

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

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

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Escherichia coli

1.0 Guideline

The maximum acceptable concentration (MAC) of Escherichia coli in public, semi-public, and private drinking water systems is none detectable per 100 mL.

Testing for E. coli *should be carried out in all drinking water systems. 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.*

Note: Further information on how to apply this guideline is outlined in section 3.0, Application of the Guideline.

2.0 Executive summary for microbiological quality of drinking water

2.1 Introduction

The information contained in this Executive summary applies to the microbiological quality of drinking water as a whole. It contains background information on microorganisms, their health effects, sources of exposure, and treatment. Information specific to bacteria is included as a separate paragraph. It is recommended that this document be read in conjunction with other documents on the microbiological quality of drinking water, including the guideline technical document on turbidity.

2.2 Background

There are three main types of microorganisms that can be found in drinking water: bacteria, viruses, and protozoa. These can exist naturally or can occur as a result of contamination from human or animal waste. Some of these are capable of causing illness in humans. Surface water sources, such as lakes, rivers, and reservoirs, are more likely to contain microorganisms than groundwater sources, unless the groundwater sources are under the direct influence of surface water.

The main goal of drinking water treatment is to remove or kill these organisms to reduce the risk of illness. Although it is impossible to completely eliminate the risk of waterborne disease, adopting a multi-barrier, source-to-tap approach to safe drinking water will reduce the numbers of microorganisms in drinking water. This approach includes the protection of source water (where possible), the use of appropriate and effective treatment methods, well-maintained distribution systems, and routine verification of drinking water safety. All drinking water supplies should be disinfected, unless specifically exempted by the responsible authority. In addition, surface water sources and groundwater sources under the direct influence of surface water should be filtered. Drinking water taken from pristine surface water sources may be exempt from filtration requirements (Health Canada, 2003). The performance of the drinking water filtration system is usually assessed by monitoring the levels of turbidity, a measure of the relative clarity of water. Turbidity is caused by matter such as clay, silt, fine organic and inorganic matter, plankton, and other microscopic organisms, which is suspended within the water. Suspended matter can protect pathogenic microorganisms from chemical and ultraviolet (UV) light disinfection.

Currently available detection methods do not allow for the routine analysis of all microorganisms that could be present in inadequately treated drinking water. Instead, microbiological quality is determined by testing drinking water for *Escherichia coli*, a bacterium that is always present in the intestines of humans and other animals and whose presence in drinking water would indicate faecal contamination of the water. The maximum acceptable concentration (MAC) of *E. coli* in drinking water is none detectable per 100 mL.

2.3 Bacteria

E. coli is a member of the total coliform group of bacteria and is the only member that is found exclusively in the faeces of humans and other animals. Its presence in water indicates not only recent faecal contamination of the water but also the possible presence of intestinal diseasecausing bacteria, viruses, and protozoa. The detection of *E. coli* should lead to the immediate issue of a boil water advisory and to corrective actions being taken. Conversely, the absence of *E. coli* in drinking water generally indicates that the water is free of intestinal disease-causing bacteria. However, because *E. coli* is not as resistant to disinfection as intestinal viruses and protozoa, its absence does not necessarily indicate that intestinal viruses and protozoa are also absent. Although it is impossible to completely eliminate the risk of waterborne disease, adopting a multi-barrier approach to safe drinking water will minimize the presence of disease-causing microorganisms, reducing the levels in drinking water to none detectable or to levels that have not been associated with disease.

While *E. coli* is the only member of the total coliform group that is found exclusively in faeces, other members of the group are found naturally in water, soil, and vegetation, as well as in faeces. Total coliform bacteria are easily destroyed during disinfection. Their presence in water leaving a drinking water treatment plant indicates a serious treatment failure and should lead to the immediate issue of a boil water advisory and to corrective actions being taken. The presence of total coliform bacteria in water in the distribution system (but not in water leaving the treatment plant) indicates that the distribution system may be vulnerable to contamination or may simply be experiencing bacterial regrowth. The source of the problem should be determined and corrective actions taken.

In semi-public and private drinking water systems, such as rural schools and homes, total coliforms can provide clues to areas of system vulnerability, indicating source contamination, as well as regrowth and/or improper treatment (if used). If they are detected, the local authority may issue a boil water advisory and recommend corrective actions.

The heterotrophic plate count (HPC) test is another method for monitoring the overall bacteriological quality of drinking water. HPC results are not an indicator of water safety and, as such, should not be used as an indicator of adverse human health effects. Each system will have a certain baseline HPC level and range, depending on site-specific characteristics; increases in concentrations above baseline levels should be corrected.

There are naturally occurring waterborne bacteria, such as *Legionella* spp. and *Aeromonas hydrophila*, with the potential to cause illnesses. The absence of *E. coli* does not necessarily indicate the absence of these organisms, and for many of these pathogens, no other

suitable microbiological indicators are currently known. However, the use of a multiple-barrier approach, including adequate treatment and a well-maintained distribution system, can reduce these bacterial pathogens to non-detectable levels or to levels that have never been associated with human illness.

2.4 Health effects

The health effects of exposure to disease-causing bacteria, viruses, and protozoa in drinking water are varied. The most common manifestation of waterborne illness is gastrointestinal upset (nausea, vomiting, and diarrhoea), and this is usually of short duration. However, in susceptible individuals such as infants, the elderly, and immunocompromised individuals, the effects may be more severe, chronic (e.g., kidney damage), or even fatal. Bacteria (e.g., *Shigella* and *Campylobacter*), viruses (e.g., norovirus and hepatitis A virus), and protozoa (e.g., *Giardia* and *Cryptosporidium*) can be responsible for severe gastrointestinal illness. Other pathogens may infect the lungs, skin, eyes, central nervous system, or liver.

If the safety of drinking water is in question to the extent that it may be a threat to public health, authorities in charge of the affected water supply should have a protocol in place for issuing, and cancelling, advice to the public about boiling their water. Surveillance for possible waterborne diseases should also be carried out. If a disease outbreak is linked to a water supply, the authorities should have a plan to quickly and effectively contain the illness.

2.5 Exposure

Drinking water contaminated with human or animal faecal wastes is just one route of exposure to disease-causing microorganisms. Outbreaks caused by contaminated drinking water have occurred, but they are relatively rare compared with outbreaks caused by contaminated food. Other significant routes of exposure include contaminated recreational waters (e.g., bathing beaches and swimming pools) and objects (e.g., doorknobs) or direct contact with infected humans or domestic animals (pets or livestock). Although surface waters and groundwater under the direct influence of surface water may contain quantities of microorganisms capable of causing illness, effective drinking water treatment can produce water that is virtually free of disease-causing microorganisms.

2.6 Treatment

The multi-barrier approach is an effective way to reduce the risk of illness from pathogens in drinking water. If possible, water supply protection programs should be the first line of defence. Microbiological water quality guidelines based on indicator organisms (e.g., *E. coli*) and treatment technologies are also part of this approach. Treatment to remove or inactivate pathogens is the best way to reduce the number of microorganisms in drinking water and should include effective filtration (unless exempted from this requirement, as described above in section 2.2) and disinfection and an adequate disinfectant residual. Filtration systems should be designed and operated to reduce turbidity levels as low as reasonably achievable without major fluctuations.

It is important to note that all chemical disinfectants (e.g., chlorine, ozone) used in drinking water can be expected to form disinfection by-products, which may affect human health. Current scientific data show that the benefits of disinfecting drinking water (reduced rates of

infectious illness) are much greater than any health risks from disinfection by-products. While every effort should be made to reduce concentrations of disinfection by-products to as low a level as reasonably achievable, any method of control used must not compromise the effectiveness of water disinfection.

3.0 Application of the guideline

E. coli is the definitive indicator of recent faecal contamination in drinking water systems and is therefore a good indicator of the possible presence of enteric pathogens of human health concern. Consequently, detection of *E. coli* in any drinking water system is unacceptable.

3.1 Public drinking water supply systems

3.1.1 Testing requirements

Residual disinfectant and turbidity should be determined on a daily basis as a minimum in water leaving a treatment plant. These recommendations do not apply to systems served by groundwater of excellent quality where disinfection is practised to increase the margin of safety. Where possible, daily testing for disinfectant residuals and turbidity should be supplemented with at least weekly tests for *E. coli* to confirm microbiological safety. In public supply distribution 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 actual sampling and testing frequencies for *E. coli*, residual disinfectant, and turbidity in treated water entering and within distribution systems will be prescribed by the responsible authority.

3.1.2 Notification

If *E. coli* is detected in a public drinking water system, the system owner should immediately notify the responsible authorities and resample and test the positive site(s). A quantitative method, as opposed to a presence–absence (P-A) test as often done initially, is suggested for re-analysis, as it provides useful information on the level of contamination. If resampling and testing confirm the presence of *E. coli* in drinking water, the owner of the waterworks system should immediately issue a boil water advisory^{*} in consultation with the responsible authorities, carry out the corrective actions described below, and cooperate with the local responsible authority in any surveillance for possible waterborne disease outbreaks (see Appendix A: Decision Tree for Routine Microbiological Testing of Public Systems). Depending on the extent of *E. coli* contamination in the first sampling — for example, positive sample results 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.

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^{*} 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, 'boil water advisory.'

3.1.3 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 of the treatment process and distribution system.
- Verify that the required disinfectant residual is present throughout the distribution system.
- Increase chlorine 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 to fish-bearing streams. The responsible authority should be consulted regarding the methods available, and 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 *E. coli*, total coliforms, disinfectant residual, and turbidity. At a minimum, one sample upstream and one downstream of the original sample site(s) plus the finished water from the treatment plant as it enters the distribution system should be tested. Other samples should be collected and tested following a sampling plan appropriate for the distribution system.
- Conduct an investigation to identify the problem and prevent its recurrence, including a measure of raw water quality (e.g., bacteriological, turbidity, colour, conductivity) and variability.
- Continue selected sampling and testing (e.g., bacteriological, 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 Health Canada's *Guidance for Issuing and Rescinding Boil Water Advisories* (Health Canada, 2001). 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.

Barring system-specific exemptions, all public supplies should be disinfected to produce microbiologically safe water and a disinfectant residual should be maintained throughout the distribution system at all times. In addition, all public supplies derived from surface water sources and groundwater under the direct influence of surface water should be treated in accordance with the guideline technical document for Turbidity (Health Canada, 2003).

3.2 Semi-public^{**} and private drinking water systems

3.2.1 Testing requirements

Testing frequencies for semi-public systems will be determined by the responsible authority and should include times when the risk of contamination is greatest — for example, spring thaw, extended heavy rains, or dry periods. Owners of 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 semi-public 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 below should be taken immediately. As a precautionary measure, some jurisdictions may recommend immediate corrective actions without waiting for confirmatory results (see Appendix B: Decision Tree for Routine Microbiological Testing of Semi-Public and Private Systems).

3.2.3 Corrective actions for disinfected supplies (surface water supplies and groundwaters under the direct influence of surface waters)

The first step, if it has not already been done, is to conduct a sanitary survey to verify the safe 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:

- Verify that a disinfectant residual is present throughout the system.
- Increase the chlorine dosage, flush the system thoroughly, and clean treated water storage tanks and domestic cisterns. Water should be dechlorinated before being discharged to fish-bearing streams. The responsible authority should be consulted regarding the methods available, and the correct procedure, for carrying out dechlorination.
- Retest to confirm that the water is safe to drink

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.

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^{**} For the purposes of this document, a semi-public 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 public supply. Examples of such facilities include schools, personal care homes, day care centres, hospitals, community wells, hotels, and restaurants. The definition of a semi-public supply may vary between jurisdictions.

Barring system-specific exemptions, all semi-public supplies should be disinfected to produce microbiologically safe water. Responsible authorities may also recommend disinfection of private supplies. In addition to disinfection, semi-public and private supplies derived from surface water sources or groundwater under the direct influence of surface waters should receive adequate filtration (or use technologies achieving equivalent quality). Drinking water taken from pristine surface water sources may be exempt from the filtration requirements (Health Canada, 2003).

3.2.4 Corrective actions for non-disinfected wells

The first step, if it has not already been done, is to conduct a sanitary survey to verify the safe 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 Health Canada's *What's in Your Well?—A Guide to Well Water Treatment and Maintenance* (Health Canada, 2004).
- Flush the system thoroughly and retest to confirm that the water is safe to drink. 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 streams. The responsible authority should be consulted regarding the methods available, and the correct procedure, for carrying out dechlorination.

If the water remains contaminated following shock chlorination, further investigation into the source of the contamination should be carried out. If the source cannot be found or cannot be 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.

It should be noted that 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 Health Canada's *Guidance for Issuing and Rescinding Boil Water Advisories* (Health Canada, 2001). An additional test should be taken after 3–4 months to ensure that the contamination has not recurred. Only a history of data can be used to confirm the long-term integrity of a supply when applied jointly with sanitory surveys. Further information on routine monitoring can be found in section 8.0, Sampling for *E. coli*.

4.0 Significance of *E. coli* in drinking water

4.1 Description

Escherichia coli is a member of the coliform group, part of the family Enterobacteriaceae, and is described as a facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacterium that possesses the enzyme β -glucuronidase.

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. Within human and animal faeces, *E. coli* is present at a concentration of approximately 10⁹ 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. Further information on illness-associated *E. coli* strains can be found in *Bacterial Waterborne Pathogens: Current and Emerging Organisms of Concern* (Health Canada, 2006a). It should be noted that 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.

Of the coliforms, *E. coli* is generally the most sensitive to environmental stresses. Its survival time 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). In general terms, *E. coli* survives for about 4–12 weeks in water containing a moderate microflora at a temperature of $15-18^{\circ}C$ (Kudryavtseva, 1972; Filip *et al.*, 1987; Edberg *et al.*, 2000). Regrowth of *E. coli* in water distribution systems is not a concern, since *E. coli* rarely grows outside the human or animal gut (Geldreich, 1996). The inability of *E. coli* to grow in water, combined with its short survival time in water environments, means that the detection of *E. coli* in a water system is a good indicator of recent faecal contamination.

5.0 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. Reasons for this include the large number of possible pathogens, the lack of inclusion of previously unknown pathogens, and the time and expense associated with routine monitoring of all pathogens. Instead, microbial indicators are used, since it is less difficult, less expensive, and less time consuming to monitor indicators than to monitor individual pathogens. Simple, inexpensive techniques 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. Newer molecular methods may provide an easy, inexpensive, and quick method for the detection of pathogens or indicators; to date, however, this is not the case.

An appropriate health-based indicator of microbial pathogens should possess several ideal qualities. The indicator should always be present when the pathogen is present and should not be detected when the pathogen is absent; it should have a life span similar to that of the pathogen of concern; it should be present in large numbers and should be readily detected by simple and inexpensive methods; and it should not multiply in the environment once it has been shed by the host. Based on these qualities, if the indicator is isolated from the water supply, this infers that pathogenic organisms *could be* present; if the indicator is absent, pathogenic organisms are *probably also* absent.

Of the contaminants that may be found in drinking water, those present in 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. It was identified as the only species in the coliform group found exclusively in the intestinal tract of humans and other warm-blooded animals and subsequently excreted in large numbers in faeces (approximately 109 per gram) (Department of National Health and Welfare, 1977). In addition to being faecal specific, *E. coli* do not usually multiply in the environment and have a life span on the same order of magnitude as those of other enteric bacterial pathogens, both of which are qualities of an ideal indicator. As mentioned previously, they are also excreted in the faeces in high numbers, making detection possible even when greatly diluted.

In contrast to the situation with *E. coli*, it was recognized that most genera in the total coliform group occur naturally in soil, vegetation, and water in addition to faeces, making them unsuitable indicators of faecal contamination. Nevertheless, total coliforms were used as a surrogate for *E. coli*, primarily because routine methods to distinguish *E. coli* from other coliform bacteria were not available. It was not until the mid-20th century that more specific methods for the thermotolerant coliforms (previously referred to as faecal coliforms), which include *E. coli* and members of the genera *Klebsiella*, *Enterobacter*, and *Citrobacter*, were developed. Although thermotolerant coliforms were more specific than total coliforms for *E. coli*, the former group contained other species that were capable of surviving and growing in water and were not faecal specific. With the advent of enzyme substrate tests, it is now possible to routinely monitor for *E. coli*.

Today, greater attention is being paid to viral and protozoan pathogens in water systems. Although E. coli is a good indicator for vegetative bacterial pathogens commonly found in treated drinking water, such as Salmonella species (Mitchell and Starzyk, 1975), it has proven to be a less effective indicator of viral and protozoan presence. In general, compared with protozoans and some viruses, E. coli and members of the coliform group do not survive as long in the environment (Edberg et al., 2000) and are more susceptible to many of the disinfectants commonly used in the drinking water industry. In contrast to the above, there is evidence that, although viruses may innately survive longer than bacteria in the environment, they may actually have a shorter life span. This is the result of their being smaller than bacteria and without defence mechanisms, so that they are more readily phagocytized by the other microflora. Consequently, some studies suggest that under certain conditions, bacterial indicators may still be good indicators for viral presence. For example, in a study by Craun et al. (1997) on groundwater consumption, it was found that the presence of coliforms correlated very well with the presence of viral gastroenteritis. An additional study conducted in southern Ontario, which looked at the quality of rural well water in terms of the presence of E. coli and any associated illness in the families, found that the occurrence of *E. coli* in the well was statistically associated with gastrointestinal illness in an individual, although the causative agents were not identified (Raina et al., 1999). Studies have also shown that bacterial indicators under specific conditions can be used to indicate protozoan pathogens. For example, one study of raw water quality showed that at high levels of thermotolerant coliforms (including E. coli), the probability of finding enteric protozoa and viruses was also very high (LeChevallier et al., 1991; Payment et al., 2000). In an additional study, an outbreak of waterborne giardiasis in a town in northern Ontario was characterized by the presence of abnormally high levels of cysts and thermotolerant coliforms in the raw water supply (Wallis et al., 1998).

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As indicated above, indicators of faecal contamination, such as *E. coli*, are good indicators of pathogens that are present and transmitted through faeces. There are other waterborne illnesses that are the result of pathogens that are not transmitted by the faecal–oral route and therefore are not found through detection of faecal indicators. No indicators are currently known for such pathogens. Implementation of a multi-barrier approach will minimize their impact.

It should be emphasized that no bacteriological analysis can replace a complete knowledge of the quality of the water at the source of supply, during treatment, and throughout a distribution system. Contamination is often intermittent and may not be revealed by the examination of a single sample. A bacteriological water analysis shows only that at the time of examination, bacteria indicating faecal pollution did or did not grow under laboratory conditions from the sample of water tested. 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 bacteriological examination.

6.0 Role of disinfectant residuals in maintaining drinking water quality

The purpose of treating drinking water is to provide a product that is microbiologically and chemically safe for consumption. In all public and semi-public systems applying disinfection, a disinfectant residual should be maintained throughout the distribution system at all times. Maintenance and monitoring of a residual disinfectant offer two benefits. First, a disinfectant residual will limit the growth of organisms within the system and may afford some protection against contamination from without; second, the disappearance of the residual provides an immediate indication of the entry of oxidizable matter into the system or of a malfunction of the treatment process. It is therefore recommended that a disinfectant residual be maintained and monitored daily throughout the entire system. The minimum disinfectant residual that needs to be maintained is determined by the responsible authority and may vary from jurisdiction to jurisdiction. It is recognized that excessive levels of disinfectant may result in taste and odour problems. If this occurs, the responsible authority may provide guidance as to the type and concentration of disinfectant residual to ensure that water remains microbiologically safe. When a residual concentration measured at a sampling point is less than that required by the responsible authority, another sample should be taken immediately. If this sample is also unsatisfactory, the line should be flushed and sampling continued until a satisfactory concentration is obtained. If the residual does not return to the allowable minimum, the disinfectant dosage should be increased. If increasing the dosage is ineffective or if excessive disinfection is required, a sanitary survey for potential sources of contamination should be made in cooperation with the responsible authority. Special samples should be taken for coliform analysis. Should all these measures prove inadequate, the responsible authority should be consulted for further advice, and action should be taken as appropriate.

7.0 Analytical methods for *E. coli*

Currently, three methods are routinely used to detect *E. coli* organisms in water: presence–absence (P-A), which is a qualitative test, and membrane filter (MF) and multiple

tube fermentation (MTF), which are both quantitative tests. A detailed description of each method is given in *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.*, 1998). A brief synopsis is given below.

All three detection methods use cultivation to detect/confirm the presence of *E. coli*. Cultivation media can be broadly categorized into two types: media containing bacterial enzymes for specific detection and confirmation of the bacterium in a single step (Feng and Hartman, 1982; Ley *et al.*, 1988) and 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 use the enzyme β -glucuronidase, a unique constitutive enzyme found in *E. coli*, *Shigella* spp., and some *Salmonella* spp., but rarely present in other coliforms (Manafi *et al.*, 1991). The most publicized methods use the β -glucuronidase activity of *E. coli* to hydrolyse 4-methylumbelliferyl- β -D-glucuronide to form 4-methylumbelliferone, which fluoresces under longwave UV light (Feng and Hartman, 1982). A distinct advantage of enzyme-based methods is that no confirmation step is required. Some enzyme-based methods, such as those that use defined substrate technology, also inhibit non-coliform bacterial growth, and thus non-coliform bacteria cannot interfere with the recovery of coliforms. Defined substrate technology 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 should be encouraged. Various enzyme-based methods have been approved by the U.S. Environmental Protection Agency as acceptable means for the detection of *E. coli* in drinking water (U.S. EPA, 1992). Enzyme-based methods have also been developed for the simultaneous enumeration of both total coliforms and *E. coli* (Edberg *et al.*, 1988).

Presumptive coliform media, such as lauryl tryptose broth, m-Endo media, or EC media (APHA *et al.*, 1998), 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* (APHA *et al.*, 1998). 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 an enzyme-based test (APHA *et al.*, 1998). The main disadvantages to using presumptive coliform media are the necessity of a confirmation step, which requires additional time to complete the analyses, and possible interference with recovery by non-coliform bacteria.

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 analyse samples in a timely manner, such as non-accredited laboratories or commercial test kits. To ensure quality control, validation samples should be sent to accredited laboratories for analysis, or, if this is not physically possible, additional samples should be analysed using the test kit for quality control purposes. The requirements for validation sampling are determined by the responsible authority. In addition, any test kits used should meet minimum requirements for accuracy and detection (sensitivity); as well, the operator must ensure that equipment is regularly calibrated and that test kits are used before their expiry dates.

7.1 Presence–absence procedure

The P-A test was developed as a more sensitive, economical, and efficient means of analysing 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 7.3), in which only one analysis bottle per sample is used. This method can be used with either enzyme-based media, such as media based on defined substrate technology, or presumptive coliform media (e.g., using lauryl tryptose broth), with follow-up *E. coli* confirmation. Commercial test kits using defined substrate technology have been developed. 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 media based on defined substrate technology 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, the P-A test is simpler than the MF and MTF procedures. Initial per-sample analysis time is less than 1 minute, and, since the allowable level of *E. coli* in drinking water is none per 100 mL, qualitative results are sufficient for protecting public health.

7.2 Membrane filter procedure

The MF procedure was introduced to bacteriological water analysis in 1951, after its capacity to produce results equivalent to those obtained by the MTF procedure was demonstrated (Clark *et al.*, 1951; Goetz and Tsuneishi, 1951). 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 the enzyme β -glucuronidase (Dufour *et al.*, 1981; Ciebin *et al.*, 1995) and incubated. The advantages of the technique were quickly recognized, as 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. When used with media containing 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.

There are some problems with the MF technique. 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., 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 -Dglucuronide 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/ fluorescence by presumptive coliforms or *E. coli*. Similarly, concentrations of HPC 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 the β -glucuronidase enzyme. Most water supplies maintaining a total chlorine residual of 0.2 mg/L had an HPC below 500 CFU/mL. Historically, some jurisdictions used background colony counts on total coliform membrane filters as a convenient and inexpensive surrogate for HPC. Background colony counts should no longer be used as a surrogate for HPC testing, but they can be useful for determining if there is interference with coliform recovery when using presumptive coliform media. Further information on HPC and background colony counts, along with their significance in drinking water, can be found in *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document* — Heterotrophic Plate Count (Health Canada, 2006b). It should also be noted that, although this method is quantitative, clumping of E. coli can lead to underestimations in concentrations. Since E. coli should not be present in treated drinking water, underestimations of concentrations are not as much of a concern as false-negative findings.

7.3 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 probable than any other number, would give the observed result; it is not an actual count of the bacteria present. 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 have been shown to inhibit the detection of *E. coli* (Waksman, 1941; Hutchison *et al.*, 1943; Means and Olson, 1981). Also, 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, the current edition of *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.*, 1998) recommends treating all tubes with turbidity, regardless of gas production,

as presumptive coliform-positive tubes. Also, in both MF and MTF methods, clumping of coliforms can lead to an underestimation of their concentrations.

Contrary to the MF procedure, the MTF procedure lacks precision and has a longer turnaround time for results; because of this, the MF procedure has largely replaced it for routine examinations of drinking water 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.

8.0 Sampling for *E. coli*

8.1 Sample size

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, practical with the MF method, will increase both the test sensitivity and the test reliability. Smaller volumes, dilutions, or other MTF combinations may be more appropriate for waters of doubtful quality.

A 500-mL sample provides sufficient volume for a coliform determination (either total coliform or *E. coli*) by one of the three methods and also for an HPC test. In addition, enough sample will remain if membrane filtration is required to complement a P-A determination provided the sample has been properly stored.

8.2 Sampling frequency

The World Health Organization lists the following factors that should be taken into account when determining sampling frequency for public 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* to confirm microbiological safety. For supplies where weekly *E. coli* testing is impractical (e.g., in small supplies), residual disinfectant determinations should be relied upon to verify microbiological safety. 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 do 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. However, it is recommended that, regardless of the population being served, a minimum of four samples per month should be examined. Table 1 is offered as a guide.

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

Table 1: Recommended sampling frequency

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, 2003). 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 above frequencies are only general guides. 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.

The general practice of basing sampling requirements on the population served recognizes that smaller water supply systems may have limited resources available for surveillance. However, because small water supplies have more facility deficiencies (McCabe *et al.*, 1970) and are responsible for more disease outbreaks than large ones (Taylor *et al.*, 1972), emphasis should also be placed on perceived problems based on sanitary surveys.

Advice on sampling of semi-public and private systems may vary from jurisdiction to jurisdiction but should include times when the risk of contamination is greatest — for example, spring thaw, heavy rains, or dry periods. New or rehabilitated wells should also be sampled initially to confirm acceptable bacteriological quality.

8.3 Location of sampling points

In public systems, the location of sampling points must be decided upon by the responsible authority. Samples should be taken at the point where the water enters a system and from representative points throughout a distribution system, although not necessarily the same points on each occasion. 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: 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 semi-public and private systems, samples are generally collected from the location(s) recommended by the responsible authority. More extensive sampling may be necessary, depending on the system and results from previous samples.

8.4 Handling of samples

Proper procedures for collecting samples must be observed to ensure that the samples are representative of the water being examined. Detailed instructions on the collection of samples for bacteriological analysis are given in *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.*, 1998).

In brief, water samples for bacteriological testing should be collected in a sterile container. Sterility is ensured if the security cap is intact immediately prior to sampling. If disinfected water is being collected, the container should already contain a neutralizing tablet or powder (e.g., sodium thiosulphate). When sampling, the sampler should have previously removed all attachments, including aerator (if necessary), washed his or her hands, disinfected the tap (if needed), allowed the water to flow for several minutes prior to collection, removed the security cap only immediately prior to collecting the sample (the cap should never be placed down on any surface), securely replaced the cap immediately after filling the bottle to the indicated level, and properly labelled the bottle and filled out accompanying submission forms, such as chain of custody paperwork when collecting samples for legal purposes. Because the way in which samples are collected has an important bearing on the results of their examination, sample collectors should be properly trained for the work.

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 an iced cooler. Ideally, the interval between collection of the sample and the beginning of its examination should not exceed 24 hours, although up to 48 hours may be acceptable for samples collected from remote areas. When delays are anticipated, a delayed incubation procedure should be employed or consideration given to on-site testing. The delayed incubation procedure, described in Standard Methods for the Examination of Water and Wastewater (APHA et al., 1998), is a modification of the standard MF technique, which permits transport of the membrane, after filtration, to a distant laboratory for incubation and completion of the test. 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 late sample contains coliforms, a repeat sample will already have been received or will be in transit. Some reports (Dutka and El-Shaarawi, 1980; McDaniels et al., 1985) support the belief that samples should be stored under refrigeration to minimize changes in populations and concentrations. Samples should be labelled with the time, date, location, type of sample (e.g., raw water, distribution system, etc.), sampler's name, and identification number (if used), along with the disinfectant residual 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.

9.0 Treatment technology

Even the most sophisticated treatment system cannot provide water that is absolutely free of disease-causing microorganisms all the time. The real goal of treatment is to reduce their presence and associated health risks to an acceptable or safe level. One measure of the safety of the water is the absence of *E. coli*. This indicates that the water is free of faecal contamination and the associated enteric pathogens. Of course, *E. coli* should not be relied on solely to indicate the microbiological safety of water, since some enteric pathogens, such as protozoa, are more resistant to water treatment techniques. The use of a multiple-barrier approach, including adequate treatment and a well-maintained distribution system, and source water protection, where possible, is the best approach to ensure water safety.

An array of options is available for treating source waters to provide high-quality drinking water. The quality of the source water will dictate the degree of treatment necessary. For public systems, options include various filtration methods and disinfection with chlorine-based compounds or alternative technologies, such as UV light or ozonation. Semi-public and private systems employ many of the same technologies, but on a smaller scale.

9.1 Municipal-scale

Barring system-specific exemptions, all public supplies, regardless of source water type, should be disinfected to produce microbiologically safe water and should maintain a disinfectant residual throughout the system at all times. In addition, all public supplies derived from surface water sources and groundwater under the direct influence of surface water should be treated in accordance with the guideline technical document for turbidity (Health Canada, 2003). In a study examining public systems (Payment *et al.*, 1985), it was shown that indicator bacteria, such as thermotolerant coliforms (which include *E. coli*), were essentially eliminated before filtration (i.e., during pre-disinfection, clarification, and coagulation), and then filtration removed most of the bacteria that had survived the earlier treatments. Post-disinfection, using chlorination or ozonation, eliminated the residual indicator bacteria. Overall, removal of total coliforms, which include *E. coli*, was greater than 6 logs in all of the treatment plants tested. This removal is sufficient to reduce the number of *E. coli* to conform to the established MAC of none detectable in 100 mL of drinking water.

The commonly used drinking water disinfectants are chlorine, chloramine, UV light, ozone, and chlorine dioxide. 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 control of protozoans. Chlorine is also less effective for inactivating organisms present in biofilms. In comparison with chlorine, chloramine is a weaker oxidant. This property is advantageous, in that the disinfectant resides longer in a distribution system. It is therefore easier to maintain a disinfectant residual, and the disinfectant is better able to penetrate into the biofilm found in the pipes and reservoirs, leading to superior coliform control (LeChevallier *et al.*, 1990). However, chloramine is less efficient at controlling a sudden pulse of contamination (Snead *et al.*, 1980), and it can lead to nitrification. UV light disinfection appears to be highly effective for inactivating many types of pathogens, including pathogenic protozoa (Wilczak *et al.*, 1996). It should be noted that when using UV light for the inactivation of *E. coli* (and other bacteria), the bacteria can undergo photo repair (Harris *et al.*, 1987; Schoenen and Kolch, 1992; Zimmer and

Slawson, 2002) and, to a lesser extent, dark repair. However, the amount of repair is not considered significant in drinking water treatment and distribution. Ozone, compared with chlorine-based disinfectants, is more efficient for the inactivation of bacteria, viruses, and protozoa. Similar to UV light, 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. 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. All chemical disinfectants used in drinking water can be expected to form unwanted disinfection by-products.

The efficacy of disinfection can be predicted based on a knowledge of the residual concentration of disinfectant, temperature, pH (for chlorine and chloramine), and contact time to first customer. This relationship is commonly referred to as the CT concept and is used by public supply systems as one tool for ensuring adequate inactivation of organisms during disinfection. CT is the product of C (the residual concentration of disinfectant, measured in mg/L) and T (the disinfectant contact time, measured in minutes). CT values for 99% inactivation of *E. coli* using chlorine, chlorine dioxide, chloramine, and ozone are provided in Table 2. In a typical treatment system, the CT provided will result in a much greater inactivation than 99%. Log inactivations using UV light disinfection are listed in Table 3. *Escherichia coli*, because of its importance as a public health indicator, has been used as a representative bacterial species. For comparison, the CT values and UV light doses for representative protozoa and viruses have been included in both tables.

Disinfectant agent	рН	<i>E. coli</i> ^a (mg·min/L)	<i>Giardia lamblia</i> ^b (mg∙min/L)	Poliovirus 1 ^a (mg·min/L)
Free chlorine	6–7	0.034-0.05	32–46°	1.1–2.5
Preformed chloramines	8–9	95–180	1470	768–3740
Chlorine dioxide	6–7	0.4-0.75	17	0.2-6.7
Ozone	6–7	0.02	1.3	0.1-0.2

^a From Hoff (1986).

^b From U.S. EPA (1999)

^c 90% inactivation CT value.

Log inactivation	E. coliª	<i>Cryptosporidium</i> ^b	Virus ^b	Giardia ^b
1	1.5-4.4	2.5	58	2.1
2	2.8-6.2	5.8	100	5.2
3	4.1–7.3	12	143	11

^a Based on five separate studies, taken from U.S. EPA (2003).

^b Based on validation studies done by U.S. EPA (2003).

From Table 2, it is apparent that, in comparison with most protozoans and viruses, coliform bacteria are easier to inactivate using the common chemical disinfectants. Also, it should be noted that chloramines have a much higher CT value than any of the other disinfectants listed. This means that, in order 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 described above. 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 viruses are more resistant.

9.2 Residential-scale

For the purposes of this document, semi-public and private supplies are considered to be residential-scale. Barring system-specific exemptions, all semi-public supplies should be disinfected to produce microbiologically safe water. Responsible authorities may also recommend disinfection of private supplies. In addition to disinfection, semi-public and private supplies derived from surface water sources or groundwater under the direct influence of surface waters should receive adequate filtration (or use technologies achieving equivalent quality).

An array of options is available for treating source waters to provide high-quality pathogen-free drinking water,, including various filtration methods and disinfection with chlorinebased compounds or alternative technologies, such as UV light or ozonation. Semi-public and private systems can employ many of these technologies , but on a smaller scale than public systems, along with other technologies, such as distillation. Semi-public and private systems using disinfection are more apt to rely on UV light and, to a lesser extent, chlorine.

These technologies have been incorporated into point-of-entry devices, which treat all water entering a building, and point-of-use devices, which treat water at only a single location — for example, at the kitchen tap in a home. Treatment technologies used in semi-public and private systems, as for those used in public systems, should achieve a 6-log reduction of *E. coli*.

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 material safety and performance of products that come into contact with drinking water. 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 to certify drinking water devices and materials as meeting NSF/ANSI standards:

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

10.0 Conclusions and Recommendations

10.1 Drinking water quality

Effective treatment including disinfection should yield water free of any coliform organisms, irrespective of the level of pollution in the source water. The presence of any type of coliform organism in treated water therefore suggests inadequate treatment and disinfection, regrowth, or infiltration in a distribution system. The presence of *E. coli* is a definite indicator of the presence of human or animal faeces. Other species in the coliform group (e.g., *Klebsiella*, *Citrobacter*, *Enterobacter*) are not restricted to faeces but occur naturally on vegetation and in soils and therefore do not necessarily indicate the presence of faecal contamination.

Routine analysis for *E. coli* should be supplemented by HPCs or by background colony counts when using lactose-based media. For enzyme-based methods using defined substrate technology, HPC or background colony count is not necessary. However, there are other reasons for measuring HPC bacteria. These can be found in Health Canada's HPC guideline technical document (Health Canada, 2006b).

Based on the above discussion, the MAC for *E. coli* in public, semi-public, and private drinking water systems is no organisms detectable per 100 mL.

10.2 Relationship with pathogenic microorganisms

When assessing the bacteriological quality of source water, testing for *E. coli* is preferred because it gives a better indication of faecal contamination. For some potential pathogenic bacteria (e.g., *Salmonella, Shigella*, and *Campylobacter jejuni*), the absence of *E. coli* in treated water is a good indication that these pathogens are probably also absent. If, however, past experience has demonstrated that the raw water could harbour pathogens for which *E. coli* are not good indicators (e.g., *Cryptosporidium parvum*, *Giardia lamblia*, *Yersinia enterocolitica*, enteric viruses), then the water should receive treatment known to remove or inactivate these pathogens. Properly treated and distributed drinking water should be essentially free of pathogenic microorganisms.

10.3 Sampling frequency and sampling size

For public systems, 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. In general, water leaving a treatment plant should be tested daily for both disinfectant residual and turbidity and tested at least weekly for *E. coli* to confirm the microbiological safety of the supply. For supplies where such action is impractical, such as small systems, disinfectant residual determinations should be relied upon. The daily sampling recommendations for disinfectant residual and turbidity do not apply to supplies served by groundwater sources of excellent quality where 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. Table 4, which reproduces Table 1 above, is offered as a guide.

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

Table 4: Recommended sampling frequency

The samples should be taken at regular intervals throughout the month. Disinfectant residual tests should be conducted when bacteriological samples are taken. The majority of samples should be taken in potential problem areas.

For semi-public and private systems, samples are collected from locations recommended by the responsible authority. Samples should be collected at times when the risk of contamination is highest — for example, spring thaw, heavy rains, or dry periods. New or rehabilitated wells should also be sampled initially to confirm acceptable bacteriological quality.

The sample volume should be sufficient to carry out all the tests required. For treated drinking water, a minimum volume of 100 mL should be examined for the coliform determination, regardless of which method is used. The maximum volume for analysis by the P-A test is usually 100 mL; however, 500 mL of sample should be collected, as an HPC and subsequent examination by the MF method can be carried out, if necessary, provided the sample has been properly stored.

10.4 Considerations for the treatment of raw supplies

Since modern water treatment technologies can produce high-quality drinking water from even heavily contaminated sources, numerical limits for the microbiological quality of raw supplies are not proposed. Nevertheless, the microbiological quality of raw water should be considered when selecting sites for new treatment plants or before performing major upgrades to existing plants. Similarly, close monitoring of the raw water quality is required so that existing treatment processes can be adjusted accordingly. In addition, measures to protect raw supplies from contamination should be implemented where feasible.

When assessing the bacteriological quality of source water, testing for *E. coli* is preferred, because it gives a good indication of faecal contamination. The presence of total coliforms or thermotolerant coliforms when *E. coli* are absent is likely due to the presence of bacteria naturally associated with soil and vegetation.

Raw water quality varies over time and between locations. The frequency of sampling for bacteriological examinations of a particular water supply should therefore be established by the surveillance agency in cooperation with the local responsible authority.

Barring system-specific exemptions, all drinking water supplies should be disinfected to produce microbiologically safe water. In addition all public supplies derived from surface water sources and groundwater under the direct influence of surface water should be treated in accordance with the guideline technical document for turbidity (Health Canada, 2003). Semi-public and private supplies using similar sources should include adequate filtration (or use technologies achieving equivalent quality) and disinfection. Drinking water taken from pristine surface water sources may be exempt from the filtration requirements (Health Canada, 2003).

It should not be inferred that these guidelines will guarantee the production of drinking water of adequate quality from every raw water source. For example, protection of the supply or partial treatment may be necessary to reduce turbidity even when the coliform counts are low. In addition, satisfaction of other water quality criteria may dictate the use of unit processes not mentioned in the above scheme.

11.0 References

APHA/AWWA/WEF (1998) Standard methods for the examination of water and wastewater. 20th edition. American Public Health Association / American Water Works Association / Water Environment Federation, Washington, DC.

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.

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.

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.

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.

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

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.

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.

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.

Geldreich, E.E. (1996) Microbial quality of water supply in distribution systems. Biological profiles in drinking water. CRC Press, Lewis Publishers, Boca Raton, FL. pp. 293, 367.

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.

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 Canada (2001) Guidelines for Canadian drinking water quality: Supporting documentation — Guidance for issuing and rescinding boil water advisories. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario (http://www.hc-sc.gc.ca/hecs-sesc/water/pdf/boil_water_advisories.pdf).

Health Canada (2003) Guidelines for Canadian drinking water quality: Supporting documentation — Turbidity. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Health Canada (2004) What's in your well? - A guide to well water treatment and maintenance. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario (http://www.hc-sc.gc.ca/hecs-sesc/water/factsheets/treatment_guide.htm).

Health Canada (2006a) Guidelines for Canadian drinking water quality: Guideline Technical Document — Bacterial waterborne pathogens: Current and emerging organisms of concern. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Health Canada (2006b) Guidelines for Canadian drinking water quality: Guideline Technical Document — Heterotrophic plate count. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

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

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.

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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.

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., 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., 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.

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.

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

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, 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.

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

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., 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.

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. Meth., 49: 31–54.

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

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.

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

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

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

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.

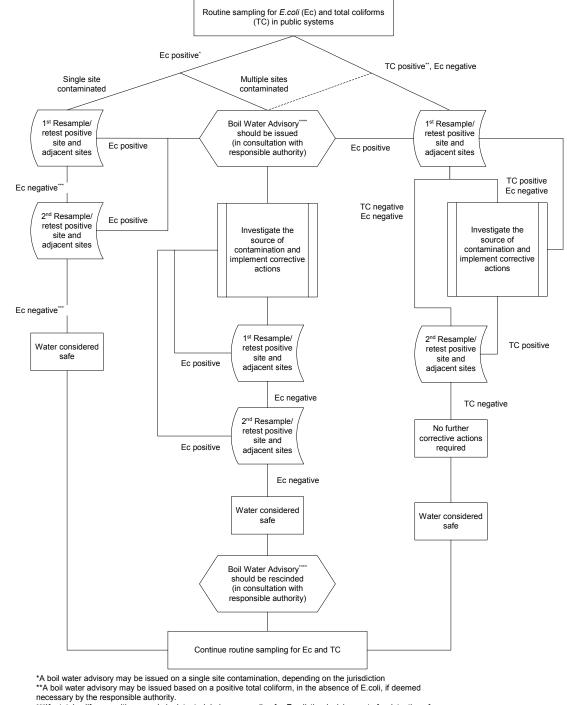
WHO (1971) International standards for drinking-water. 3rd edition. World Health Organization, Geneva.

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

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

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.

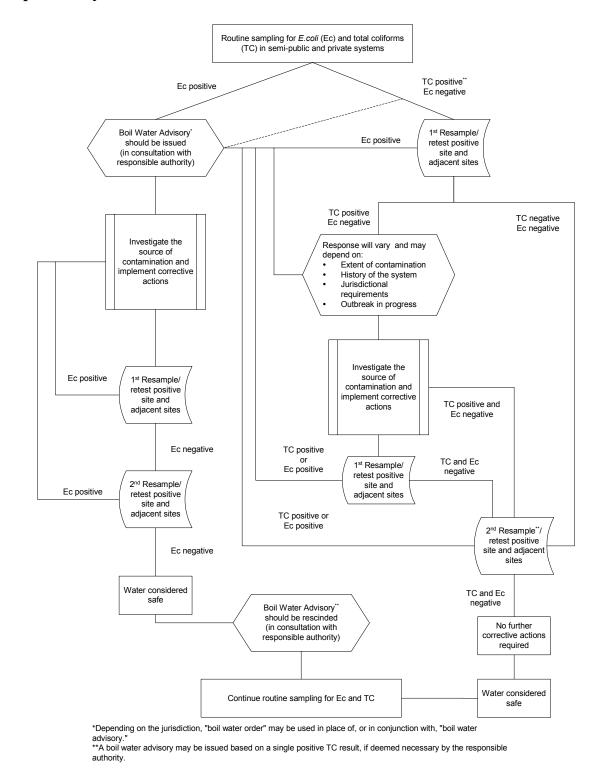
Zimmer, J.L. and Slawson, R.M. (2002) Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. Appl. Environ. Microbiol., 68(7): 3293–3299.



Appendix A: Decision tree for routine microbiological testing of public systems

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."

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Appendix B: Decision tree for routine microbiological testing of semi-public and private systems

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Appendix C: List of acronyms

ANSI	American National Standards Institute
CFU	colony-forming unit
HPC	heterotrophic plate count
MAC	maximum acceptable concentration
MF	membrane filter
MPN	most probable number
MTF	multiple tube fermentation
NSF	NSF International
P-A	presence-absence
UV	ultraviolet