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Guidance on the use of Enterococci bacteria as indicators in Canadian drinking water supplies

Guidance Document for Public Consultation

Consultation period
ends January 25, 2019

Canada

Document for Public Consultation

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Guidance on the use of Enterococci bacteria as indicators in Canadian drinking water supplies

Purpose of consultation

This document has been developed with the intent to provide regulatory authorities and decision-makers with guidance on the use of enterococci as a bacteriological indicator in Canadian drinking water supplies.

The document is being made available for a 60-day public consultation period. The purpose of this consultation is to solicit comments on the proposed guidance document. Comments are appreciated, with accompanying rationale, where required. Comments can be sent to Health Canada via email at HC.water-eau.SC@canada.ca. If this is not feasible, comments may be sent by mail to the Water and Air Quality Bureau, Health Canada, 269 Laurier Avenue West, A.L. 4903D, Ottawa, Ontario K1A 0K9. All comments must be received before January 25, 2019

Comments received as part of this consultation will be shared with members of the Federal-Provincial-Territorial Committee on Drinking Water (CDW), along with the name and affiliation of their author. Authors who do not want their name and affiliation to be shared with CDW should provide a statement to this effect along with their comments.

It should be noted that this guidance document will be revised following evaluation of comments received, and a final guidance document will be posted. This document should be considered as a draft for comment only.

Guidance on the use of Enterococci bacteria as indicators in Canadian drinking water supplies

Executive summary

Enterococci are a bacteriological indicator of faecal contamination that can be used in assessing drinking water safety. They can be included in a drinking water monitoring program to provide information on the quality of the source water, the adequacy of treatment and the delivery of safe drinking water to the consumer.

Health Canada recently completed its review of enterococci in drinking water. This guidance document describes the significance, sampling and treatment considerations for the use of enterococci as a bacteriological indicator in the context of drinking water quality and safety. During its spring 2017 meeting, the Federal-Provincial-Territorial Committee on Drinking Water reviewed the guidance document on enterococci in Canadian drinking water supplies and gave its endorsement for this document to undergo public consultation.

Assessment

Enterococci tests can be a useful tool to complement the information provided by the microbiological parameters that are traditionally used in drinking water monitoring, *E. coli* and total coliforms. Enterococci may persist longer and be carried further than *E. coli* in the environment. Thus enterococci may indicate faecal contamination in water that might otherwise be missed. Adding enterococci to a monitoring program may provide enhanced information to the bacteriological monitoring programs already in place. The intent of this document is to provide stakeholders, such as provincial and territorial regulatory authorities, decision makers, water system owners and consultants with guidance on the use of enterococci in a monitoring program with the objectives of identifying and minimizing microbiological risks in Canadian water systems.

International considerations

Drinking water quality guidelines, standards and/or guidance established by foreign governments or international agencies may vary due to the science available at the time of assessment, as well as the utilization of different policies and approaches. Enterococci are widely used for assessing water quality in many parts of the world, but are not used as frequently as other indicators such as *E. coli*. The World Health Organization states that the detection of enterococci should lead to consideration of further action, but has not established a guideline value. The European Union's Drinking Water Directive includes enterococci as a parameter for audit monitoring with a standard of zero enterococci per 100 mL of water, but does not require frequent monitoring. The Australian Drinking Water Guidelines do not include a guideline value for enterococci, but indicate that enterococci can be used to assess source water quality, the adequacy of treatment, post-treatment ingress into the distribution system and the delivery of safe drinking water at consumer taps. The United States Environmental Protection Agency Ground Water Rule lists enterococci as one of three state-specified bacterial indicators of faecal contamination, alongside *E. coli* and coliphages.

Part A. Guidance on the use of Enterococci bacteria as indicators in Canadian drinking water supplies

This document provides information on enterococci as a faecal indicator bacteria in the context of drinking water quality and safety.

In Canada, provincial and territorial drinking water regulations typically require verification monitoring for *E. coli* and total coliforms for all drinking water systems that supply potable water to the public. *E. coli* is a valuable indicator of faecal contamination, but, like any indicator, it also has limitations. *E. coli* is more sensitive to environmental stresses and disinfection than enteric viruses, protozoa and some bacterial pathogens.

Using a broader range of faecal indicators rather than just *E. coli* enhances the potential to identify and thus trigger responses to issues.

The enterococci group has demonstrated some advantages that may enhance our ability to reliably detect faecal contamination in water environments. Enterococci are widely used as tools for assessing water quality in many parts of the world. They are relatively abundant in human and animal faeces, easily cultured, and have been correlated with human health outcomes in fresh and marine waters. They have been used as faecal indicator bacteria for recreational water, drinking water and water re-use applications.

Enterococci are members of the bacterial genus *Enterococcus*, a group which comprises 30-plus species. Several species, such as *Enterococcus faecalis* and *Enterococcus faecium*, are members of the microbial gut community and are excreted in the faeces of humans and animals. Enterococci can be found in water environments polluted by sewage or faecal wastes from humans and animals. Species have also been detected in a variety of environmental habitats.

Enterococci have shown evidence of more robust survival against stressors in aquatic environments and disinfectants commonly used in the drinking water industry (chlorine, monochloramine, ultraviolet [UV] light) when compared to *E. coli*. They may persist longer and be transported further than *E. coli* in some water environments, and so may point to water quality deficiencies potentially not captured with traditional faecal indicator monitoring.

Enterococci can be employed as a verification indicator to provide additional useful information on the quality of the drinking water source, the adequacy of drinking water treatment and the microbial condition of the distribution system. They can be part of routine testing to complement information provided by other microbiological verification parameters: *E. coli* and total coliforms. Enterococci testing can also be used during an audit of a drinking water system in order to gain more information about the microbiological quality in the system and whether potential vulnerabilities exist.

Research suggests that monitoring for enterococci may be particularly useful for untreated groundwater wells and in drinking water distribution systems. Enterococci may survive longer than *E. coli* in some contaminated groundwater supplies. Therefore, for untreated groundwater systems, the presence of enterococci can indicate the potential vulnerability of the source water. Enterococci may also persist longer than *E. coli* where drinking water disinfection is inadequate. In the distribution system, the presence of enterococci can indicate a failure of a treatment barrier in place or signify a potential pathway of entry for faecal contamination. It has been suggested that the enterococci group may be a useful indicator of bacteriological water quality in newly installed or repaired water mains or in areas with low-flow such as dead-ends.

The incentive for using enterococci in drinking water management strategies is that it is a useful tool to verify the effectiveness of system barriers in place and to potentially detect water quality deficiencies that may not be identified when using only *E. coli* as the indicator of faecal contamination.

It is important that drinking water authorities understand that verification indicators provide a check of the performance of the barriers in place to achieve safe drinking water. They do not provide a gauge of the amount of possible illness from exposure to pathogens in drinking water. Although *E. coli* is considered at this time to be the primary faecal indicator, the information provided by enterococci monitoring can improve a utility's understanding of their drinking water system and help them further achieve their objectives of minimizing risks and assuring drinking water quality.

As is the case with other microbial indicators, our knowledge of the biology and ecology of enterococci is incomplete. The existence of environmental habitats serving as potential sources of indicator bacteria is a challenge connected to both enterococci and *E. coli* as indicators of faecal contamination. This highlights the need for continued study to fill in the gaps in our current understanding of these organisms as faecal indicators.

Decisions about the usefulness of including enterococci monitoring as part of a multi-barrier or water safety plan approach for a drinking water system should be determined by the responsible authority. The number and frequency of samples required for enterococci testing will vary depending on the expected goals of the application. If the goal is routine testing for verification of the adequacy of treatment and/or the microbial condition of the distribution system, sampling at a frequency similar to that used for other microbiological indicators such as *E. coli* and total coliforms is recommended. If the goal is testing for enterococci during an audit of a drinking water system, a lower number of samples may be required on an annual basis. Further information on the role of *E. coli* and total coliforms in water quality management can be found in the guideline technical documents on *E. coli* and total coliforms.

Well designed, operated and properly maintained drinking water systems are capable of producing drinking water with no detectable enterococci. Therefore, there should be no enterococci detected in a drinking water system. If enterococci are detected in a drinking water system, confirmation, notification and corrective actions identical to those outlined in the guideline technical document for *E. coli* should be put in place. In order to make correct and timely public health decisions, it is important to use validated or standardized detection methods. Verification steps required as part of the analytical methods may prolong the time needed to complete the analyses. As with the positive detection of any microbiological indicator, any decisions regarding corrective actions necessary should also be informed by the assessment of the integrity and physical condition of the drinking water system and the verification of available operational indicators (e.g. disinfectant residual, turbidity).

The use of a broader range of indicators lends additional public health protection in light of uncertainties around individual indicators. There is good evidence that both enterococci and *E. coli* indicate faecal contamination, but their presence is not always correlated with one another. Given that enterococci are good indicators of faecal pollution and that they are somewhat more robust and may be transported further than *E. coli*, including this parameter in a monitoring program may provide enhanced or comparative information to *E. coli* and total coliforms monitoring currently in place. Ongoing operations, monitoring and treatment optimization will help ensure water utilities achieve water quality goals and maximize public health protection. Maintaining current knowledge of best practices and remaining aware of advancements in the drinking water industry are important aspects of a multi-barrier or water safety plan approach to

ensure water safety.

Part B. Supporting information

B.1 Description, sources, and exposure

B.1.1 Description and sources

Enterococci are members of the bacterial genus, *Enterococcus*. They are Gram-positive, round-shaped bacteria that occur singly, in pairs or short chains.

The classification of enterococci has evolved over the years. Enterococci were first considered a specific division of the genus *Streptococcus* that met criteria of being able to: grow between 10 and 45°C, survive heating for 30 minutes at 60°C, and grow at pH 9.6 and at 6.5% sodium chloride. Enterococci have also been referred to as faecal streptococci since they were initially found in intestines of animals. The development of molecular methods led to the designation of *Enterococcus* as a new genus in 1984 (Fisher and Phillips, 2009).

The genus is currently thought to comprise 30-plus species classified into 5–6 major groups (*E. faecalis, E. faecium, E. avium, E. gallinarum, E. italicus, and E. cecorum*) (Svec and Devriese, 2009; Byappanahalli et al., 2012a). However, much uncertainty still exists regarding the true number of species, their groupings and the scope of their habitats (Del Mar Lleò et al., 2005). Some members are predominantly faecal species, while others are found more frequently in the environment. The terms, enterococci, faecal streptococci and intestinal enterococci are encountered in the drinking water literature, but for practical purposes these can be considered largely synonymous and interchangeable (Del Mar Lleò et al., 2005; Byappanahalli et al., 2012). Standard test methods for enterococci are not specific for faecal species and detect species found naturally in the environment (APHA et al., 2012).

Enterococci occur naturally in the intestines of humans and a range of animals, including mammals, birds, reptiles and insects. Concentrations in human and animal faeces typically fall in the range from 10^3-10^7 cells per gram (Ashbolt et al., 2001; Leclerc et al., 2001; Ervin et al., 2013). Numbers in animal species can vary considerably and some studies have found higher enterococci numbers in the faeces of farm animals and domestic pets than in human faeces (Ervin et al., 2013; Masters et al., 2015). In general, numbers of enterococci in human and animal faeces are lower than *E. coli* numbers by one to a few orders of magnitude (on a $\log_{10}$ scale) (Donnison, 1992; Cabral, 2010; Ervin et al., 2013, Boehm and Sassoubre, 2014).

E. faecalis and *E. faecium* are the predominant species encountered in faeces and sewage. Other species commonly isolated from faecal material, but in lower numbers include: *E. durans*, *E. hirae*, *E. gallinarum*, and *E. avium* (Poucher et al., 1991; Moore et al., 2008; Staley et al., 2014).

Enterococci have also been detected in diverse environmental habitats (Byappanahalli et al., 2012a; Staley, 2014). These have included plants, flowers, vegetables, cereals and grasses (Mundt et al., 1962; Müller et al., 2001; Ott et al., 2001; Sánchez Valenzuela et al., 2012); freshwater and marine water sand, soil and sediments (Obiri-Danso and Jones, 2000; Ran et al., 2013); and mats of the green algae *Cladophora*, decaying seaweed and submerged aquatic vegetation (Anderson et al., 1997; Whitman et al., 2003; Badgley et al., 2010; Byappanahalli et al., 2012a). Certain members of the genus have, to date, been principally detected in environment habitats. For example, *E. camelliae*, *E. casseliflavus*, *E. mundtii*, and *E. sulfureous* are all found

among plants (Collins et al., 1986; Moore et al., 2008; Byappanahalli et al., 2012a).

However, there have been reports of principally environmental species being isolated from human or animal feces (Splichalova et al., 2015; Medeiros et al., 2017; Beukers et al., 2017). As well there have been numerous reports of the principal faecal species being detected in environmental samples in the absence of obvious sources of faecal contamination (Müller et al., 2001; Sánchez Valenzuela et al., 2012; Byappanahalli et al., 2012a).

There is an accumulating body of evidence that, in favourable environments outside the gastrointestinal tract, certain strains of faecal indicator bacteria may be capable of growth, multiplication and ultimately adaptation to become naturalized (Ferguson and Signoretto, 2011; Ran et al., 2013). A naturalized population is one that has evolved to exist in the environment independently of faecal contamination. Over time, research has shown that environmental habitats serving as potential sources of indicator bacteria is a challenge connected to all of the groups that have been traditionally viewed as drinking water faecal indicators (total coliforms, thermotolerant coliforms, *E. coli*, enterococci) (Whitman et al., 2003; Byappanahalli et al., 2012a). This has complicated the interpretation when detecting these organisms, as it can no longer be presumed true that any one group is exclusively associated with faecal wastes. Still, they remain valuable indicators of potential vulnerabilities to faecal contamination and of the performance of drinking water barriers in place.

Only a few studies have characterized the enterococci species detected in municipal scale drinking water supplies and groundwater supplies. Available reports have consistently identified that the faecal species *E. faecalis* and *E. faecium* are the two species detected most often in these sources (Sinton and Donnison, 1994; Celico et al., 2004; Grammenou et al., 2006; Jackson et al., 2012; Peter et al., 2012). Other enterococci species including those considered as either of predominantly faecal or environmental origin have been detected, but typically less often (Sinton and Donnison, 1994; Jackson et al., 2012). Data from some surface water and groundwater investigations have demonstrated that other enterococci species may at times be encountered at frequencies similar to or exceeding those of *E. faecium or E. faecalis* (Moore et al., 2008; Suzuki et al., 2012; Furtula et al., 2013). Moore et al. (2008) observed that the largely environment-associated species *E. casseliflavus* was the dominant species in urban runoff, while *E. faecium*, *E. faecalis* and *E. hirae* were dominant in sewage samples. Traditional culturing methods are not really designed to identify enterococci from different sources (Byappanahalli et al., 2012a).

Enterococci can persist in water from as little as a few hours to as long as a few weeks. In more favourable habitats that provide nutrients and protection from environmental stresses (e.g., soils, sand, and masses of aquatic vegetative material), survival of enterococci can be prolonged to the scale of months (Davies et al., 1995; Pote et al., 2009). For instance, enterococci have been shown to survive for over 6 months in sun-dried algal mats that were subsequently stored at 4°C (Whitman et al., 2003).

Historically, enterococci have been regarded as being more resistant to environmental stresses, such as increased salinity or desiccation, than the traditional faecal indicator bacteria, *E. coli* or thermotolerant coliforms (WHO, 1997, 2004). It has been proposed that this may be due in part to their thicker Gram-positive cell wall (Byappanahalli et al., 2012a). A number of studies have reported enterococci being capable of surviving longer than *E. coli* in marine waters (Lessard and Sieburth, 1983; Sinton et al., 1994; Bordalo et al., 2002; Fujioka and Yoneyama, 2002; Sinton et al., 2002). In fresh waters, differential survival rates have been observed (Sinton et al., 2002; Anderson et al., 2005; Deller et al., 2006; Fisher et al., 2012). A review of groundwater studies concluded that rates of inactivation for enterococci were in general slower than those for *E. coli* or faecal coliforms and similar to those of coliform bacteria (John and Rose,

2005). Enterococci have shown a greater tolerance to desiccation than *E. coli* in some investigations (Byappanahalli and Fujoka, 2004; Mika et al., 2009), which probably explain their abundance and ubiquity in certain tropical soils (Byappanahalli et al., 2012b).

Under the stresses of the water environment, enterococci can enter a viable but non-culturable (VBNC) state where they do not grow on laboratory media, but are otherwise alive and capable of resuscitation when conditions become favourable (Boehm and Sassoubre, 2014; Ramsey et al., 2014). The VBNC state is a primary survival strategy for bacteria that has been observed with numerous species, including those relevant to drinking water (Ramsey et al., 2014). Overall, enterococci can be considered to have a life span on the same order as most waterborne bacterial pathogens. It is generally agreed, however, that enterococci do not survive as long in the environment as waterborne pathogenic protozoa or viruses (Sinton et al., 2002; Medema et al., 2003).

B.1.2 Exposure

Concentrations of enterococci in temperate lakes and rivers are typically less than 10^3 colony forming units (CFU) per 100 mL (Jenkins et al., 2005; Ran et al., 2013). Concentrations can be elevated in response to rainfall events, with surface water samples containing 10^4–10^5 CFU/100 mL having been recorded (Haack et al., 2003; Wilkes et al., 2009; Nnane et al., 2011). Values exceeding 10^2 CFU per 100 mL have been reported for contaminated groundwater sources (Atherholt et al., 2003; Schneeburger et al., 2014). In addition, counts in excess of 10^4/g dry weight have been reported in beach substrates, such as algae (Whitman et al., 2003).

More data is needed pertaining to the detection of enterococci in the treated water and distribution systems of municipal-scale drinking water supplies in Canada. In the EU, Member States' drinking water reports for large systems have shown that although the majority of states report greater than 99% compliance with respect to microbiological parameters under the Drinking Water Directive, a large number of water supply zones report at least one incidence of non-compliance related to enterococci and *E. coli* sampling (KWR, 2012; European Commission, 2015). Non-compliance reports for enterococci among individual water supply zones were in sufficient numbers and sufficiently distinct from those for *E. coli*, to conclude that without enterococci monitoring, some potential issues with water quality might not have been recognized.

Within the literature, studies involving municipal drinking water distribution systems have noted the detection of enterococci in samples in the absence of *E. coli*, or reported more frequent detection in samples collected from dead ends or where residual chlorine concentrations were greater than 0.1 mg/L (Mendez et al., 2004; Batté et al., 2006). This supports the usefulness of enterococci as an indicator of water quality problems. Post-treatment contamination (e.g., through cross-connections, back siphonage, low pressure events, contamination of storage reservoirs) and contamination of mains from repairs have been identified as causes of distribution system contamination linked to illness. It has been suggested that because of their more robust survival capabilities, enterococci may be a useful indicator of sanitary quality for newly installed or repaired water mains (WHO, 1970, 1997, 2011).

The occurrence and survival of enterococci species in distribution system biofilms have also been examined. Detection of enterococci in biofilms in full-scale municipal systems was infrequent and in low numbers when present (Lee and Kim, 2003; Batté et al., 2006; Långmark et al., 2007). Although in some instances enterococci can be detected in biofilms in distribution systems, they do not seem to be a significant component of the biofilm matrix.

There is some data available regarding detection of enterococci (and other faecal indicators) at the point of drinking water consumption. In the United Kingdom (UK), samples to

assess regulatory compliance are taken from consumer's taps. Data from drinking water authorities for the UK and Ireland for the years 2010-2014 showed that for large public supplies, very few samples per year were non-compliant with the regulatory standard of 0 enterococci per 100 mL (DWI, 2016; DWQR, 2016a, 2016b; EPA, 2016a, 2016b; Northern Ireland Water, 2016). Across the various jurisdictions over this time period, the number of non-compliant samples per country per year ranged from a minimum of 0 to a maximum of 11. The data also did show that although the number of samples collected for enterococci were typically less than those collected for *E. coli*, enterococci was at times detected at a greater relative frequency (DWI, 2016; DWQR, 2016a, 2016b; EPA, 2016a, 2016b; Northern Ireland Water, 2016). This highlights the additional water quality information which could be afforded with the inclusion of enterococci monitoring.

More data is also needed pertaining to enterococci detection in small drinking water systems in Canada. Small systems are more vulnerable to waterborne disease outbreaks than larger municipal water systems (NRC, 1997; Schuster et al., 2005; NCCPH, 2011: Murphy et al., 2016). A groundwater monitoring program in Quebec has collected data on the faecal indicator testing for non-disinfected groundwater wells (Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques, 2016). Results of samples from 223 non-disinfected groundwater drinking stations collected from 2010 to 2014 showed that the overall percentage of tests positive for enterococci was nearly identical to the percentage positive for *E. coli* (2.38% vs. 2.36% respectively).

Reviews of drinking water reports prepared by EU Member States and drinking water authorities for England and Ireland revealed that small systems were more frequently non-compliant for enterococci testing than were larger utilities (KWR, 2012; European Commission, 2015; DWI, 2016; EPA, 2016a, 2016b).

Some groundwater investigations have shown evidence of greater persistence of enterococci compared to the indicators faecal coliforms or *E. coli* in contaminated aquifers (Sinton and Donnison, 1994; Roser et al., 2005; Naclerio et al., 2008; Schneeberger et al., 2014). During a study of the impacts of two residential septic systems on the quality of nearby groundwater aquifers, Schneeberger et al. (2014) observed that enterococci concentrations declined more gradually with increasing distance from the contamination source than *E. coli* concentrations. Enterococci were also detected in higher numbers than *E. coli* in groundwater samples collected between15–30 m and 30–40 m from the septic drainfields (Schneeberger et al., 2014). Because of their robust survival capabilities, enterococci may survive longer and be transported further than *E. coli* in some water environments, including some groundwater supplies.

In a Quebec study involving the detection of bacteriological indicators in municipal groundwater wells, total coliforms were found to correlate with *E. coli* and enterococci contamination (Payment and Locas, 2005). The data for wells categorized as having intermediate or poor water quality showed that in samples where enterococci were detected, total coliforms were also detected and in greater numbers. Similar findings were noted in studies of groundwater supplies having known sources of faecal contamination (Atherholt et al., 2003). In another study conducted in Quebec on the impacts of manure application practices in the watershed on groundwater quality, the frequency of detection of enterococci and *E. coli* among all groundwater samples (n=1,260) was 5.8% and 1.5% respectively (Government of Quebec, 2004). For both indicators, the frequency of detection in groundwater samples from watersheds where manure was applied was not significantly different from that reported for samples from the non-impacted control areas.

During an examination of rural drinking water systems in Alabama, Wedgworth et al. (2015) observed that the number of samples that were positive for enterococci was significantly greater than that for *E. coli* at all locations sampled (well, post-treatment, post-storage, in-line and end line). Total coliform detection was observed to correlate with enterococci detection at all locations in this study. In groundwater sources with sporadic or low levels of contamination, total coliforms, *E. coli* or enterococci may each be detected on their own (Atherholt et al., 2003; Locas et al., 2007, 2008).

Numerous studies have investigated linkages between the presence of indicator bacteria and the detection of specific faecal bacterial, viral or protozoan pathogens in surface water sources. Individual studies have occasionally observed a correlation between the presence of either enterococci or *E. coli* (or other indicators) and the detection of a specific pathogen, however the relationships are generally weak (Brookes et al., 2005, Wilkes et al., 2009). Studies in groundwater sources have been fewer in number and have shown weak correlations at best (Borchardt et al., 2003; Locas et al., 2007, 2008; Pitkänen et al., 2011; Hynds et al., 2014). Researchers studying microbiological quality in Canadian drinking water municipal wells observed that total coliforms and, to a lesser extent, *E. coli* were better indicators than enterococci in predicting the presence of human enteric viruses (Locas et al., 2007, 2008). Reviews of the body of evidence on this subject have concluded that neither enterococci nor *E. coli* has shown a greater likelihood of correlating with the presence of faecal pathogens (Payment and Locas, 2011; Wu et al., 2011).

Direct correlations between indicator concentrations and the type or concentration of any pathogen should not be expected. Within a watershed, indicators and pathogens may come from multiple different sources, and once discharged into water sources experience different dilution, transport, and inactivation rates (Wilkes et al., 2009; Payment and Locas, 2011). Despite the lack of direct correlations with specific pathogens, the presence of enterococci in surface or groundwater sources generally indicates faecal contamination and thus a potential health risk, regardless of whether or not specific pathogens are observed (Wu et al., 2011).

The enterococcal surface protein (*esp*) gene found in *Enterococcus faecalis* and *E. faecium* has been explored as a potential marker of sewage pollution in recreational waters (Byappanahalli et al., 2008; Harwood et al., 2014). However, the detection of the gene in both animal faecal sources and environmental samples has limited the interest in any further exploration of this marker as a reliable microbial source tracking tool for human faecal wastes (Byappanahalli et al., 2008; Staley et al., 2014; Harwood et al., 2014).

B.2 Health effects

Although enterococci are members of the natural faecal flora, some have also been implicated in opportunistic infections. Infections with enterococci have occurred at locations outside the intestines under conditions where immunity has been suppressed, where tissue injuries have occurred or where the body's normal flora has been disrupted as a result of use of prescription antibiotics (Tendolkar et al., 2003). Consumption of treated drinking water has not been reported as a route of exposure leading to infection.

B.2.1 Risk assessment

A health-based risk assessment for enterococci is not considered appropriate since enterococci are used only as indicator organisms. Risk assessments have been done for specific

microbiological organisms that have health implications, such as enteric viruses and the enteric protozoa *Cryptosporidium* and *Giardia* (Health Canada, 2011, 2012a).

Although the presence of enterococci is not necessarily associated with the presence of specific pathogens, studies involving recreational waters have reported linkages between gastrointestinal and acute febrile respiratory illnesses and enterococci levels (Kay et al., 1994; Fleisher et al., 1996; Wade et al., 2006, 2010; Heaney et al., 2012, 2014). As well, in some studies of the incidence of gastrointestinal illness and indicator presence in small drinking water supplies, data on the presence of enterococci fit the statistical regression models better than the *E. coli* data (Borchardt et al., 2003; Risebro et al., 2012).

The adoption of a risk-based approach, such as a multi-barrier or a water safety plan approach, is essential to the effective management of drinking water systems (CCME, 2004; WHO, 2011; Health Canada, 2013; Government of Alberta, 2015). Current drinking water guidelines encourage the adoption of a multi-barrier approach to produce clean, safe, and reliable drinking water. This approach includes the protection of source water, the use of appropriate and consistently effective treatment, a well-maintained distribution system, qualified personnel, routine verification of drinking water quality, and communication and public education.

E. coli and total coliforms are bacterial indicators that are used to verify water safety and changes in water quality, respectively. Monitoring for these indicators is one of the measures used to determine groundwater vulnerability, surface water quality, and to verify that water has been adequately treated and safely distributed. Enterococci are a bacteriological indicator of faecal contamination that can also be used under a multi-barrier approach for verification that the drinking water system is producing water that is microbiologically acceptable.

As part of a risk management plan, together with monitoring for other microbial indicators (*E. coli*, total coliforms) and disinfectant residual and turbidity testing, enterococci monitoring results can be used to assess:

- Source water microbiological quality, faecal impacts, possible sources of contamination and quality changes.
- Adequacy of drinking water treatment, the microbial condition of water in the distribution system and the delivery of safe drinking water to the consumer.

Enterococci testing may also be used for investigative purposes in order to better understand a water system. The presence of any enterococci in water leaving a treatment plant or in any treated water immediately post-treatment signifies inadequate treatment. In non-disinfected wells, the presence of enterococci indicates the presence of recent faecal contamination or a potential pathway of faecal contamination. The presence of enterococci in the distribution and storage system, when water tested immediately post-treatment is free of enterococci, suggests that post-treatment contamination has occurred.

B.3 Analytical methods

Most culture media methods for enumerating enterococci are based on the detection of the activity of the enzyme β- glucosidase (esculinase) which is present in the large majority of enterococci species and strains. Methods also take advantage of biochemical characteristics specific to the *Enterococcus* genus and use media additives and incubation temperatures to inhibit the growth of background microorganisms and differentiate enterococci from other Gram-positive bacteria.

Media have been designed to identify enterococci in water samples without identifying the species (Leclerc et al., 1996; APHA et al., 2012). Although some species have environmental origins and have been found in the absence of faecal contamination, the presence of any enterococci in water systems provides evidence of a potential pathway of faecal contamination. Multiple types of tests can be used; however, variability exists among these tests in their sensitivity for detection and quantification. It is important to use validated or standardized methods to make correct and timely public health decisions (Leclerc et al., 1996). Additional verification or confirmation steps may be required which will prolong the time required to complete the analyses (APHA et al., 2012).

B.3.1 Culture-based methods

Standard Methods for the Examination of Water and Wastewater lists methods for enumeration of enterococci via the membrane filtration (MF) technique, the multiple tube fermentation (MTF) technique and a fluorogenic substrate test (APHA et al., 2012). Membrane filtration methods are considered to represent the current "gold standard" for enterococci water quality assessments (Byappanahalli et al., 2012a). The MTF approach is recommended as an option when waters are highly turbid (APHA et al., 2012). Formulations of the fluorogenic substrate are available commercially for use in a MTF, multi-well or presence-absence format (APHA et al., 2012), ISO Method 7899-2 is the specified method of analysis for enterococci under the EU Council Directive (though member states can use alternative equivalent methods) (EU, 1998; ISO, 2000). A list of published standardized methods is provided in Table 1.

Table 1. Published standardized methods for the enumeration of enterococci in drinking water.

Organization and method	Media	Basis for detection	Enterococci detection criteria	Time to obtain results
Multiple tube fermentation (MTF)				
Standard Methods 9230 B	Azide dextrose broth and esculin azide agar (2-step method)	β-glucosidase enzyme	Turbid tubes producing black colonies with black-brown halos	48 h
Membrane filtration (MF)				
Standard Methods 9230 C	mE-EIA (2-step method)	β-glucosidase enzyme	Pink to red colonies with black-brown halo	48 h
	mEI (1-step method)	β-glucosidase enzyme	Colonies ≥ 0.5mm with blue halo	24 h
	mEnterococcus (1-step method)	TTC substrate dye metabolism	Light and dark red colonies	48 h
ISO 7899-2	mE-EIA (2-step method)	β-glucosidase enzyme	Pink to red colonies with black-brown halo	48 h
Fluorogenic substrate test (MTF, multi-well, presence-absence)				
Standard Methods 9230 D	Commercial fluorogenic substrate media (1 step method)	β-glucosidase enzyme	Fluorescence under UV light (366 nm)	24 h

Comparison studies between the commercial fluorogenic substrate test and different MF methods have been conducted in various water matrices (fresh and marine bathing waters, surface water, and treated drinking water). Correlation coefficients ranging from 0.68 to 0.93 have been reported (Fricker and Fricker, 1996; Abbot et al., 1998; Eckner, 1998; Kinzelman et al., 2003). In some studies the commercial test was shown to be of similar or greater sensitivity (Fricker and Fricker, 1996; Eckner, 1998; Kinzelman et al., 2003); whereas in others the MF methods were shown to be more sensitive (Adcock and Saint, 2001; Heiber et al., 1998; Maheux et al., 2009).

A study compared the ability of three β-glucosidase-based commercially available test methods to detect 110 different *Enterococcus* strains from diverse origins (Maheux et al., 2009). Detection performances reported for a commercial fluorogenic substrate method and two membrane filtration methods were 68.3%, 83.2% and 88.1% respectively. With strains of the primary faecal species *E. faecalis* and *E. faecium*, detection performance for all three methods was close to 90% or greater (Maheux et al., 2009). In a comparative analysis of these same three methods for the detection of enterococci in well water samples collected in the Quebec City region, rates of detection reported were 3.0%, 5.5% and 11.5% respectively (Maheux et al., 2012).

B.3.2 Molecular methods

Molecular methods for the detection of enterococci in natural waters have been developed by the United States Environmental Protection Agency (U.S. EPA) but are not currently approved. The U.S. EPA (2015a, 2015b) has validated two quantitative polymerase chain reaction (qPCR) methods for detecting DNA from enterococci bacteria in natural waters: Method 1609.1 and Method 1611.1.

Molecular methods for drinking water are under development but have not been approved for drinking water compliance monitoring. The most significant challenge associated with drinking water analysis is the stricter limit for indicator organism presence, and therefore the need for method sensitivity at very low concentrations. More work is needed in this area to develop standardized methods that can be used accurately, reliably and affordably. A molecular method for the detection of enterococci has been developed by Maheux et al. (2011) that combines microbial particle concentration and recovery, whole genome amplification and real-time PCR detection of ribosomal RNA gene targets. The authors reported detection of as few as 4.5 enterococci cells per 100 mL in less than 5 h with this method and further stated it should theoretically be able to detect 1 CFU of enterococci per 100 mL. Another molecular method has been described by Pitkanen et al. (2013), in which ribosomal RNA is used as the qPCR target used instead of the ribosomal RNA gene. Further validation is needed, but the study data suggest advantages of increased detection sensitivity and the ability to detect viable enterococci when compared to DNA-based qPCR assays (Pitkanen et al., 2013).

Current PCR methods using ribosomal RNA gene targets are capable of detecting the *Enterococcus* genus, but are not able to distinguish individual species (Ryu et al., 2013). Park et al. (2016) described a method using multiple species-specific primers that is capable of identifying several *Enterococcus* species.

With all of these methods, additional studies are needed to confirm the results and the acceptability of the procedures to assess water quality.

B.3.3 Sampling for enterococci

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., 2012). Ideally, the interval between collection of the sample and the beginning of its examination should not exceed 24 hours (Bartram and Rees, 2000), and analysis within 8 hours is recognized as the preferred time interval (Bartram and Rees, 2000; APHA et al., 2012). In remote areas, up to 48 hours may be an acceptable time interval; however, the implications of the extended holding time should be discussed with the responsible authorities. When delays are anticipated, onsite testing (e.g., with commercialized test methods) may be considered. Utilities should first consult with authorities about the acceptability of this practice. A minimum volume of 100 mL of water should be examined to obtain a reliable estimate of the number of organisms (using MTF, MF or presence-absence) at the expected low levels in treated drinking water. Examination of larger volumes, such as in groundwater with very low levels of contamination, can increase both the sensitivity and the reliability of the test. Smaller volumes, dilutions, or other MTF combinations may be more appropriate for waters of poor quality.

When determining sampling frequency requirements for municipal scale systems, the application of a universal sampling formula is made impossible by basic differences in factors such as source water quality, adequacy and capacity of treatment, and size and complexity of the distribution system (WHO, 1971, 1976, 2004). Instead, the sampling frequency and location of sampling points should be determined by the responsible authority after due consideration of local conditions—for example, variations in raw water quality and a history of the treated water quality. The sampling frequency should meet all jurisdictional requirements.

The number of samples for enterococci testing can also vary depending on the expected goals of the monitoring strategy. If the monitoring goal is routine faecal indicator testing to verify the adequacy of drinking water treatment and the microbial condition of the distribution system, sampling at a frequency similar to that used for other microbiological indicators such as *E. coli* and total coliforms is recommended. Further information on the role of *E. coli* and total coliforms in drinking water quality management can be found in the guideline technical documents on *E. coli* and total coliforms (Health Canada, 2012b, 2012c).

If the monitoring goal is for periodic inspections of drinking water systems, sampling and testing for enterococci can be conducted less frequently. As an example, the EU drinking water directive specifies minimum sampling frequency requirements for enterococci when used as an audit parameter to periodically determine a system's compliance. Minimum sampling frequencies are based on the volume of water distributed or produced by a water system per day, with the number of samples increasing in accordance with the size of production. Specified frequencies range from 1 sample per year for supplies producing >100 to ≤ 1000 m^3 per day to >10 samples per year for supplies producing > 100,000 m^3 per day (EU, 1998).

There are limitations that need to be considered when interpreting the sampling results. Simulation studies have shown that it is very difficult to detect a contamination event in a distribution system unless the contamination occurs in a main, in a reservoir, at the treatment plant, or is of long duration at a high concentration (Speight et al., 2004; van Lieverloo, 2007). In addition, with microbiological indicator testing, the low rate of positive samples means that it can be difficult to see statistically significant differences in positive rates, for example, before and after a corrective action, unless a very large number of samples are evaluated (Rosen et al., 2009). Hargy et al. (2010) demonstrated that large sample volumes (20L) were useful in improving the detection of total coliforms compared to 100 mL samples. The results suggested that sample volume (and not water quality) was a better indicator of the detected presence of total coliforms. There are limited demonstrations evaluating effective monitoring strategies, and additional statistical and field work are needed that simultaneously consider the parameters of

sample volume, monitoring frequency, detection method, false/true positives and negatives, and cost. These limitations highlight the importance of implementing a source-to-tap multi-barrier approach or water safety plan approach for determining the microbiological quality of the drinking water.

The usefulness of including enterococci monitoring as part of a multi-barrier approach for a drinking water system should be determined by the responsible authority. For small systems, additional guidance may also need to be considered. Smaller water supplies may have more deficiencies, but also more limited resources available for monitoring. Emphasis should also be placed on identified problems based on source-to-tap assessments, including sanitary surveys.

B.4 Treatment technology

The primary goal of treatment is to reduce the presence of disease-causing organisms and associated health risks to an acceptable or safe level.

There has been limited data published on the effectiveness of various drinking water treatment and disinfection technologies specific to the removal and inactivation of enterococci. Overall, physical removal methods (including coagulation, flocculation, sedimentation, slow or rapid sand filtration and direct filtration with or without filtration aid) can accomplish 1–4 log removal of indicator organisms (*E. coli*, coliforms, enterococci) (Payment et al., 1985; Smeets et al., 2006). Membrane filtration technologies are also capable of removing 4 log to greater than 6 log of bacteria (NSF, 2002; Smeets et al., 2006). Disinfectants commonly used in the drinking water industry, such as chlorine, chloramine, chlorine dioxide, ozone, and UV light, are known to be effective against the enterococci group. Each of the above agents has demonstrated the ability to produce greater than a 4 log inactivation of enterococci in laboratory-scale experiments.

Scientific studies have provided some evidence of the enterococci group being more resistant to inactivation with chlorine, monochloramine and UV light as compared to *E. coli*; however the limited data makes it difficult to draw strong conclusions (Chang et al., 1985; Harris et al., 1987; Rice et al., 1993). Still, it is generally accepted that enterococci responses to disinfection are of the same order of magnitude (Hijnen et al., 2011). Overall, the evidence shows that enterococci are much more sensitive to chlorination than the enteric protozoans *Giardia* and *Cryptosporidium*, and more sensitive to UV inactivation than certain enteric viruses. Therefore, water that is treated to meet the guidelines for enteric viruses and enteric protozoa should have an acceptable bacteriological quality, including achieving enterococci concentrations of none detectable per 100 mL of water leaving the treatment plant. Further information on inactivation of specific protozoan and viral pathogens can be found in the guideline technical documents on protozoa and enteric viruses (Health Canada, 2011, 2012a).

In distribution systems, a disinfection residual is needed to protect against bacterial regrowth and serve as a sentinel for water quality changes. Regular monitoring of distribution system water quality (e.g., microbial indicators, disinfectant residual, turbidity, pH) and having operations and maintenance programs in place (water mains cleaning, cross-connection control, replacements and repairs) are important for ensuring drinking water is distributed to consumers with minimum loss of quality (Kirmeyer et al., 2001, 2014).

Residential-scale treatment is also applicable to small drinking water systems. Surface water is not recommended as a residential-scale water supply unless it is properly filtered and disinfected and monitored for water quality. Well water supplies can also be contaminated and may require treatment. Various options are available for treating source waters to provide high-

quality pathogen-free drinking water. These include systems that rely on chlorine, UV light or filtration. Health Canada does not recommend specific brands of residential-scale drinking water treatment devices, but it strongly recommends that consumers look for a mark or label indicating that the device has been certified by an accredited certification body as meeting the appropriate NSF International (NSF)/American National Standards Institute (ANSI) standard. These standards have been designed to safeguard drinking water by helping to ensure the safety of material and performance of products that come into contact with drinking water. Treatment devices should be inspected and serviced in accordance with the maintenance schedule and manufacturer's recommendations.

B.5 International considerations

Drinking water quality guidelines, standards and/or guidance established by foreign governments or international agencies may vary due to the science available at the time of assessment, as well as the utilization of different policies and approaches.

The European Union (EU) Drinking Water Directive has included enterococci (originally faecal streptococci) as a microbiological drinking water parameter since 1980 (EU, 1980). The current EU Drinking water directive outlines the legislative requirements for all its Member States (EU, 1998). Under the Directive, enterococci are categorized as a parameter for audit monitoring – to provide information as to whether or not the parametric values are being complied with. These are tested less frequently than check (i.e., routine monitoring) parameters. The EU standard for enterococci is a value of zero per 100mL (EU, 1998).

The WHO Guidelines for Drinking Water Quality, Fourth edition (WHO, 2011) contain fact sheets on numerous microbial indicators, including intestinal enterococci. The guidance from WHO specifies that the intestinal enterococci group can be used as an index of faecal pollution. The presence of the group provides evidence of recent faecal contamination, and detection of intestinal enterococci should lead to consideration of further action. No guideline values are specified.

The Australian Drinking Water Guidelines provide fact sheets on numerous microbial indicators, including intestinal enterococci, but no guideline values are specified. It is discussed that for application in practice, enterococci can be used to assess source water quality, the adequacy of treatment, post-treatment ingress into the distribution system and the delivery of safe drinking water at consumer taps (NHMRC, 2011).

The U.S. EPA Ground Water Rule (U.S. EPA, 2006) lists enterococci as one of three state-specified bacterial indicators of faecal contamination, alongside *E. coli* and coliphages. Under the rule, untreated ground water systems having a total coliform-positive sample must collect at least one sample from each ground water source and test for one of the state-specified indicators (referred to as triggered source water monitoring). Should a triggered sample test positive for the faecal indicator, the system must notify state authorities and the public and take corrective actions.

B.6 Research gaps

There has been a wealth of important research conducted that has advanced our knowledge of the *Enterococcus* genus and its applicability as an indicator of faecal contamination. Despite this, there are numerous gaps in the information that still need to be filled.

The value of enterococci as indicator organisms, their comparative or enhancing value to *E. coli* and total coliforms monitoring programs could be explored through pilot-scale monitoring programs and focused laboratory-based studies. Any exploration of the practical use of enterococci as an indicator by regulators could be enriched by partnership with university researchers who could provide the expertise for focused treatment, enumeration and characterization studies.

Regulators or utilities may wish to evaluate the potential of enterococci as primary indicator organisms through a comparison study with existing indicators (e.g., total coliforms and *E. coli*). A pilot-study could involve split sampling of current regulatory samples (total coliform and *E. coli*) followed by evaluation at a certified laboratory. Samples should be taken over the course of the year to observe seasonal variation and from multiple regions to ensure geographical diversity. Samples from raw and treated water from both ground and surface water should be taken and include a variety of representative water quality types. A sufficient number of samples should be taken to provide meaningful statistical interpretation of the data.

Although there have been several informative studies on enterococci survival under various treatment conditions, there is still a need for focused studies that compare the relative survival rates for *E. coli* and enterococci under various drinking water treatment technologies with a variety of water qualities.

The performance of the recommended culture based methods for drinking water samples in various Canadian water quality types should also be evaluated. Further, the continued development of rapid and easy molecular methods for enterococci detection under a variety of water quality conditions would also be useful.

Other important areas for future research involve improving our understanding of: the *Enterococcus* genome and its functional and taxonomic aspects; the identity and diversity of *Enterococcus* species found in various drinking water supplies; how enterococci could inform microbial risk assessment, and; the extent to which the non-enteric environment can serve as sources of enterococci.

Part C. References and acronyms

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C.2 List of acronyms

ANSI	American National Standards Institute
CFU	colony forming units
DNA	deoxyribonucleic acid
EU	European Union
ISO	International Organization for Standardization
MF	membrane filtration
MTF	multiple tube fermentation
NSF	NSF International
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
U.S. EPA	United States Environmental Protection Agency
UV	ultraviolet
VBNC	viable but non-culturable
WHO	World Health Organization