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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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Total Coliforms

1.0 Guideline

The maximum acceptable concentration (MAC) of total coliforms in water leaving a treatment plant in a public system and throughout semi-public and private supply systems is none detectable per 100 mL.

For distribution systems in public supplies where fewer than 10 samples are collected in a given sampling period, no sample should contain total coliform bacteria. In distribution systems where greater than 10 samples are collected in a given sampling period, no consecutive samples from the same site or not more than 10% of samples should show the presence of total coliform bacteria.

Testing for total coliforms should be carried out in all drinking water systems. The number, frequency, and location of samples for total coliform testing will vary according to the type and size of the system and jurisdictional requirements.

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 the 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 disease-causing 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 bacterial regrowth and/or inadequate treatment (if used). If they are detected in drinking water, the local authority responsible for drinking water may issue a boil water advisory and recommend corrective actions. It is important to note that decisions concerning boil water advisories should be made at the local level based upon site-specific knowledge and conditions.
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 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 waters 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 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

#### 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 disinfection residuals and turbidity should be supplemented with at least weekly tests for total coliforms. In public distribution systems, the number of samples collected for total coliform testing should reflect the size of the population being served, with a minimum of four samples per month. The actual sampling and testing frequencies for total coliforms, residual disinfectant, and turbidity in treated water entering and within distribution systems will be prescribed by the responsible authority.

**3.1.2 Notification**

The presence of any total coliform bacteria in water leaving a treatment plant indicates a serious breach in treatment and is therefore unacceptable. This situation should be corrected immediately. In a distribution system, no consecutive samples from the same site or not more than 10% of samples in a given sampling period, based on a minimum of 10 samples, should show the presence of total coliform bacteria. In distribution systems where fewer than 10 samples are collected, no sample should contain total coliform bacteria. The presence of total coliforms in a distribution system indicates water quality degradation, possibly via regrowth or post-treatment contamination, and should therefore be investigated.

If the above conditions are exceeded, the system owner should notify all responsible authorities and immediately reanalyse the coliform-positive sample(s) and resample and test the positive site(s) to confirm the presence or absence of both *E. coli* and total coliforms (see Appendix A: Decision Tree for Routine Microbiological Testing of Public Systems). A quantitative method is suggested for the reanalysis, as it provides useful information on the level of contamination at each site. Actions required if *E. coli* presence is confirmed are outlined in...
Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — Escherichia coli (Health Canada, 2006a). If total coliforms (in the absence of E. coli) are confirmed, some or all of the corrective actions listed below may be necessary.

3.1.3 Corrective actions

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

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

Knowledge of the history of the system enables qualified personnel to consider appropriate actions when occasional low levels or frequencies of total coliforms are detected in the absence of E. coli. This level and frequency of occurrence will vary according to the historical records for that system.

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

- Verify the integrity of the treatment process and distribution system.
- Verify that the required disinfectant residual is present throughout the distribution system.
- Increase the chlorine dosage, flush the water mains, clean treated water storage tanks (municipal reservoirs and domestic cisterns), and check for the presence of cross-connections and pressure losses. Water should be dechlorinated before being discharged 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 sites adjacent to the site(s) of the positive sample(s). Tests performed should include total coliforms, E. coli, disinfectant residual, and turbidity. At a minimum, one sample upstream and one downstream 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, colour, turbidity, 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. Quantitative tests are better than presence–absence (P-A) tests for carrying out this task.
If enhanced health surveillance indicates that a waterborne outbreak may be occurring or if conditions exist that could result in a waterborne outbreak, then the necessity of issuing a boil water advisory should be immediately discussed with senior operations personnel at the water utility and with the responsible authority. In the event that an incident that may have contaminated the distribution system or interfered with treatment is known to the owner, consumers may be notified immediately to boil the drinking water. 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 that demonstrate full system-wide integrity (including acceptable bacteriological quality, disinfection residuals, and/or turbidity). Additional negative results may be required by the local responsible authority. Further information on boil water advisories can be found in Health Canada’s Guidance for Issuing and Rescinding Boil Water Advisories (Health Canada, 2001).

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

Sampling frequencies for semi-public systems will be determined by the authority having jurisdiction for the system and should include times when the risk of contamination is greatest — for example, spring thaw, extended heavy rains, or dry periods. Owners of private supplies should be encouraged to have their water tested for total coliforms during these same periods. New or rehabilitated wells should also be tested before use to confirm microbiological safety.

3.2.2 Notification

No samples from semi-public or private drinking water supplies should contain coliforms. If a sample contains total coliform bacteria, it should be immediately reanalysed and the positive site resampled and tested to confirm the presence or absence of both E. coli and total

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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, ‘boil water advisory’.

2 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.
coliforms. If resampling confirms that the system is contaminated with *E. coli*, the actions required are outlined in *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — Escherichia coli* (Health Canada, 2006a).

Responses to total coliform-positive samples in the absence of *E. coli* can vary from jurisdiction to jurisdiction. As a precautionary measure, some jurisdictions will always advise the owner to boil the drinking water or use an alternative safe source as an interim measure until corrective actions are taken. In other jurisdictions, advice on interim measures is site-specific and dependent on such factors as concentration of total coliform bacteria, historical water quality data, the health status of the users, and delays in investigation. Regardless of whether or not a boil water advisory is issued, the source of the contamination needs to be identified, and appropriate actions need to be taken (see Appendix B: Decision Tree for Routine Microbiological Testing of Semi-Public and Private Systems). These may include some or all of the corrective actions outlined in sections 3.2.3 and 3.2.4.

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

The first step 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 total coliforms are detected after implementing the corrective actions noted above, a boil water advisory should be issued, if not already in place. Alternatively, a source of water known to be safe should be used until the situation is corrected. The presence of total coliforms after corrective actions suggests that the system remains vulnerable to contamination. If the problem cannot be corrected, additional treatment or a new source of drinking water should be considered.

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 total coliforms are detected after implementing the corrective actions noted above, a boil water advisory should be issued, if not already in place. Alternatively, a source of water known to be safe should be used until the situation is corrected. The presence of total coliforms after shock chlorination and flushing suggests that the well remains vulnerable to contamination. If the problem cannot be reasonably identified or corrected, an appropriate disinfection device or well reconstruction or replacement should be considered.

It should be noted that a single negative total coliform result in a semi-public or private system is not necessarily indicative of a safe water supply. A minimum of two consecutive total coliform negative samples should be obtained. An additional test should be taken after 3–4 months to ensure that the contamination has not recurred. Only a history of data can be used to confirm the long-term integrity of a supply when applied jointly with sanitary surveys. Further information on routine monitoring can be found in section 8.0, Sampling for Total Coliforms

4.0 Significance of total coliforms in drinking water

4.1 Description

Total coliforms belong within the family Enterobacteriaceae and have been defined in the 20th edition of *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.*, 1998) as follows:

1. all facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35°C;
2. many facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that develop red colonies with a metallic (golden) sheen within 24 hours at 35°C on an Endo-type medium containing lactose; or
3. all bacteria possessing the enzyme $\beta$-galactosidase, which cleaves a chromogenic substrate (e.g., ortho-nitrophenyl-$\beta$-D-galactopyranoside), resulting in release of a chromogen (ortho-nitrophenol).
These definitions are not to be regarded as identical; rather, they refer to three groups that are roughly equivalent. All three groups contain various species of the genera *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, and many others (Leclerc *et al.*, 2001). Some members of these groups are naturally occurring in the environment and are of faecal origin, while others are found exclusively in the environment (Table 1).

Although not included in the coliform group, members of the genus *Aeromonas* can ferment lactose and possess β-galactosidase; therefore, they can yield false-positive total coliform reactions. *Aeromonas* species are ubiquitous in the environment, having been found in lakes, rivers, marine waters, sewage effluents, and drinking waters, among other places (Allen *et al.*, 1983; Nakano *et al.*, 1990; Poffe and Op de Beeck, 1991; Payment *et al.*, 1993; Ashbolt *et al.*, 1995; Bernagozzi *et al.*, 1995; Chauret *et al.*, 2001; El-Taweel and Shaban, 2001). False positives resulting from the presence of *Aeromonas* can be excluded using the cytochrome oxidase test.

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

<table>
<thead>
<tr>
<th>ONPG</th>
<th>Faecal origin</th>
<th>Non-faecal origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Budvicia</em></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Citrobacter</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Erwinia</em></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Leclercia</em></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Serratia</em></td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*a* Adapted from Leclerc *et al.* (2001).

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

A subset of the total coliform group, known as the thermotolerant coliforms (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. Thermotolerant coliforms are distinguished from total coliforms by their ability to tolerate elevated incubation temperatures during culturing. By definition, thermotolerant coliforms include the portion of the total coliform group capable of forming gas within 24 hours at 44.5°C or that produce a blue colony on m-FC broth within 24 hours at 44.5°C (APHA *et al.*, 1998). This group includes members of the genera *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter*. Recent advances in *E. coli* detection methods have made the need for thermotolerant coliform testing in drinking water quality management redundant.
4.2 Sources

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

The presence of total coliforms in a distribution system, as opposed to the natural environment, results from inadequately treated source water, allowing total coliforms to pass through the treatment system into the distribution system; subsequent regrowth; or intrusion of the organisms into the water post-treatment. A study done by Kirmeyer *et al*., (1999) showed that coliforms could be detected surrounding distribution system pipelines; therefore, post-treatment contamination could result from numerous problems, such as pipe leaks with negative pressure events, pipe breaks, inadequate cleaning and disinfection after repairs, and cross-connections, including backflow, with non-potable water. In addition, surges in water mains from activities such as hydrant tests and fire-fighting may result in the sloughing of biofilm and a subsequent rise in total coliform bacterial counts.

After the introduction of total coliforms into the distribution system, their survival and possible growth depend on many factors, including (but not limited to) water temperature, retention time of the water, type and concentration of disinfectant (if present), presence of nutrients, specifically the assimilable organic carbon and the biodegradable dissolved organic carbon concentrations, pipe material characteristics, and presence of sediments. Not all members of the coliform group persist in water for the same length of time under identical conditions (APHA *et al*., 1998). *E. coli*, for example, is generally the most sensitive to environmental stresses and does not usually grow outside the human or animal gut (Geldreich, 1996). *Klebsiella*, *Citrobacter*, and *Enterobacter*, on the other hand, are more likely to persist in the environment and, under favourable conditions, can multiply in water. For example, in water distribution systems, *Klebsiella* was able to survive and even grow in drinking water biofilms on the interior surface of water mains and in storage tanks (LeChevallier *et al*., 1987; LeChevallier and McFeters, 1990; Edberg *et al*., 1994). Total coliform presence in biofilms may result in resistance to disinfection and other eradication measures (Martin *et al*., 1982; Geldreich and Rice, 1987).

5.0 Role of total coliforms in maintaining drinking water quality

As early as the late 19th century, *E. coli* was recognized as the only species in the coliform group found exclusively in the intestinal tract of humans and other warm-blooded animals. At the time, detection methods for *E. coli* were impractical for routine monitoring.
As a result, total coliforms were used as a surrogate for \textit{E. coli} to indicate faecal contamination of drinking water supplies. It was recognized even then that total coliforms were not faecal specific; at the time, however, the majority of the total coliforms in drinking water were indeed \textit{E. coli}.

Total coliforms continued as an indicator of faecal contamination for a large part of the 20th century. It was not until the mid-20th century that more specific methods for the thermotolerant coliforms, which include \textit{E. coli} and members of the genera \textit{Klebsiella}, \textit{Enterobacter}, and \textit{Citrobacter}, were developed. The use of the thermotolerant coliform test became widespread as a surrogate for \textit{E. coli}, but it soon became evident that the majority of these organisms isolated from distribution systems were primarily members of the genus \textit{Klebsiella} (Edberg \textit{et al.}, 2000).

Now, with the availability of enzyme-based methods for \textit{E. coli} testing, drinking water purveyors have the tools necessary to routinely test for this faecal-specific bacteria. Consequently, testing for the less specific thermotolerant coliforms is no longer recommended. Also, additional studies have confirmed that total coliforms are not good indicators of faecal contamination. For example, a study done comparing water systems for the presence of outbreaks and violations of the Total Coliform Rule found no significant difference in total coliform violations between areas with and without outbreaks of waterborne illness (Nwachuku \textit{et al.}, 2002). There is some research supporting a link between the presence of viruses and total coliforms in groundwater, but further information is needed (Abbaspazdegan \textit{et al.}, 2003).

### 5.1 Public systems

As operational indicators, total coliforms provide information on the adequacy of drinking water treatment and on the microbial condition of the distribution system. For example, the presence of any total coliform bacteria in water leaving a treatment plant or in any treated water immediately post-treatment signifies inadequate treatment and is unacceptable and should be corrected immediately. If total coliforms are found in the distribution system, but water tested immediately post-treatment is free of total coliforms, this suggests that regrowth or post-treatment contamination has occurred. Numerous studies (LeChevallier \textit{et al.}, 1987; LeChevallier and McFeters, 1990; Edberg \textit{et al.}, 1994) have documented that \textit{Enterobacter} and \textit{Klebsiella} frequently colonize the interior surfaces of water mains and storage tanks when conditions are favourable. Nevertheless, the occurrence of coliforms apparently as a result of regrowth should not be ignored. Corrective action in such cases is required in order to maintain the usefulness of total coliforms as an indicator of the overall quality of the water. It should be noted that, in the absence of \textit{E. coli}, the presence of total coliforms in the distribution system is of no immediate public health significance. However, their presence should prompt further actions.

This position has been adopted by other countries as well. The Drinking Water Inspectorate of England and Wales has included in its regulations a mandatory value of zero coliforms per 100 mL in water leaving treatment works, a mandatory value of zero coliforms per 100 mL in 95\% of samples for water in service reservoirs, and a non-mandatory value of zero coliforms per 100 mL at the consumer’s tap. In these regulations, non-mandatory values do not need to be met, but exceedances need to be investigated and actions taken only if they represent a health risk (DWI, 2000). These regulations are based on the European Union’s Council Directive on the quality of water intended for human consumption (Council of the European Union, 1998).
5.2 Semi-public and private systems

In disinfected semi-public and private systems, total coliforms are also considered operational indicators. Their presence provides evidence of the inadequacy of disinfection or deterioration of water quality in the system. The presence of total coliforms in non-disinfected wells indicates that the well is either prone to surface water infiltration and therefore at risk of faecal contamination or that bacterial regrowth is occurring within the well or plumbing system. Implementation of corrective actions, such as shock chlorination and flushing, provides valuable information on the source of the total coliform bacteria. Regrowth problems should be solved following these actions. The continued presence of total coliforms is probably the result of infiltration, indicating that the system is vulnerable to contamination with pathogenic microorganisms. The extent of the contamination can also be used to aid in determining the cause of the contamination, interim protective measures, and the necessary corrective actions. Examples of corrective actions are outlined in section 3.2, Semi-Public and Private Drinking Water Systems.

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 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 total coliforms

In Canada, three methods are currently used for routine monitoring of total coliforms in water: presence–absence (P-A), membrane filter (MF), and multiple tube fermentation (MTF).
Total Coliforms (February 2006)

procedures. A detailed description of these methods can be found in *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 1998).

All three detection methods use cultivation to detect/confirm the presence of total coliforms. Cultivation media can be broadly categorized into two types: media containing bacterial enzymes for specific detection and confirmation of the total coliforms 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 total coliforms.

Methods that detect and confirm the presence of total coliforms in a single step are based on the use of the enzyme β-galactosidase. By definition, all bacteria possessing the enzyme β-galactosidase belong to the total coliform group (APHA et al., 1998). One publicized method uses the β-galactosidase activity of total coliforms to hydrolyse a chromogenic substrate — for example, *ortho*-nitrophenyl-β-D-galactopyranoside — in the media to release *ortho*-nitrophenol (yellow), turning the media yellow. The change in medium colour to yellow therefore indicates a positive total coliform result. These new enzyme-based methods are also capable of the simultaneous detection of both total coliforms and *E. coli* (Edberg et al., 1988).

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 bacteria 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 total coliforms, can utilize vital nutrients from the media (Rompré et al., 2002). For these reasons, the use of enzyme-based methods should be encouraged.

Presumptive coliform media, such as lauryl tryptose broth, m-Endo media, or LES Endo media (APHA et al., 1998), can be used for total coliform detection. When using lactose-based media for the P-A test or the MTF procedure, the formation of acid and/or gas following incubation for up to 24 hours at 35°C constitutes a positive presumptive test for total coliforms. Additional tests may be required to confirm the presence of total coliforms. Using the MF procedure, colony characteristics, such as colour and surface sheen, are used for presumptive identification (APHA et al., 1998). Additional methods for the verification of total coliforms recovered by the MF technique have been described (Evans et al., 1981b; Standridge and Delfino, 1982; LeChevallier et al., 1983b).

All analyses for total coliforms should be carried out as directed by the responsible authority. In many cases, the responsible authority will recommend or require the use of accredited laboratories. In some cases, it may be necessary to use other means to 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 is a qualitative procedure that was developed as a sensitive, economical, and efficient means of analysing drinking water samples (Clark and Vlassoff, 1973). Essentially, it is
a modification of the MTF procedure (see section 7.3), in which only one analysis bottle per sample is used. It is therefore recommended only for the examination of a water supply for which a sequential or consecutive series of samples has been collected. Based on a typical 100-mL water sample, the detection limit of the procedure is 1 organism per 100 mL. This sensitivity is equal to that of the classical MTF and MF methods. This method has also been shown to detect injured coliforms within the 24-hour response time (Rompré et al., 2002). 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 confirmation. Commercial test kits using defined substrate technology have been developed.

In comparative tests, the P-A method was shown to be at least as sensitive as the MF technique for the recovery of coliforms in drinking water samples (Clark, 1980; Jacobs et al., 1986; Pipes et al., 1986). Also, a nationwide evaluation in the United States demonstrated no statistical difference in the number of coliform-positive samples obtained by the standard MTF method compared with the P-A procedure using methods based on defined substrate technology (Edberg et al., 1989). Technically, the P-A test is simpler than the MF and MTF procedures, with an initial per-sample analysis time of less than 1 minute. The qualitative nature and the need to confirm positive results (for lactose broth only) are the only shortcomings of the P-A test. Since total coliforms are operational indicators, quantitative results using MF or MTF analysis will be more informative.

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). It is a quantitative procedure that uses membrane filters with pore sizes sufficient to retain the target organisms. The water sample is filtered through the membrane, which is then transferred to an appropriate growth medium for identification and quantitation. This procedure has the advantages of being able to examine larger volumes of water than with MTF, as well as having an increased sensitivity and reliability and requiring significantly reduced time, labour, equipment, space, and materials. These qualities have made the MF technique the method of choice in some jurisdictions for the routine enumeration of coliforms in drinking water. One disadvantage of the MF procedure that should be noted is that it cannot be used on highly turbid water samples. The particulate matter concentrated by the filter can interfere with colony development and with the production of surface sheens used for visual detection of coliforms.

A major concern, for this and other methods that use stressful selective media (i.e., media that contain inhibitory chemicals for non-target organisms), is an inability to enumerate coliform bacteria that have been subjected to sublethal injury (e.g., by chlorination) in the treatment plant or distribution system. Stressed organisms are often not able to grow on the selective coliform media but can recover through a resuscitation process. Experiments have shown that as many as 90% of the total coliforms present may be injured (Clark et al., 1951). One significant improvement in the MF technique has been the development of a new medium (m-T7) for the enhanced recovery of stressed coliforms in drinking water (LeChevallier et al., 1983a). Evaluation of media using routine drinking water samples (LeChevallier et al., 1983b; McFeters et al., 1986) and surface water samples (McFeters et al., 1986; Freier and Hartman,
1987) showed a higher coliform recovery on the m-T7 medium compared with the m-Endo medium. In all the above cases, chlorine was used as the stressing agent. Work using monochloraminated samples (Rice et al., 1987) and ozonated samples (Adams et al., 1989) showed that m-T7 performed no better than m-Endo agar in enumerating *E. coli* and *Citrobacter freundii*.

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

### 7.3 Multiple tube fermentation procedure

The MTF procedure, in comparison with the MF procedure, lacks precision, is more difficult to perform, and takes longer to produce results; because of this, the latter has largely replaced it for routine examinations of drinking water. However, the MTF procedure is still of value when conditions render the MF technique unusable — for example, with turbid, coloured, or grossly contaminated water — and as a comparative procedure.

In the MTF procedure, 10-fold dilutions of water to be tested are added to tubes containing the appropriate media (5 or 10 tubes per dilution) and incubated. Both enzyme-based methods and presumptive coliform media can be used. For drinking water, dilution should be unnecessary because of the expected low counts. With enzyme-based methods, a confirmation step is not necessary. As mentioned above, media containing the coliform-specific enzyme β-galactosidase undergo a specified colour change to signify a positive confirmed total coliform result. Using presumptive media, additional tests to confirm the presence of total coliforms are required. For example, the presence of total coliforms can be confirmed using a brilliant green lactose bile broth. The formation of gas in this fermentation tube at any time within 48 hours at 35°C constitutes a positive confirmation test (Rompré et al., 2002). Regardless of media type, results are reported as a most probable number (MPN). The MPN is only a statistical estimate of...
the number of bacteria that, more probable than any other number, would give the observed result; it is not an actual count of the bacteria present. Commercial kits are available for MPN determinations. The most widely publicized kits use a multi-well plate containing specific media and the enzyme $\beta$-galactosidase. A water sample is added to the plate. The wells that contain total coliforms undergo a specified colour change. The number of positive wells is then used to calculate the MPN.

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

8.0 Sampling for total coliforms

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 total coliforms. For supplies where weekly total coliform 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 disinfection 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 2 is offered as a guide.

Table 2: Recommended sampling frequency

<table>
<thead>
<tr>
<th>Population served</th>
<th>Minimum number of samples per month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 5000</td>
<td>At least 4</td>
</tr>
<tr>
<td>5000–90 000</td>
<td>1 per 1000 persons</td>
</tr>
<tr>
<td>90 000+</td>
<td>90 + (1 per 10 000 persons)</td>
</tr>
</tbody>
</table>

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. 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 the 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 the system and from representative points throughout the 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 any accompanying chain of custody paperwork. 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 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.

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 coliform bacteria. This indicates that the water is free of faecal contamination and the associated enteric pathogens. Of course, coliforms alone should not be relied on 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 technology

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 total coliforms, 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 was greater than 6 logs in all of the treatment plants tested. This removal is sufficient to reduce the number of total coliforms to conform to the established MAC of none detectable in 100 mL of drinking water (see section 10.0).

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 3. 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 4. E. 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.
Table 3: CT values for 99% inactivation at 5°C

<table>
<thead>
<tr>
<th>Disinfectant agent</th>
<th>pH</th>
<th>$E. \text{coli}^a$ (mg/min/L)</th>
<th>$Giardia \text{lamblia}^b$ (mg/min/L)</th>
<th>Poliovirus $1^c$ (mg/min/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free chlorine</td>
<td>6–7</td>
<td>0.034–0.05</td>
<td>32–46$^c$</td>
<td>1.1–2.5</td>
</tr>
<tr>
<td>Preformed chloramines</td>
<td>8–9</td>
<td>95–180</td>
<td>1470</td>
<td>768–3740</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>6–7</td>
<td>0.4–0.75</td>
<td>17</td>
<td>0.2–6.7</td>
</tr>
<tr>
<td>Ozone</td>
<td>6–7</td>
<td>0.02</td>
<td>1.3</td>
<td>0.1–0.2</td>
</tr>
</tbody>
</table>

$^a$ From Hoff (1986).
$^c$ 90% inactivation CT value.

From Table 3, it is apparent that, in comparison with most protozoans and viruses, coliform bacteria are easier to inactivate using the common chemical disinfectants. Also, 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 4) shows that, of the representative organisms, bacteria (in this instance, $E. \text{coli}$) and protozoa require comparable doses of UV light to achieve the same level of inactivation, whereas viruses are much more resistant.

Table 4: UV dose (mJ/cm²) required for inactivation

<table>
<thead>
<tr>
<th>Log inactivation</th>
<th>$E. \text{coli}^b$</th>
<th>Cryptosporidium$^b$</th>
<th>Virus$^b$</th>
<th>$Giardia^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5–4.4</td>
<td>2.5</td>
<td>58</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>2.8–6.2</td>
<td>5.8</td>
<td>100</td>
<td>5.2</td>
</tr>
<tr>
<td>3</td>
<td>4.1–7.3</td>
<td>12</td>
<td>143</td>
<td>11</td>
</tr>
</tbody>
</table>

$^a$ Based on five separate studies, taken from U.S. EPA (2003).
$^b$ Based on validation studies done by U.S. EPA (2003).

9.2 Residential

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 chlorine-based 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

10.1.1 Public drinking water supply systems

Effective treatment including disinfection should yield water free of any coliform organisms, no matter how polluted the source water may have been. The presence of any type of coliform organism in treated water leaving a plant therefore suggests inadequate treatment and disinfection and is unacceptable. If the water leaving a treatment plant has tested negative for total coliforms but total coliforms are present in the distribution system, this suggests regrowth or infiltration into the system. Although the presence of total coliforms is not a reliable indicator of the presence of faecal contamination, the cause of their presence should be investigated and further action taken if necessary. In a distribution system, public health decisions should not be based solely on the presence of total coliforms, in the absence of *E. coli*, unless the investigation indicates a problem that results in a threat to public health.

Routine analysis for coliform bacteria should be supplemented by HPCs or by background colony counts on the total coliform membrane filters when using lactose-based media. For enzyme-based methods using defined substrate technology, HPC or background colony count determinations are not necessary. However, there are other reasons for measuring HPC bacteria. These can be found in Health Canada’s guideline technical document for HPC (Health Canada, 2006b).
Based on the above discussion, the MAC for total coliforms in public drinking water systems, in water leaving a treatment plant is no organisms detectable per 100 mL. In a distribution system of a public drinking water supply, total coliforms are an indicator of water quality and are not used solely for public health decisions; however, their presence should prompt further actions.

10.1.2 **Semi-public and private drinking water supply systems**

The presence of total coliforms in semi-public and private systems may result from an inadequacy in treatment and disinfection, regrowth in the distribution system, or infiltration into the system through either the source water or the distribution system.

The MAC for coliforms in semi-public and private drinking water systems is no organisms detectable per 100 mL. Because total coliforms are not uniformly distributed in water and are subject to considerable variation in terms of their significance, responses to the presence of total coliforms in the absence of *E. coli* will vary from jurisdiction to jurisdiction, and the water will still be considered to conform to the MAC.

10.2 **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. As a minimum, water leaving a treatment plant should be tested daily for disinfectant residual and turbidity and tested at least weekly for total coliforms. For supplies where weekly total coliform testing is impractical (e.g., in small supplies), residual disinfectant determinations should be relied upon to verify microbiological safety. Small supplies should also routinely carry out sanitary surveys as an additional action to verify the safety of the system. The daily sampling recommendations for disinfection 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 5, which reproduces Table 2 above, is offered as a guide.

### Table 5: Recommended sampling frequency

<table>
<thead>
<tr>
<th>Population served</th>
<th>Minimum number of samples per month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 5000</td>
<td>At least 4</td>
</tr>
<tr>
<td>5000–90 000</td>
<td>1 per 1000 persons</td>
</tr>
<tr>
<td>90 000+</td>
<td>90 + (1 per 10 000 persons)</td>
</tr>
</tbody>
</table>

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. The routine analysis for coliform bacteria should be supplemented by HPCs or by background colony counts on the total coliform membrane filters when using lactose-based media.

10.3 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 for the indication of faecal contamination. The presence of total 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 all public systems 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. It is recognized, however, that excessive levels of disinfectant may result in taste and odour problems. In these cases, the responsible authority may provide guidance as to the type and concentration of disinfectant residual to ensure that water remains microbiologically safe.

In addition to disinfection, 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 this guideline 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 coliforms are absent. In addition, satisfaction of other water quality criteria may dictate the use of unit processes not mentioned in the above scheme.

### 11.0 References


Total Coliforms (February 2006)


*Guidelines for Canadian Drinking Water Quality: Guideline Technical Document*
Total Coliforms (February 2006)


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


Total Coliforms (February 2006)


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

Routine sampling for E.coli (Ec) and total coliforms (TC) in public systems

1st Resample/retest positive site and adjacent sites

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Single site contaminated

Ec positive

2nd Resample/retest positive site and adjacent sites

Ec positive

1st Resample/retest positive site and adjacent sites

Boil Water Advisory**** should be issued (in consultation with responsible authority)

Ec positive

Investigate the source of contamination and implement corrective actions

Ec negative***

Multiple sites contaminated

TC positive**, Ec negative

2nd Resample/retest positive site and adjacent sites

Ec positive

Investigate the source of contamination and implement corrective actions

TC negative Ec negative

2nd Resample/retest positive site and adjacent sites

Ec positive

1st Resample/retest positive site and adjacent sites

Ec negative

Water considered safe

2nd Resample/retest positive site and adjacent sites

Ec positive

Ec negative

No further corrective actions required

Ec negative***

TC negative

Water considered safe

Ec positive

Continue routine sampling for Ec and TC

* A boil water advisory may be issued on a single site contamination, depending on the jurisdiction.
** 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 semi-public and private systems

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*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

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANSI</td>
<td>American National Standards Institute</td>
</tr>
<tr>
<td>CFU</td>
<td>colony-forming unit</td>
</tr>
<tr>
<td>HPC</td>
<td>heterotrophic plate count</td>
</tr>
<tr>
<td>MAC</td>
<td>maximum acceptable concentration</td>
</tr>
<tr>
<td>MF</td>
<td>membrane filter</td>
</tr>
<tr>
<td>MPN</td>
<td>most probable number</td>
</tr>
<tr>
<td>MTF</td>
<td>multiple tube fermentation</td>
</tr>
<tr>
<td>NSF</td>
<td>NSF International</td>
</tr>
<tr>
<td>P-A</td>
<td>presence–absence</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
</tbody>
</table>