1988). A defined substrate technology for the enumeration of microbial indicators of environmental pollution. Yale J. Biol. Med., 61: 389–399.

Edberg, S.C., Allen, M.J. and Smith, D.B. (1988). National field evaluation of a defined substrate method for the simultaneous evaluation of total coliforms and *Escherichia coli* from drinking water: comparison with the standard multiple tube technique. Appl. Environ. Microbiol., 54: 1595–1601.

Edberg, S.C., Rice, E.W., Karlin, R.J. and Allen, M.J. (2000). *Escherichia coli*: the best biological drinking water indicator for public health protection. J. Appl. Microbiol., 88: 106S–116S.

Evans, T.M., Waarvick, C.E., Seidler, R.J. and LeChevallier, M.W. (1981). Failure of the most probable number technique to detect coliforms in drinking water and raw water supplies. Appl. Environ. Microbiol., 41: 130–138.

Fass, S., Dincher, M.L., Reasoner, D.J., Gatel, D. and Block, J.C. (1996). Fate of *Escherichia coli* experimentally injected in a drinking water distribution pilot system. Water Res., 30: 2215–2221.

Feng, P.C.S. and Hartman, P.A. (1982). Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol., 43: 1320–1329.

Filip, Z., Kaddu Mulindwa, D. and Milde, G. (1987). Survival and adhesion of some pathogenic and facultative pathogenic microorganisms in ground water. Water Sci. Technol., 19(7): 1189–1190.

Foppen, J.W.A. and Schijven, J.F. (2006). Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. Water Res. 40: 401–426.

Fricker, C.R., Bullock, S., Murrin, K. and Niemela, S.I. (2008a). Use of the ISO 9308-1 procedure for the detection of *Escherichia coli* in water utilizing two incubation temperatures and two confirmation procedures and comparison with defined substrate technology. J. Water Health, 6: 389–397.

Fricker, C.R., DeSarno, M., Warden, P.S. and Eldred, B.J. (2008b). False negative β -D-glucuronidase reactions in membrane lactose glucuronide agar medium used for the simultaneous detection of coliforms and *Escherichia coli* from water. Lett. Appl. Microbiol., 47: 539–542.

Fricker, C.R., Warden, P.S. and Eldred, B.J. (2010). Understanding the cause of false negative β -d-glucuronidase reactions in culture media containing fermentable carbohydrate. Lett. Appl. Microbiol. 50 (547-551).

Geldreich, E.E. (1996). Biological profiles in drinking water. In: Microbial quality of water supply in distribution systems. E.E. Geldreich (ed.). CRC Press, Lewis Publishers, Boca Raton, FL. pp. 104–144.

Geldreich, E.E., Nash, H.D., Reasoner, D.J. and Taylor, R.H. (1972). The necessity of controlling bacterial populations in potable waters: community water supply. J. Am. Water Works Assoc., 64: 596–602.

Goetz, A. and Tsuneishi, N. (1951). Application of molecular filter membrane to bacteriological analysis of water. J. Am. Water Works Assoc., 43: 943–969.

Hardina, C.M. and Fujioka, R.S. (1991). Soil: the environmental source of *Escherichia coli* and enterococci in Hawaii's streams. Environ. Toxicol. Water Qual., 6: 185–195.

Harris, D.G., Adams, V.D., Sorensen, D.L. and Curtis, M.S. (1987). Ultra-violet inactivation of selected bacteria and viruses with photoreactivation of bacteria. Water Res., 21: 687–692.

Health and Welfare Canada (1993). Water treatment principles and applications: a manual for the production of drinking water. Canadian Water and Wastewater Association, Ottawa, Ontario.

Health Canada (2009). Guidelines for Canadian drinking water quality: guidance for issuing and rescinding boil water advisories. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. Available at: www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/boil_water-eau ebullition/index-eng.php

Health Canada (2011). Guidelines for Canadian drinking water quality: guideline technical document — enteric viruses. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H129-6/2011E). Available at: www.healthcanada.gc.ca/waterquality

Health Canada (2012a). Guidelines for Canadian drinking water quality: guideline technical document — total coliforms. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H144-8/2013E-PDF). Available at: www.healthcanada.gc.ca/waterquality

Health Canada (2012b). Guidance on the use of heterotrophic plate counts in Canadian drinking water supplies. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario (Catalogue No. H144-6/2013E-PDF). Available at: www.healthcanada.gc.ca/waterquality

Health Canada (2012c). Guidelines for Canadian drinking water quality: guideline technical document — enteric protozoa: *Giardia* and *Cryptosporidium*. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H129-23/2013E-PDF). Available at: www.healthcanada.gc.ca/waterquality

Health Canada (2013a). Guidance on waterborne bacterial pathogens. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No. H144-10/2013E-PDF). Available at: www.healthcanada.gc.ca/waterquality

Health Canada (2013b). Guidelines for Canadian drinking water quality: guideline technical document — turbidity. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No. H144-9/2013E-PDF). Available at: www.healthcanada.gc.ca/waterquality

Heuvel, A.V., McDermott, C., Pillsbury, R., Sandrin, T., Kinzelman, J., Ferguson, J., Sadowsky, M., Byappanahalli, M., Whitman, R. and Kleinheinz, G.T. (2010). The green alga, *Cladophora*, promotes *Escherichia coli* growth and contamination of recreational waters in Lake Michigan. Journal of Environmental Quality, 39 (1): 333-344.

Hijnen, W.A., Beerendonk, E., Smeets, P. and Medema, G.J. (2004). Elimination of micro-organisms by drinking water treatment processes—a review, 1st edition. Kiwa N.V. Water Research, Nieuwegein, The Netherlands.

Hijnen, W.A., Beerendonk, E.F. and Medema, G.J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cyst in water: A review. Water Res., 40: 3–22.

Hoff, J.C. (1986). Inactivation of microbial agents by chemical disinfectants. U.S. Environmental Protection Agency, Cincinnati, OH (EPA/600/S2-86/067).

Hörman, A., Rimhanen-Finne, R., Maunula, L., von Bonsdorff, C., Torvela, N., Heikinheimo, A. and Hänninen, M. (2004). *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., noroviruses, and indicator organisms in surface water in southwestern Finland, 2000–2001. Appl. Environ. Microbiol., 70(1): 87–95.

Hunter, P.R., Chalmers, R.M., Hughes, S. and Syed, Q. (2005). Self-reported diarrhea in a control group: a strong association with reporting of low-pressure events in tap water. Clin. Infect. Dis., 40: e32–e34.

Hutchison, D., Weaver, R.H. and Scherago, M. (1943). The incidence and significance of microorganisms antagonistic to *Escherichia coli* in water. J. Bacteriol., 45: 29.

Ishii, S., Ksoll, W.B., Hicks, R.E. and Sadowsky, M.J. (2006). Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. Appl. Environ. Microbiol., 72(1): 612–621.

ISO (2006). Water quality – Sampling for microbiological analysis. ISO 19458: 2006.

Jacobs, N.J., Zeigler, W.L., Reed, F.C., Stukel, T.A. and Rice, E.W. (1986). Comparison of membrane filter, multiple-fermentation-tube, and presence—absence techniques for detecting total coliforms in small community water systems. Appl. Environ. Microbiol., 51: 1007–1012.

Jiménez, L., Muniz, I., Toranzos, G.A. and Hazen, T.C. (1989). Survival and activity of *Salmonella typhimurium* and *Escherichia coli* in tropical freshwater. J. Appl. Bacteriol., 67: 61–69.

Jokinen, C.C., Schreier, H., Mauro, W., Taboada, E., Isaac-Renton, J.L., Topp, E., Edge, T., Thomas, J.E. and Gannon, V.P.J. (2010). The occurrence and sources of *Campylobacter* spp., *Salmonella enterica*, and *Escherichia coli* O157:H7 in the Salmon River, British Columbia, Canada. J. Water Health, 8: 374–386.

Juhna, T., Birzniece, D., Larsson, S., Zulenkov, D., Sharipo, A., Azevedo, N.F., Ménard-Szczebara, F., Castagnet, S., Féliers, C. and Keevil, C.W. (2007). Detection of *Escherichia coli* in biofilms from pipe samples and coupons in drinking water distribution networks. Appl. Environ. Microbiol., 73: 7456–7464.

Karim, M.R., Manshadi, F.D., Karpiscak, M.M. and Gerba, C.P. (2004). The persistence and removal of enteric pathogens in constructed wetlands. Water Res. 38: 1831–1837.

Kudryavtseva, B.M. (1972). An experimental approach to the establishment of zones of hygienic protection of underground water sources on the basis of sanitary-bacteriological indices. J. Hyg. Epidemiol. Microbiol. Immunol., 18: 503–511.

LeChevallier, M.W. (2003). Conditions favouring coliform and HPC bacterial growth in drinking-water and on water contact surfaces. In: Heterotrophic plate counts and drinking-water safety. The significance of HPCs for water quality and human health. J. Bartram, J. Cotruvo, M. Exner, C. Fricker, and A. Glasmacher (eds.). IWA Publishing, London, UK. pp. 177–198.

LeChevallier, M.W. and Au, K.K. (2004). Water treatment and pathogen control: Process efficiency in achieving safe drinking water. IWA Publishing, London, UK, on behalf of the World Health Organization, Geneva.

LeChevallier, M.W., Cameron, S.C. and McFeters, G.A. (1983). New medium for the improved recovery of coliform bacteria from drinking water. Appl. Environ. Microbiol., 45: 484–492.

LeChevallier, M.W., Babcock, T.M. and Lee, R.G. (1987). Examination and characterization of distribution system biofilms. Appl. Environ. Microbiol. 53: 2714–2724.

LeChevallier, M.W., Lowry, C.D. and Lee, R.G. (1990). Disinfection of biofilms in a model distribution system. J. Am. Water Works Assoc., 82(7): 87–99.

LeChevallier, M.W., Norton, W.D. and Lee, R.G. (1991). Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. Appl. Environ. Microbiol., 57(9): 2610–2616.

Leclerc, H., Mossel, D.A.A., Edberg, S.C. and Struijk, C.B. (2001). Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. Annu. Rev. Microbiol., 55: 201–234.

Lehtola, M.J., Torvinen, E., Kusnetsov, J., Pitkänen, T., Maunula, L., von Bonsdorff, C.H., Martikainen, P.J., Wilks, S.A., Keevil, C.W. and Miettinen, I.T. (2007). Survival of *Mycobacterium avium*, *Legionella pneumophila*, *Escherichia coli*, and Caliciviruses in drinking water-associated biofilms grown under high-shear turbulent flow. Appl. Environ. Microbiol., 43: 2854–2859.

Ley, A.N., Bowers, R.J. and Wolfe, S. (1988). Indoxyl-β-glucuronide, a novel chromogenic reagent for the specific detection and enumeration of *Escherichia coli* in environmental samples. Can. J. Microbiol., 34: 690–693.

Locas, A., Barthe, C., Barbeau, B., Carrière, A., and Payment, P. (2007). Virus occurrence in municipal groundwater sources in Quebec, Canada. Can. J. Microbiol., 53 (6): 688-694.

Locas, A., Barthe, C., Margolin, A.B., and Payment, P. (2008). Groundwater microbiological quality in Canadian drinking water municipal wells. Can. J. Microbiol., 54 (6):472-478.

Manafi, M., Kneifel, W. and Bascomb, S. (1991). Fluorogenic and chromogenic substrates used in bacterial diagnostics. Microbiol. Rev., 55: 335–348.

Martins, M.T. Rivera, I.G., Clark,, D.L., Stewart, M.H., Wolfe, R.L. and Olson, B.H. (1993). Distribution of *uidA* gene sequences in *Escherichia coli* isolates in water sources and comparison with the expression of beta-glucuronidase activity in 4-methylumbelliferyl-beta-D-glucuronide media. Appl. Environ. Microbiol. 59(7):2271.

McCabe, L.J., Symons, J.M., Lee, R.D. and Robeck, G.G. (1970). Survey of community water supply systems. J. Am. Water Works Assoc., 62: 670–687.

McDaniels, A.E., Bordner, R.H., Gartside, P.S., Haines, J.R. and Kristen, P. (1985). Holding effects on coliform enumeration in drinking water samples. Appl. Environ. Microbiol., 50: 755–762.

McFeters, G.A., Cameron, S.C. and LeChevallier, M.W. (1982). Influence of diluents, media, and membrane filters on detection of injured water-borne coliform bacteria. Appl. Environ. Microbiol., 43: 97–103.

Means, E.G. and Olson, B.H. (1981). Coliform inhibition by bacteriocin-like substances in drinking water distribution systems. Appl. Environ. Microbiol., 42: 506–512.

Medema, G.J., Bahar, M. and Schets, F.M. (1997). Survival of *Cryptosporidium parvum*, *Escherichia coli*, faecal enterococci and *Clostridium perfringens* in river water: influence of temperature and autochtonous microorganisms. Water Sci. Tech., 35(11–12): 249–252.

Mitchell, D.O. and Starzyk, M.J. (1975). Survival of *Salmonella* and other indicator microorganisms. Can. J. Microbiol., 21: 1420–1421.

Narasimhan, R., Brereton J., Abbaszadegan, M., Alum, A. and Ghatpande, P. (2004). Sample collection procedures and locations for bacterial compliance monitoring. Awwa Research Foundation, Denver, CO.

NHMRC (2011). Australian drinking water quality guidelines paper 6. National Health and Medical Research Council, Natural Resource Management Ministerial Council, Canberra, Commonwealth of Australia.

Olstadt, J., Schauer, J.J., Standridge, J. and Kluender, S. (2007). A comparison of ten USEPA approved total coliform/*E. coli* tests. J. Water Health, 5:267–282.

Payment, P. and Franco, E. (1993). *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. J. Appl. Environ. Microbiol., 59(8): 2418–2424.

Payment, P. and Pintar, K. (2006). Waterborne pathogens: a critical assessment of methods, results and data analysis. Rev. Sci. Eau, 19: 233–245.

Payment, P., Trudel, M. and Plante, R. (1985). Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants. Appl. Environ. Microbiol., 49(6): 1418–1428.

Payment, P., Siemiatycki, J., Richardson, L., Renaud, G., Franco, E. and Prévost, M. (1997). A prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water. Int. J. Environ. Health Res., 7: 5–31.

Payment, P., Berte, A., Prevost, M., Menard, B. and Barbeau, B. (2000). Occurrence of pathogenic microorganisms in the Saint Lawrence River (Canada) and comparison of health risks for populations using it as their source of drinking water. Can. J. Microbiol., 46(6): 565–576.

Pipes, W.O., Minnigh, H.A., Moyer, B. and Troy, M.A. (1986). Comparison of Clark's presence–absence test and the membrane filter method for coliform detection in potable water samples. Appl. Environ. Microbiol., 52: 439–443.

Power, M.L., Littlefield-Wyer, J., Gordon, D.M., Veal, D.A. and Slade, M.B. (2005). Phenotypic and genotypic characterization of encapsulated *Escherichia coli* isolated from blooms in two Australian lakes. Environ. Microbiol., 7: 631–640.

Raina, P.S., Pollari, F.L., Teare, G.F., Goss, M.J., Barry, D.A.J. and Wilson, J.B. (1999). The relationship between *E. coli* indicator bacteria in well-water and gastrointestinal illnesses in rural families. Can. J. Public Health, 90(3): 172–175.

Rompré, A., Servais, P., Baudart, J., de-Roubin, M. and Laurent, P. (2002). Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J. Microbiol. Methods, 49: 31–54.

Rose, J.B., Darbin, H. and Gerba, C.P. (1988). Correlations of the protozoa, *Cryptosporidium* and *Giardi*a, with water quality variables in a watershed. Water Sci. Technol., 20(11/12):271–276.

Rose, J.B, Gerba, C.P. and Jakubowski, W. (1991). Survey of potable water supplies for *Cryptosporidium* and *Giardia*. Environ. Sci. Technol., 25(8):1393–1400.

Schaffter, N. and Parriaux, A. (2002). Pathogenic-bacterial water contamination in mountainous catchments. Water Res. 36: 131–139.

Schoenen, D. and Kolch, A. (1992). Photoreactivation of *E. coli* depending on light intensity after UV irradiation. Zentralbl. Hyg., 192: 565–570.

Schuster, C.J., Ellis, A.G., Robertson, W.J., Charron, D.F., Aramini, J.J., Marshall, B.J. and Medeiros, D.T. (2005). Infectious disease outbreaks related to drinking water in Canada, 1974-2001. Can. J. Public Health, 96 (4):254-255.

Snead, M.C., Olivieri, V.P., Kawata, K. and Krusé, C.W. (1980). The effectiveness of chlorine residuals in inactivation of bacteria and viruses introduced by post treatment contamination. Water Res., 14: 403.

Speight, V.L., Kalsbeek, W.D. and DiGiano, F.A. (2004). Randomized stratified sampling methodology for water quality in distribution systems. J. Water Resour. Plann. Manag., 130: 330–338.

Stevens, M. N., Ashbolt, N.J. and Cunliffe, D. (2001). Microbial indicators of drinking water quality – a NHMRC discussion paper. National Health and Medical Research Council, Canberra, Australia.

Sunderland, D., Graczyk, T.K., Tamang, L. and Breysse, P.N. (2007). Impact of bathers on levels of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts in recreational beach waters. Water Res., 41: 3483–3489.

Taylor, A., Craun, G.F., Faich, G.A., McCabe, L.J. and Gangarosa, E.J. (1972). Outbreaks of waterborne disease in the United States, 1961–1970. J. Infect. Dis., 125: 329–331.

U.S. EPA (1991). Guidance manual for compliance with the filtration and disinfection requirements for public water systems using surface water sources. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA (1992). National primary drinking water regulations: analytical techniques; coliform bacteria — final rule. U.S. Environmental Protection Agency. Fed. Regist., 57(112): 24744.

Escherichia coli (March 2012)

U.S. EPA (1999). EPA guidance manual: disinfection profiling and benchmarking. U.S. Environmental Protection Agency, Washington, DC (EPA-815-R-99-013).

U.S. EPA (2003). Ultraviolet disinfection guidance manual (draft). Office of Water, U.S. Environmental Protection Agency, Washington, DC (EPA 815).

U.S EPA (2010). National primary drinking water regulations: revisions to the total coliform rule; proposed rule. U.S. Environmental Protection Agency, Washington, DC (EPA-HQ-OW-2008-0878).

van Lieverloo, J.H.M., Mesman, G.A.M., Bakker, G.L., Baggelaar, P,K., Hamed, A. and Medema, G. (2007). Probability of detecting and quantifying fecal contamination of drinking water by periodically sampling for *E. coli*: a simulation model study. Water Res., 41: 4299–4308.

Waksman, S.A. (1941). Antagonistic relations of microorganisms. Bacteriol. Rev., 5: 231.

Wallis, P.M., Primrose, B. and Robertson, W.J. (1998). Outbreak of waterborne giardiasis caused by sewage contamination of drinking water. Environ. Health Rev., 42(2): 44–51.

WHO (1971). International standards for drinking-water, 3rd edition. World Health Organization, Geneva, Switzerland.

WHO (1976). Surveillance of drinking-water quality. World Health Organization, Geneva, Switzerland. (WHO Monograph Series No. 63).

WHO (2004). Guidelines for drinking-water quality, 3rd edition. Vol. 1. Recommendations. World Health Organization, Geneva, Switzerland.

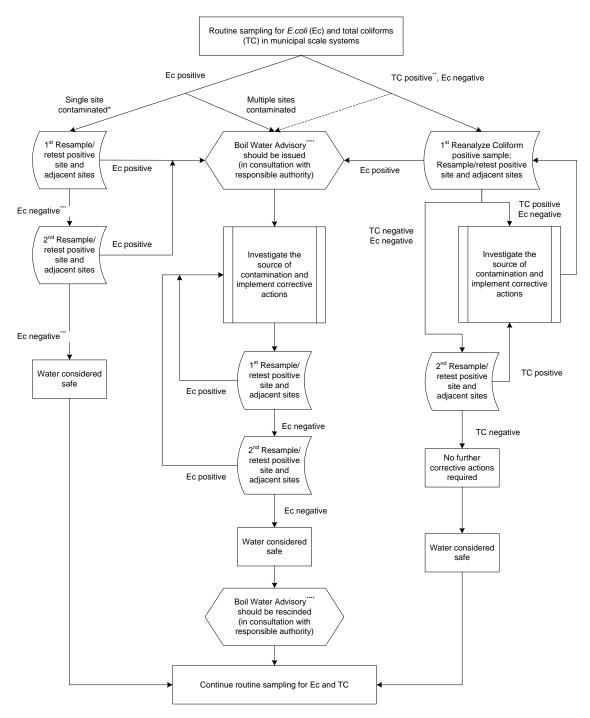
Wilczak, A., Jacangelo, J.G., Marcinko, J.P., Odell, L.H., Kirmeyer, G.J. and Wolfe, R.L. (1996). Occurrence of nitrification in chloraminated distribution systems. J. Am. Water Works Assoc., 88(7): 74–85.

Williams, M.M. and Braun-Howland, E.B. (2003). Growth of *Escherichia coli* in model distribution system biofilms exposed to hypochlorous acid or monochloramine. Appl. Environ. Microbiol., 69: 5463–5471.

Winfield, M.D. and Groisman, E.A. (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Appl. Environ. Microbiol., 69(7): 3687–3694.

Zimmer, J.L. and Slawson, R.M. (2002). Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. Appl. Environ. Microbiol., 68(7): 3293–3299.

Appendix A: Decision Tree for Routine Microbiological Testing of Municipal **Scale Systems**



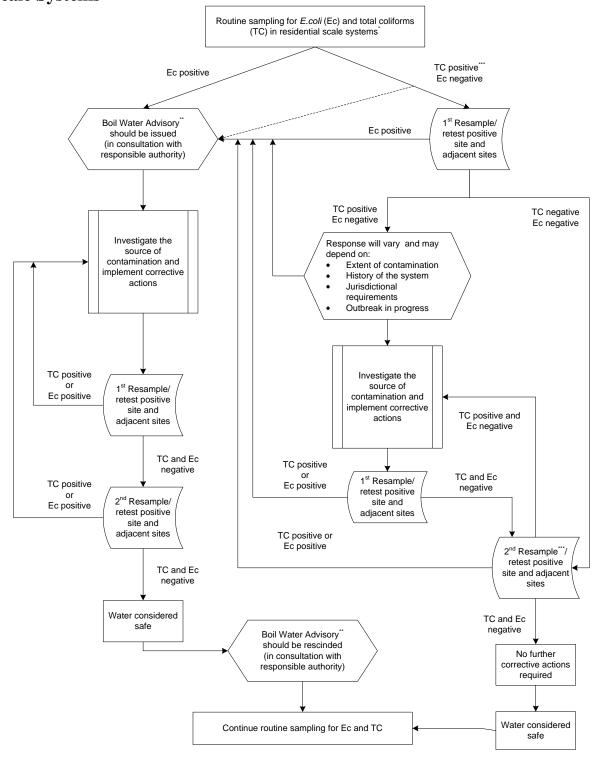
^{*}A boil water advisory may be issued on a single site contamination if deemed necessary by the responsible authority

^{**}A boil water advisory may be issued based on a positive total coliform, in the absence of *E.coli*, if deemed necessary by the responsible authority.
***If a total coliform positive sample is detected during resampling for *E.coli*, the decision route for detection of a total coliform positive sample, in

the absence of *E.coli*, should be followed (right-hand side of the decision tree).

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

Appendix B: Decision Tree for Routine Microbiological Testing of Residential **Scale Systems**



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

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

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

Appendix C: List of Acronyms

AOC assimilable organic carbon

ANSI American National Standards Institute

CFU colony-forming unit

EPA Environmental Protection Agency (U.S.)

GUDI groundwater under the direct influence of surface water

HPC heterotrophic plate count

MAC maximum acceptable concentration
MCL maximum contaminant level (U.S.)
MCLG maximum contaminant level goal (U.S.)

MF membrane filtration
MPN most probable number
MTF multiple tube fermentation

NSF NSF International
P-A presence—absence
QA quality assurance
QC quality control
UV ultraviolet