



GUIDELINES FOR
CANADIAN
RECREATIONAL
WATER
QUALITY

**MICROBIOLOGICAL
SAMPLING AND ANALYSIS**

Guideline Technical Document



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Échantillonnage et analyse microbiologiques – Document technique*

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MICROBIOLOGICAL SAMPLING AND ANALYSIS

Foreword

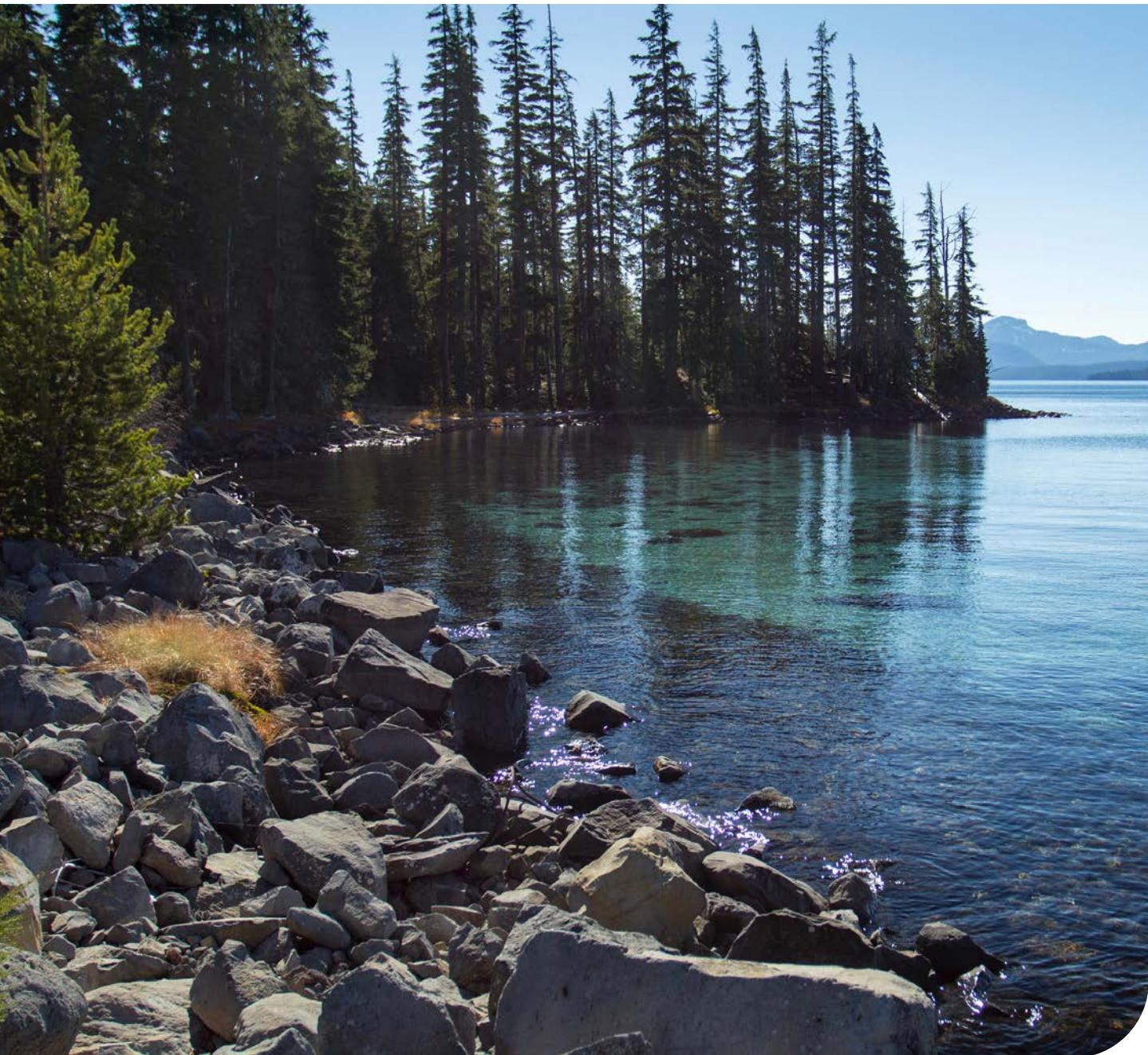
The Guidelines for Canadian Recreational Water Quality consist of multiple guideline technical documents that consider the various factors that could interfere with the safety of recreational waters from a human health perspective. This includes technical documents on understanding and managing risks in recreational waters; fecal indicator organisms; microbiological sampling and analysis; cyanobacteria and their toxins; physical, aesthetic and chemical characteristics; and microbiological pathogens and other biological hazards. These documents provide guideline values for specific parameters used to monitor water quality hazards and recommend science-based risk management strategies.

Recreational waters are any natural fresh, marine or estuarine bodies of water that are used for recreational purposes; this includes lakes, rivers and human-made systems (such as stormwater ponds and artificial lakes) that are filled with untreated natural waters. Jurisdictions may choose to apply these guidelines to other natural waters for which limited treatment is applied (for example, short-term use of disinfection for an athletic event). Applying the guidelines in these scenarios should be done with caution. Some disease-causing microorganisms (such as protozoan pathogens) are more difficult to disinfect than fecal indicator organisms and may still be present even if disinfection has reduced the fecal indicators to acceptable levels.

Recreational water activities that could present a human health risk through intentional or incidental immersion and ingestion include primary contact activities (such as swimming, wading, windsurfing and waterskiing) and secondary contact activities (such as canoeing, boating and fishing).

Each guideline technical document has been established based on current, published scientific research related to health effects, aesthetics and beach management considerations. The responsibility for recreational water quality generally falls under provincial and territorial jurisdiction, so the policies and approaches, as well as the resulting management decisions, may vary between jurisdictions. The guideline technical documents are intended to inform decisions by provincial, territorial and local authorities that are responsible for the management of recreational waters.

This document includes information on sampling and analysis for microbiological parameters. For a complete list of the guideline technical documents available, please refer to the Guidelines for Canadian Recreational Water Quality summary document available on the [Canada.ca](https://www.canada.ca) website (Health Canada, 2024).





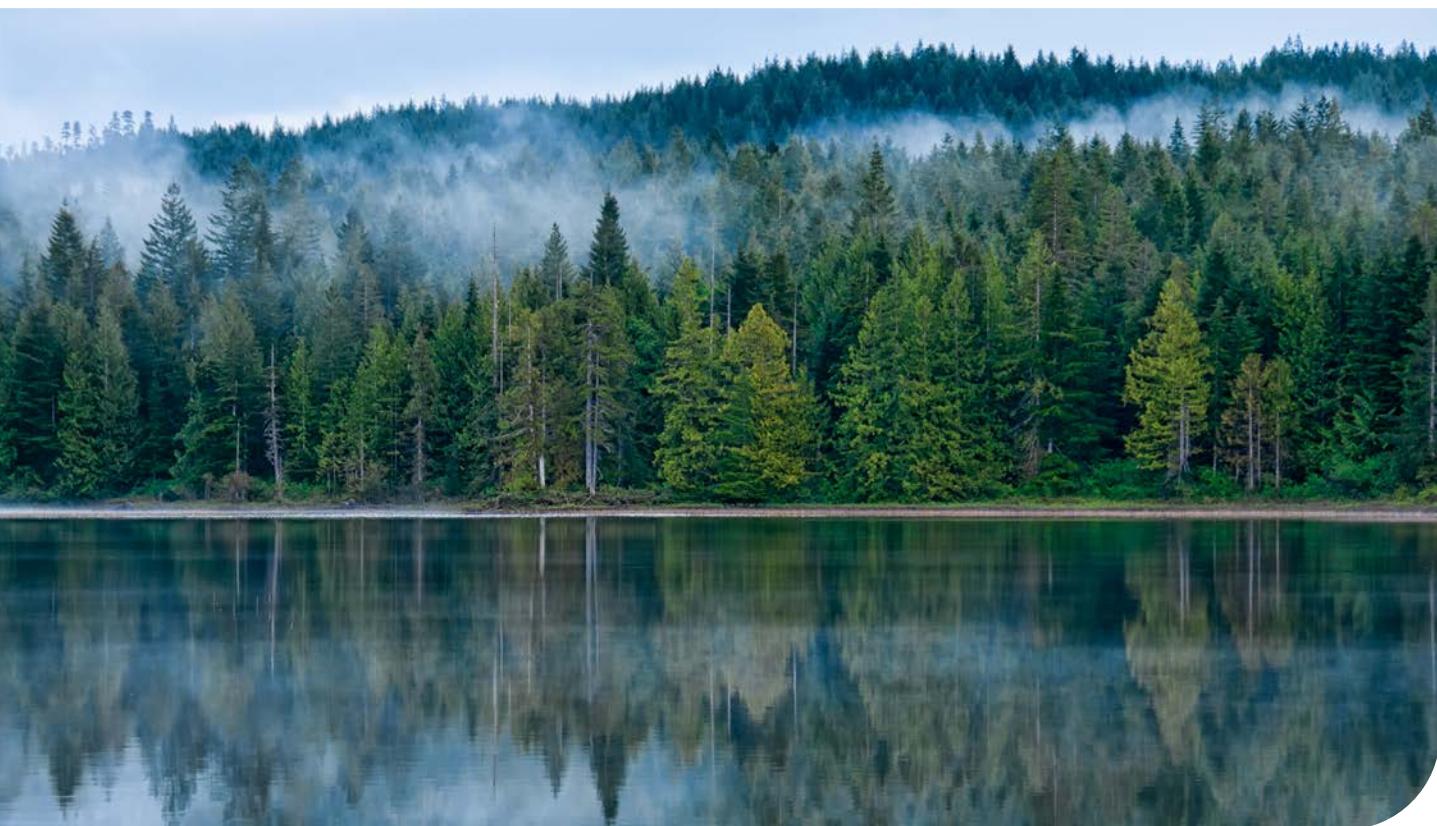
1.0 OVERVIEW OF MICROBIOLOGICAL SAMPLING AND ANALYSIS

Monitoring of recreational water quality is an important component of a preventive risk management approach. Its main functions are to characterize water quality, confirm the effectiveness of risk management activities already in place and guide future mitigation actions. Having a well-structured and well-planned monitoring program for recreational water areas is essential for managers and authorities to assess risks, inform public health decisions and communicate water quality information to the public. The priority microbiological hazards for most areas are fecal wastes that are introduced into the water by humans and animals, and harmful cyanobacterial blooms.

Routine water sampling and analysis for the primary indicators of fecal contamination—*Escherichia coli* (*E. coli*, fresh water) and enterococci (marine and fresh water)—is used to inform day-to-day management decisions (such as the issuing of swimming advisories) and for determining the overall suitability of an area for recreational water use. Additional data collected during on-site investigations (for example, environmental conditions, area activities) provide support for interpreting and predicting water quality results. Other fecal indicators can be included in monitoring programs or used in source-tracking studies to provide further information on the sources of fecal contamination that helps refine the understanding of health risks. Sampling and analysis of sand and sediments may also be conducted during investigations, as it is known that these matrices can harbour microorganisms, including fecal indicators and pathogens, from fecal matter and other environmental sources. The use of standard procedures for the collection, transport and analysis of samples is critical to obtain the most accurate assessment of water quality. Standardized culture-based and polymerase-chain-reaction-(PCR) based methods for the quantification of fecal indicators are available. However, the choice of analytical methods will depend on factors such as monitoring program requirements, laboratory capability and capacity, beach-specific considerations (such as source water characteristics) and jurisdictional requirements.

Pathogenic microorganisms (that is, pathogenic bacteria, protozoa, viruses and fungi) may be present in recreational waters. Routine monitoring for these microorganisms is not recommended due to the complexity and costs associated with analysis (see section 3.3). Testing for specific pathogens may be conducted for investigative purposes—for example, in response to an outbreak of waterborne illness. Primary indicators of fecal contamination are used to show the potential presence of fecal pathogens (WHO, 2000b; Hussain, 2019). Microbial source tracking information can also aid in interpreting fecal indicator results (Schoen et al., 2011; Alberta Health, 2022).

Cyanobacteria blooms are also a significant hazard for some recreational water bodies. Information on parameters and monitoring methods used in cyanobacterial management plans can be found in the technical documents on cyanobacteria and their toxins developed for the Guidelines for Canadian Recreational Water Quality (Health Canada, 2022) and the Guidelines for Canadian Drinking Water Quality (Health Canada, 2017). Different methods and techniques are used to collect samples for cyanobacterial identification or for toxin analysis. For detailed information, refer to Water Quality Research Australia (2009), APHA et al. (2023), and Welker and Raymond (2021).





2.0 MICROBIOLOGICAL SAMPLING

Accurately describing the water quality at sites within the recreational water area (for example, areas of peak user density, areas adjacent to known or potential sources of fecal pollution) is the goal of routine microbiological sampling. Multiple factors can influence the temporal and spatial variability of fecal indicator bacteria at beaches. These include climatic and environmental factors (such as storm events, temperature and area hydrodynamics), sources of fecal indicators (such as fecal pollution, beach sand and sediments), bather activities and site features that can prevent or promote mixing (for example, breakwaters, jetties) (U.S. EPA, 2010). Each beach has its own unique characteristics depending on the factors that predominate at any given time. Beach monitoring plans should therefore be designed considering specific knowledge of the beach area and the variability in water quality that can be encountered at that location. Guidance on the development of monitoring programs for beaches can be found in the *Guidelines for Canadian Recreational Water Quality—Understanding and Managing Risks in Recreational Waters* technical document (Health Canada, 2023b).

When sampling, all collection procedures and laboratory analyses should be carried out as directed by the responsible authority. It is important to use validated or standardized methods to support informed and timely public health decisions. Beach managers should consult with the responsible authority to confirm any specific requirements, such as the use of accredited laboratories for sample analysis. When purchasing laboratory services, beach managers should consult with the analytical laboratory on issues such as method performance; quality assurance/quality control programs; chain of custody needs; requirements for the collection, labelling, transport and holding time of samples; and the turnaround time for results.

Sterile sample containers (such as sterile glass or plastic bottles and presterilized plastic bags with wire closures) should be prepared and provided by the analytical laboratory. The sample volume should accommodate the full complement of tests required and reanalysis, if necessary. Bottles capable of holding volumes of 250 mL–500 mL should be adequate for most routine water analyses (WHO, 2000a; Robertson et al., 2003). Collection of greater volumes of water (for example, 1 L, 10 L or more) may be necessary under certain circumstances, such as during targeted testing for waterborne pathogens or alternative indicators (WHO, 2000a; U.S. EPA, 2018).

Laboratories should provide the sample submission/chain of custody forms necessary to accompany the samples and should identify the requirements for completing the forms and labelling samples. This may include instructions about using waterproof pens and labels, pre-labelling containers prior to collection to prevent errors, and how to avoid damaging the forms and labels during transport to the laboratory.

Regardless of the method used, the use of sterilized equipment and aseptic technique is of utmost importance in minimizing the risk of accidental contamination of the sample.

2.1 Sample collection—water

The beach monitoring plan developed by the responsible authority should identify instructions for the location, time of day and frequency of sample collection. Detailed instructions on sample collection are available elsewhere (U.S. EPA, 1997; APHA et al., 2023; ISO, 2021b). Some basic aspects are discussed here.

Where currents are present, samples should be obtained from the upstream side of the sampler, boat or other platforms. When sampling in the surf zone, samples should be collected during incoming waves or surges of water. Disturbances of foreshore sand and sediments can resuspend fecal indicator microorganisms, inflating microbiological estimates. Actions should be taken to minimize the collection of suspended sands and sediments. For example, when sampling in calm waters, samplers should avoid kicking up bottom sediments while moving to the sampling location or wait until the sediment has settled. When collecting samples, samplers should wear clean, disposable gloves or have clean, sanitized hands.

Sample containers should be kept closed until just before collection. Care should be taken at all times to avoid touching the inside of the bottle or bottle cap, the bottle mouth and neck or the inside of plastic bags with hands or other surfaces. When collecting a sample, the bottle cap or perforated plastic bag closure is removed and the bottle or bag is lowered into the water with the opening pointed downward and away from the sampler. At the appropriate depth, the bottle is turned upwards into the current or the bag is opened and allowed to fill, ensuring that the surface of the water is not collected. An air space of at least 2.5 cm should be left to allow for proper mixing before analysis; a small portion of water can be poured out, if necessary. The bottle is recapped or the bag is closed and sealed using the closures.



The distance below the surface where the water sample is taken from is less critical than the overall depth of the water or the “depth zone” where sampling is conducted (U.S. EPA, 2010). Water samples collected in shallow depth zones (such as ankle or knee depth) will often have higher numbers of fecal indicator bacteria than those collected in deeper depth zones (such as chest depth) (Edge and Hill, 2007; Edge et al., 2010; U.S. EPA, 2010). Responsible authorities should identify the required sampling depth zone, and water samples should be consistently collected at this required depth zone.

Sampling 15 cm to 30 cm below the surface of the water provides an adequate measure of water quality and human exposure (U.S. EPA, 2005c, 2010) and can limit collection of debris floating along the surface that might interfere with testing. Standard methods (APHA et al., 2023) recommend sample collection at 30 cm below the surface. Recreational monitoring and method valuation studies conducted by the United States Environmental Protection Agency (U.S. EPA) also utilized a 30 cm distance below the surface of the water (Haugland et al., 2005; U.S. EPA, 2005c).

2.1.1 Additional data

Collecting additional data at the time of sampling can be valuable for identifying beach-specific factors that influence water quality and for predicting when water quality targets may be exceeded. Common parameters include wind direction and speed, wave height, turbidity, water temperature, antecedent rainfall and nearby elements that can affect water quality (for example, animal counts, bather density) (Francy et al., 2013a; U.S. EPA, 2016). Environmental Health and Safety Surveys (EHSS), the equivalent of the U.S. EPA’s beach sanitary surveys, provide a vehicle for collecting consistent ancillary data, as well as pre- and post-season data review to guide future actions (Health Canada, 2023b). EHSS data can be further utilized to develop and test water quality predictive models (U.S. EPA, 2008; Kinzelman et al., 2012; Health Canada, 2023b). Literature on the development and use of predictive modelling tools and free modelling software are available (Francy et al., 2013a; U.S. EPA, 2016, 2022).

2.1.2 Composite sampling

Composite sampling is an alternative approach to traditional sampling that provides a way to assess the water quality at multiple points along a beach without increasing the number of laboratory analyses required, potentially minimizing the associated costs.

When contemplating integrating a composite sampling technique into a beach monitoring program, it is important that beach managers understand the variability in concentrations of fecal indicator bacteria at different locations along the intended stretch of beach. Beaches can have significant spatial variability in fecal pollution sources and indicator bacteria concentrations (Edge et al., 2010). Hotspots (sampling locations with persistently high fecal indicator bacteria concentrations) are problematic for composite sampling strategies (Kinzelman et al., 2006; Edge et al., 2010). Poor water quality results from these areas can be masked when a sample is combined into a composite (Kinzelman et al., 2006). As a result, samples from a hotspot should not be composited with other samples (Kinzelman et al., 2006).

Beach managers should also understand how composite sampling analysis differs from the analysis of individual samples. Composite sampling adds an extra layer of uncertainty to the process of estimating water quality: a combined sample is estimating the average indicator density over all samples, and this estimate is used to characterize the water quality for the whole beach. This extra step has the potential to introduce error to the final water quality results. Increasing the number of samples that form the composite can offset the additional uncertainty and improve the precision of the results (U.S. EPA, 2005c, 2010).

A composite sample produces an estimate of an arithmetic mean as opposed to the geometric mean that is commonly used in recreational water quality trend assessments. The U.S. EPA's environmental monitoring for public access and community tracking (EMPACT) report (U.S. EPA, 2005c) outlines the differences between arithmetic and geometric means. The authors note that, according to mathematical principles, the arithmetic mean will always be equal to or greater than the geometric mean (U.S. EPA, 2005c). Additionally, in log-normal data distributions, the arithmetic mean and the geometric mean are related, and the latter can be estimated when the variance of indicator densities is known (U.S. EPA, 2005c). The results of composite samples can be used as a conservative estimate of water quality or they can be converted to an estimate of the geometric mean (provided the variance is known) (U.S. EPA, 2010).

The feasibility of composite sampling at a given beach can be determined by spatial distribution studies, by examination of historical microbiological data and from information on beach characteristics provided by an EHSS (Health Canada, 2023b). If a composite sampling technique is being considered, beach managers should contact the responsible authority to discuss any requirements that may apply. Consultation with laboratories about services for preparing and analyzing composite samples is also advised. Further details on composite sampling and its application in recreational water monitoring programs can be found elsewhere (Kinzelman et al., 2006; Bertke, 2007; Reicherts and Emerson, 2010; U.S. EPA, 2005c, 2010).



2.2 Sample collection—sand and sediments

There are no accepted standard methods for the collection of beach sand samples for the enumeration of microorganisms. However, research methods are described in the scientific literature that have been successfully used for sample collection. As sand is heterogeneous in nature, samples should be collected at numerous representative locations, including potential hotspots, to best characterize the beach (Brandão, 2019). The World Health Organization (WHO) recommends using sterile sampling spoons (or core samplers) to collect several shallow aliquots from the surface in the target area (up to 10 cm deep) (WHO, 2021). Other published methods describe the use of sterilized sampling spoons, shovels and corers, probes or similar sterilized devices for sampling the sand and pore water at beaches (Vogel et al., 2017). Beach-specific characteristics can affect the results when using different collection methods (Vogel et al., 2017). Responsible authorities and beach managers should consult the literature to determine which methods may be most suitable for their needs. For a review of methods used in research settings, refer to Vogel et al. (2017). Best practices for sample collection can be found in standardized or other published methods relating to the collection, preservation and handling of water, sediment or soil samples (APHA et al., 2023; Wollum, 2018; ISO, 2021b).

Shaking by hand, sonication and blenders have been used to elute fecal indicator bacteria from sand particles for enumeration (Whitman and Nevers, 2003; Kinzelman et al., 2004; Boehm et al., 2009). The WHO (2021) recommends extraction of microorganisms from beach sand as described in Boehm et al. (2009).

For guidance specific to the sampling of sediments, standard methods are available (APHA et al., 2023; ISO, 2021b).

2.3 Sample transport, preservation and storage

Once collected, samples should be kept cool but unfrozen (that is, 4°C to 10°C) and in the absence of light until the time of analysis (APHA et al., 2023; U.S. EPA, 2021). Insulated coolers containing frozen ice packs may be used to transport samples to the laboratory. Samples should be protected from direct contact with ice packs and arranged to prevent tipping. Once at the laboratory, samples should be stored in the refrigerator and analyzed as soon as possible upon receipt (APHA et al., 2023).

2.3.1 Holding time limitations

For samples analyzed using culture-based methods, the preferred holding time from collection to the completion of analysis is less than 8 hours (APHA et al., 2023; U.S. EPA, 2021). Guidance documents on water quality assessment recommend a maximum holding time of 24 hours (WHO, 2000a; Payment et al., 2003). Although limited data exists on changes in microbial concentration due to storage (Pope et al., 2003; Thapa et al., 2020), the implications of a long holding time should be discussed with the analytical laboratory. Specifically, it is important to consider the likelihood or impact of inaccurate results when using culture-based methods of analysis due to declines in the fecal bacterial indicator count that can occur during extended storage. This should be weighed against the impact of samples not being submitted at all if a beach manager is unable to have samples delivered within the holding time limits. If samples cannot be analyzed within the required holding time, field testing with commercialized test methods or the use of delayed incubation procedures should be considered (APHA et al., 2023).

When using the U.S. EPA's quantitative polymerase-chain-reaction (qPCR) methods (see section 3.1.2), samples should not be held longer than 6 hours between collection and initiation of analyses (U.S. EPA, 2015a, 2015b). Adhering to the holding time limits is necessary for the production of valid data (U.S. EPA, 2015a, 2015b) and to gain the maximum benefit of the more rapid qPCR method. If samples cannot be delivered to meet the holding time limits, alternative approaches for improving the timeliness of water quality decisions can be considered, such as predictive models (Health Canada, 2023b) or automated enzymatic methods—see section 3.1.3.



3.0 METHODS FOR MICROBIOLOGICAL ANALYSIS

3.1 Recommended indicators of fecal contamination

3.1.1 Culture-based methods

Standardized and U.S. EPA-approved methods available for the detection of *E. coli* and enterococci in recreational water are summarized in Table 1. Authorities may approve other methods for use in their jurisdictions (Public Health Ontario, 2022; CEAEQ, 2023). The two main culture-based approaches used for the enumeration of fecal indicator bacteria are the most probable number (MPN) technique and the membrane filtration (MF) technique. The MPN technique involves dilution or partitioning of the sample into tubes or wells. The samples are then incubated and MPN statistics are used to calculate the number of bacteria in the sample based on the number of partitions giving positive test reactions. With the MF technique, samples are filtered, the filter is placed on a diagnostic growth medium containing differential and selective agents and, after incubation, the number of resulting target colonies are counted.

Table 1. Standardized and U.S. EPA-approved culture-based methods for the detection of *E. coli* and enterococci in recreational water

| Method | Media | Format | Turnaround time | U.S. EPA-approved method ^b |
|--|---------------------------------|---------|-----------------|---------------------------------------|
| <i>E. coli</i> | | | | |
| SM 9223 B ^a ISO 9308-2:2012 ^b | Colilert-18 [®] medium | DST-MPN | 18 h | Y |
| SM 9223 B ^a | Colilert [®] medium | DST-MPN | 24 h | Y |
| SM 9223 B ^a | Colisure [®] medium | DST-MPN | 24 h | N |
| ISO 9308-3 ^b | MUG/EC medium | MTF-MPN | 36–72 h | N |
| SM 9213D ^a U.S. EPA 1103.1 ^c | mTEC agar (2-step) | MF | 24 h | Y |
| SM 9213D ^a U.S. EPA 1603 ^c | Modified mTEC agar | MF | 24 h | Y |
| U.S. EPA 1604 ^c | MI Medium | MF | 24 h | Y |

| Method | Media | Format | Turnaround time | U.S. EPA-approved method ^b |
|--|---|---------|----------------------|---------------------------------------|
| E. coli | | | | |
| U.S. EPA—N/A ^{c,d} | KwikCount EC medium | MF | 8–10 h | Y |
| U.S. EPA—N/A ^c | m-ColiBlue24® broth | MF | 24 h | Y |
| Enterococci | | | | |
| SM 9230 D ^a | Enterolert® medium | DST-MPN | 24 h | Y |
| ISO 7899–1:2000 ^b | MUD-SF media | MTF-MPN | 36–72 h | N |
| SM 9230 B ^a | Azide dextrose broth-bile esculin azide agar (2-step) | MTF-MPN | 48–72 h ^e | Y |
| SM 9230 C ^a U.S. EPA 1600 ^c | mEl agar | MF | 24 h | Y |
| ISO 7899–2:2000 ^b | Slanetz and Bartley agar-bile esculin azide agar (2-step) | MF | 46 h | N |
| SM 9230 C ^a U.S. EPA 1106.1 ^c | mE agar-EIA media (2-step) | MF | 48 h | Y |
| SM 9230 C ^a | mEnterococcus agar | MF | 48 h | Y |

^a APHA et al., 2023; ^bU.S. EPA, 2021; ^cISO, 2021a; ^dApproved for freshwater only; ^eConfirmed enterococci and/or fecal streptococci

SM—standard methods; EC—*Escherichia coli*; DST—defined substrate technology; MF—membrane filtration; MTF—multiple tube fermentation; MPN—most probable number



Most MPN and MF methods are based on the detection of specific enzymes considered to be characteristic of the target microorganisms. Chromogenic or fluorogenic substrates are incorporated into the growth media. When these are metabolized by the target microorganism, they confer a unique property (such as the presence of a colour or fluorescence) to the developing colony or surrounding media that is diagnostic. Some commercial methods use defined substrate technology, a reagent system where the substrate also serves as the principal source of carbon and energy for the target bacteria.

There are several advantages and disadvantages associated with the use of culture-based methods (see Table 2).

Table 2. Advantages and disadvantages of culture-based methods

| Method | References |
|---|---|
| Advantages | |
| Easy-to-perform methods that require basic bacteriology laboratory facilities and trained personnel. | WHO, 2000a, 2000b; Köster et al., 2003; Payment et al., 2003 |
| Provide an acceptable measure of microorganism viability. | WHO, 2000a; Köster et al., 2003; Payment et al., 2003 |
| Have demonstrated an association with gastrointestinal illness at beaches impacted by human fecal contamination. | <i>E. coli</i> : Dufour, 1984; U.S. EPA, 1986; Wiedenmann et al., 2006; Marion et al., 2010 <i>Enterococci</i> : Cabelli, 1983; Wiedenmann et al., 2006; Wade et al., 2006, 2008, 2010 |
| Less costly than PCR methods (start-up equipment, testing costs). | Shrestha and Dorevitch, 2020; Saleem et al., 2022 |
| Disadvantages | |
| Do not detect microorganisms in the viable but non-culturable state, potentially underestimating microorganism concentrations. | Del Mar Lleó et al., 1998; Zimmerman et al., 2009 |
| Time lapse between sample collection and results availability (> 18 hours) delays management decisions; results may no longer reflect current water quality. | Whitman et al., 1999; U.S. EPA, 2005c; Boehm, 2007; Nevers and Whitman, 2010 |
| Enzyme-based detection methods are less specific than molecular methods that detect ribosomal deoxyribonucleic acid (DNA) or ribonucleic acid (rRNA) targets. | Zhang et al., 2015; Maheux et al., 2017 |
| Interference from turbid samples and overgrowth of non-target microorganisms can lead to underestimation of target. | Kinzelman et al., 2003; Sercu et al., 2011; Nevers et al., 2014 |

Enterococci are the preferred indicator of fecal contamination for marine waters, whereas either *E. coli* or enterococci can be used for testing in fresh waters. Correlations between *E. coli* and enterococci concentrations by culture-based methods can vary from location to location (Kinzelman et al., 2003; Francy et al., 2013b; Nevers et al., 2013). Studies measuring both indicators at beaches have demonstrated the potential for more exceedances of the U.S. EPA's recommended single sample maximum limit or beach action value (BAV) for enterococci (61 colony-forming unit (cfu)/100 mL or 70 cfu/100 mL, respectively) compared with the U.S. EPA's recommended *E. coli* beach action value of 235 cfu/100 mL (Kinzelman et al., 2003; Dorevitch et al., 2011; Francy et al., 2013b; Nevers et al., 2013; Byappanahalli et al., 2018; Campbell and Kleinheinz, 2020). Beach managers that consider changing the fecal indicator that is routinely monitored should conduct side-by-side testing of the current and proposed indicators prior to implementation.

3.1.2 Polymerase-chain-reaction (PCR) based methods

There is growing interest in the use of PCR-based methods to analyze fecal indicator bacteria in recreational waters. Testing using this molecular approach requires specialized equipment and highly trained personnel. Responsible authorities and beach managers that explore the use of PCR methods in recreational water monitoring programs are advised to consult with laboratories on the requirements for implementing analytical test procedures. Consultation with other responsible authorities, beach managers or recreational water quality professionals with experience in this area may help to identify actions that have proven to be successful in other communities.

3.1.2.1 Quantitative PCR (qPCR) method

Quantitative PCR methods for monitoring fecal indicator bacteria at beaches can provide water quality information within several hours (Haugland et al., 2021). The U.S. EPA has validated two qPCR methods for the quantification of *Enterococcus* in recreational waters (Table 3).

Table 3. Standardized qPCR methods for the detection of enterococci in recreational water

| Organization—method | Name |
|-------------------------------|--|
| U.S. EPA, 1609.1 ^a | TaqMan® qPCR with internal amplification control assay |
| U.S. EPA, 1611.1 ^a | TaqMan® qPCR Assay |

^a U.S. EPA, 2019c



These qPCR methods are based on the detection of a target sequence from the large subunit rRNA (23S rRNA) gene found in all known species of *Enterococcus* in water (U.S. EPA, 2015a, 2015b). To summarize, the method involves filtration of water samples, extraction of total DNA and detection/quantification of enterococci sequences by real-time qPCR. Calibrator samples containing defined quantities of *Enterococcus* cells and a sample processing control are extracted and analyzed in the same manner. To determine the performance of the qPCR assays and instrument, laboratories also generate standard curves from PCR analysis of *Enterococcus* DNA standards. The resulting ratio of target sequences from the test samples compared with the calibrator cells (adjusted for differences in DNA recovery) are then used to estimate the enterococci cell equivalents (called calibrator cell equivalents) in the water samples. Two acceptable U.S. EPA methods are available: Method 1611.1 and Method 1609.1. The U.S. EPA more strongly endorses the use of Method 1609.1 (Nappier et al., 2019). It has been applied successfully in multiple investigative studies (Shrestha and Dorevitch, 2019, 2020; Campbell and Kleinheinz, 2020; Saleem et al., 2022). A “Draft Method C” for enumeration of *E. coli* by qPCR has been developed and preliminarily validated by the U.S. EPA but has not been approved for use (Lane et al., 2020; Haugland et al., 2021).

Quantitative PCR is now a well-established technique for rapidly measuring the microbiological quality of recreational waters. Since a validated method for enterococci was established, several jurisdictions in the U.S. and Canada have implemented qPCR technology in their beach monitoring and public notification programs (Griffith and Weisberg, 2011; Dorevitch et al., 2017; Byappanahalli et al., 2018; Alberta Health, 2022). Some U.S. jurisdictions are using the U.S. EPA’s “Draft Method C” or alternative EPA-approved qPCR methods to measure *E. coli* levels at beaches (Kinzelman et al., 2011; EGLE, 2019). Thus, a cumulative body of knowledge and experience with qPCR methods has developed that can be leveraged to educate and assist prospective new users.

While qPCR methods offer several advantages over culture-based methods, they also have disadvantages (see Table 4).

Table 4. Advantages and disadvantages of qPCR methods

| Method | References |
|---|---|
| Advantages | |
| Results are available within 2 to 6 hours of analyzing the sample, creating opportunities to communicate the results on the same day. | Lavender and Kinzelman, 2009; Griffith and Weisberg, 2011; Sheth et al., 2016; Campbell and Kleinheinz, 2020; Shrestha and Dorevitch, 2020; Saleem et al., 2022 |
| DNA or RNA sequences are a more specific attribute for microorganism detection than enzyme-expression. | Frahm et al., 1998; Frahm and Obst, 2003; Zhang et al., 2015; Maheux et al., 2017 |
| Enterococci qPCR method showed a stronger association with gastrointestinal illness than the enterococci culture-based method at beaches impacted by human fecal contamination. | Wade et al., 2006, 2008, 2010, 2022 |
| Detect both culturable and non-culturable microorganisms. | Haugland et al., 2005; Higgins et al., 2007; Viau and Peccia, 2009 |
| Ability to freeze membrane filters for future analysis. | U.S. EPA, 2015a, 2015b; Kinzelman et al., 2020; Shrestha and Dorevitch, 2020 |
| Instrumentation and training can also be applied to microbial source tracking studies. | Harwood et al., 2014; Lane et al., 2020 |
| DNA extracts can be further used for microbial source tracking (MST) qPCR assays to provide information about fecal sources. | Alberta Health, 2022 |
| Disadvantages | |
| Complex method with requirements for advanced technology, calibration procedures and trained personnel. | Griffith and Weisberg, 2011; Shanks et al., 2012; Ferretti et al., 2013; Haugland et al., 2016; Byappanahalli et al., 2018; Aw et al., 2019; Sivaganesan et al., 2019; Lane et al., 2020; Shrestha and Dorevitch, 2020; Saleem et al., 2022 |
| More costly than culture-based methods. | Byappanahalli et al., 2018; Lane et al., 2020; Shrestha and Dorevitch, 2020; Saleem et al., 2022 |
| Methods do not discriminate between targets originating from viable or non-viable cells, potentially overestimating microorganism concentrations. | Haugland et al., 2005 |
| Water samples may contain substances that interfere with detection and result in underestimation of target. | Cao et al., 2012; Haugland et al., 2012; Sivaganesan et al., 2019 |
| Methods require educating stakeholders on the meaning of unfamiliar reporting units. | Ferretti et al., 2013; Shrestha and Dorevitch, 2020 |
| Predictable relationship to gastrointestinal illness has not been established for <i>E. coli</i> qPCR methods. | U.S. EPA, 2012c; Shrestha and Dorevitch, 2019 |



Quantitative PCR methods are more complex and labour-intensive than traditional culture-based methods (Ferretti et al., 2013; Shrestha and Dorevitch, 2020). Requirements for equipment include dedicated workstations with laminar flow hoods, devices that are used in DNA extraction and analysis steps (bead beater, microcentrifuge, visible and UV light spectrophotometers, PCR thermocycler) and a freezer capable of reaching -80°C for long-term storage of stocks, standards and samples (U.S. EPA, 2015a, 2015b). Laboratory analysts need training on the use of qPCR using U.S. EPA methods (Shanks et al., 2012; Aw et al., 2019; Lane et al., 2020). Laboratories using U.S. EPA methods must also meet quality acceptance criteria for calibration model (for example, standard curve) parameters, positive and negative controls and matrix interference to ensure the production of high-quality data (U.S. EPA, 2015a, 2015b; Sivaganesan et al., 2019). Studies have confirmed that, with proper training, laboratories can attain a high level of confidence in implementing qPCR technologies in a beach monitoring program (Haugland et al., 2016; Aw et al., 2019; Lane et al., 2020; Saleem et al., 2022).

Site-specific studies to evaluate method performance and identify sample interference issues are recommended prior to adopting qPCR methods and their associated BAVs (U.S. EPA, 2012c; Ferretti et al., 2013; Campbell and Kleinheinz, 2020; Shrestha and Dorevitch, 2020; Saleem et al., 2022). Studies should include side-by-side testing of water samples using qPCR and culture-based methods to help beach managers understand the differences in method results and the potential impacts on beach management decisions (Dorevitch et al., 2017; Campbell and Kleinheinz, 2020; Saleem et al., 2022). The U.S. EPA recommends that studies to demonstrate method performance consist of at least 10 samples taken on different days, represent conditions where recreation is expected to occur and include samples following heavy rain events (U.S. EPA, 2013). In peer-reviewed publications describing the implementation of qPCRbased notification programs at beaches, one to multiple samples per week were collected over periods ranging from 6 to 14 weeks (that is, the entire beach season) (Lavender and Kinzelman, 2009; Sheth et al., 2016; Dorevitch et al., 2017; Byappanahalli et al., 2018; Saleem et al., 2022).

Quantitative PCR and culture-based detection techniques focus on different attributes of the fecal indicator (U.S. EPA, 2012c). Culture-based methods detect viable microorganisms that can grow in culture. Quantitative PCR methods detect DNA sequences which can come from viable and non-viable forms of the microorganism. The results produced by these methods are different and are not directly interchangeable (U.S. EPA, 2012c). Sources of fecal pollution, environmental factors and site characteristics can affect the ratios of viable, non-viable and non-culturable microorganisms in recreational waters and this relationship will differ from location to location (Lavender and Kinzelman, 2009; Raith et al., 2014; Campbell and Kleinheinz, 2020). Some studies have reported an increase in the

number of samples exceeding U.S. EPA BAVs when using qPCR measurements compared with culture-based methods (Sheth et al., 2016; Campbell and Kleinheinz, 2020), whereas others reported more exceedances using culture-based methods (Nevers et al., 2013; Dorevitch et al., 2017; Byappanahalli et al., 2018).

The presence of interfering substances in natural bodies of water is also a significant concern when considering implementation of qPCR methods. Certain substances can interfere with PCR by inhibiting polymerase function or preventing primers from binding, leading to reduced efficiency and underestimation of results (Haugland et al., 2012; U.S. EPA, 2012c). Examples of substances that can cause interference include humic and tannic acids, calcium, iron and iron-containing compounds, certain types of clay particles, silts and coral sands (Haugland et al., 2012; Nappier et al., 2019). Inhibition during analysis may result in failed quality control criteria, leading to a sample being rejected (Lane et al., 2020). Thus, beach managers must consider management actions to take in the event of an invalid sample (Lane et al., 2020). Studies have reported issues with sample inhibition at rates ranging from 1% to 11% (Sivaganesan et al., 2019; Haugland et al., 2016; Byappanahalli et al., 2018; Shrestha and Dorevitch, 2019; Campbell and Kleinheinz, 2020; Lane et al., 2020). The U.S. EPA has introduced method adaptations to better estimate and control interference. These include updated assays to estimate DNA recovery and identify inhibition, and the use of newer reagents based on more sophisticated chemistry (Nappier et al., 2019; U.S. EPA, 2015a, 2015b). Diluting the sample DNA extracts (such as a 5-fold dilution) is also recommended as an approach to lessening interference in water samples that may fail quality control criteria (U.S. EPA, 2013, 2015a, 2015b; Nappier et al., 2019). Additional guidance for conducting qPCR assays is available in Bustin et al. (2009).

The timeliness of sample collection and transport, laboratory analysis and communicating results to the public is important for enabling same-day beach water monitoring and notifications using qPCR methods (Griffith and Weisberg, 2011; Ferretti et al., 2013; Shrestha and Dorevitch, 2020). The most notable advantage of the *Enterococcus* qPCR method is the increased public health protection that is anticipated by providing more timely notifications to swimmers of elevated levels of fecal indicator bacteria (U.S. EPA, 2012c). Saleem et al. (2023) showed that the faster, same-day analysis provided by the U.S. EPA's 1609.1 qPCR method for *Enterococcus* reduced the number of incorrect beach postings and beach days lost compared to a culture-based *E. coli* method at two Toronto beaches. Producing same-day notifications may be difficult for beaches that are located more than an hour away from a qPCRcapable laboratory. Test turnaround times should also be discussed with the analytical laboratory to ensure monitoring results can be relayed, or updated, in a timely manner. High-use beaches serving large populations and having a short travel distance to a centralized laboratory may be in the best position to explore the



use of rapid molecular methods (Griffith and Weisberg, 2011; Ferretti et al., 2013; Shrestha and Dorevitch, 2020). Beaches that monitor for fecal bacterial indicators daily are ideally suited to take advantage of the speed in obtaining results provided by qPCR (Griffith and Weisberg, 2011). Beaches located near river mouths or other sources of fecal pollution that can rapidly impact beach water quality would also benefit from the speed of qPCR and same-day water quality data for beach notifications (Saleem et al., 2022, 2023).

The experiences of other responsible authorities that have switched their beach monitoring and notification programs from culture-based to qPCR-based methods can also be informative. For example, the transition for the Chicago Park District's beaches is described in Dorevitch et al. (2017) and Shrestha and Dorevitch (2020). When switching to qPCR methods, it is also important that beach managers develop a communications plan to educate beach users about the new monitoring technology and the advantages it provides when used in a beach monitoring program.

3.1.2.2 Digital PCR (dPCR)

Digital PCR (dPCR) is a rapidly emerging technology for determining the quantity of DNA in a water sample. In contrast to traditional PCR approaches, where DNA is measured in a single tube, in dPCR the DNA sample is partitioned into thousands to millions of smaller reactions (that is, in small chambers on a chip for chamber digital PCR or water-in-oil droplets for droplet digital PCR) (Cao et al., 2015; Nappier et al., 2019). The partitioning process results in the DNA target being present in some partitions and absent in others (Cao et al., 2015). PCR amplification occurs only in a portion of these partitions and the frequency of positive reactions is used with Poisson statistics to estimate the concentration of target DNA (Cao et al., 2015; Nappier et al., 2019). A key advantage of dPCR methods over qPCR is the elimination of the need to generate standard curves, which require additional labour and are a potential source of method variability (Cao et al., 2015). This method also offers the potential for reduced susceptibility to some interferences, a superior ability to quantify multiple targets (Cao et al., 2015, 2016a; Wang et al., 2016; Nappier et al., 2019), and the potential for greater sensitivity, precision and reproducibility (Tiwari et al., 2022). A limitation of dPCR is that the capital and consumable costs are greater than those for a qPCR system (Cao et al., 2016b; Tiwari et al., 2022). Studies comparing dPCR with qPCR and culture-based methods for the detection of enterococci in recreational water samples have demonstrated good correlations between method results (Cao et al., 2015; Wang et al., 2016; Staley et al., 2018; Crain et al., 2021). Development of a validated dPCR method for use in beach monitoring programs requires additional work. Guidance on standards for conducting dPCR assays is available (The dMIQE Group, 2020). More detailed information on dPCR advantages, limitations and applications in water quality monitoring can be found in Cao et al. (2016b) and Tiwari et al. (2022).

3.1.3 Automated enzymatic methods

One area of active research is the development of automated or semi-automated enzyme-based detection systems to reduce the sample-to result time when monitoring fecal pollution in water. Methods are predominantly based on fluorogenic measurement of the activity of the β -D-glucuronidase enzyme within the sample, either after a short incubation period or directly without a culture step. The concentration of *E. coli* or degree of fecal pollution is then estimated from the fluorogenic signal. Time to results varies depending on the type of device and the concentration of the target bacteria but can range from 15 minutes up to 18 hours (Demeter et al., 2020).

Bench-top and field portable systems are available that provide automatic incubation and measurement of manually collected samples (Bramburger et al., 2015; Schang et al., 2016; Angelescu et al., 2019). Fully automated systems that permit autonomous on-site monitoring and same-day or even near-real time reporting have also been developed. Field studies have demonstrated the usefulness of rapid online technologies for identifying fecal pollution source and peak episodes of fecal contamination in surface water (Burnet et al., 2019a, 2019b) and for the rapid assessment of recreational water quality (Angelescu et al., 2019; Cazals et al., 2020). However, additional investigation is needed to better understand the links between fecal pollution, health risks and the results generated by automated enzyme detection methods (Angelescu et al., 2019; Burnet et al., 2019a, 2019b; Cazals et al., 2020; Demeter et al., 2020). Further work on validating and standardizing these methods is also needed (Demeter et al., 2020). For reviews of the use of technologies for monitoring of fecal pollution in water by enzymatic methods, refer to Demeter et al. (2020) and Briciu-Burghina and Regan (2023).

When considering rapid online technologies, beach managers need to assess factors like costs, time to results, method robustness, power and communication capabilities, maintenance requirements and infrastructure needed for the installation of on-site sensors (Demeter et al., 2020; Briciu-Burghina and Regan, 2023). The comparatively high capital costs of automated online measurement technologies are a noted limitation (Burnet et al., 2019a; Cazals et al., 2020). Before selecting an automated detection method, site-specific studies to evaluate its performance should be carried out. This should include side-by-side testing of water samples using the automated device and standard culture-based methods. Method results should be compared and, if necessary, site-specific relationships between enzymatic activity and target bacteria concentrations determined.



3.1.4 Fecal pollution source tracking methods

A toolbox of methods is available for fecal source tracking (FST). The choice of FST target and method depends on the scale and objectives of the investigative study (Stoeckel, 2005). FST methods can be useful for detecting sources of pollution such as human sewage and fecal contamination originating from ruminants, canine and avian species impacting recreational areas or for assisting with waterborne illness outbreak investigations (Goodwin et al., 2016; Shanks and Mattioli, 2018; Ballesté et al., 2020; McKee et al., 2020). The guideline technical document *Understanding and Managing Risks in Recreational Waters* provides general information on FST approaches (Health Canada, 2023b), including microbial, chemical and other biological source tracking markers. Among the most commonly used FST approaches are microbial source tracking (MST) methods, which utilize attributes of host specific microorganisms to identify and quantify fecal sources (Harwood et al., 2014). Scientific literature provides details on methodological approaches that have been used for FST/MST (for example, Stoeckel, 2005; U.S. EPA, 2005b; Badgley and Hagedorn, 2015; Edge et al., 2021) as well as reviews of MST methods and their performance (Boehm et al., 2013; Harwood et al., 2014).

The U.S. EPA has validated two qPCR-based MST methods for the characterization of human sources of fecal pollution in fresh and marine waters, employing human-associated gene sequences from *Bacteroides*-like microorganisms (U.S. EPA, 2019a, 2019b). Integrating qPCR-based MST methodologies into a qPCR-based fecal indicator monitoring program can be a cost-effective approach to adding information about the origin of fecal pollution at beaches. The province of Alberta applies MST qPCR assays for human- or ruminant-specific *Bacteroides* genetic markers at beaches exceeding established enterococci qPCR recreational water quality benchmarks (Alberta Health, 2022). The information is used to assess fecal pollution sources and make source-based risk management decisions (Alberta Health, 2022).

3.2 Alternative indicators of fecal pollution

Testing for other fecal indicator organisms (coliphages, *Bacteroides* spp., bacteriophages of *Bacteroides* spp., *Clostridium perfringens*) can be considered to provide additional information on the fecal contamination associated with a recreational area. Standardized methods for the detection of these microorganisms are summarized in Table 5. The use of fecal indicator organisms in MST applications is discussed in section 3.1.4.

Table 5. Standardized methods for the detection of coliphages, *Bacteroides* spp., bacteriophages of *Bacteroides* spp. and *Clostridium perfringens* in recreational water

| Organization—method | Name |
|---|---|
| Bacteriophages of <i>Bacteroides</i> spp. | |
| ISO 10704–4:2001 ^a | Enumeration of bacteriophages infecting <i>Bacteroides fragilis</i> |
| <i>Clostridium perfringens</i> | |
| ISO 14189:2013 ^a | Enumeration of <i>Clostridium perfringens</i> —Method using membrane filtration |
| Coliphages | |
| ISO 10705:1:1995 ^a | Enumeration of F-specific RNA bacteriophages |
| ISO 10705–2:2000 ^a | Enumeration of somatic coliphages |
| SM 9224 B-F ^b | Detection of coliphages |
| U.S. EPA 1602 ^c | Method 1602: Male-specific (F+) and somatic coliphage in water by single agar layer procedure |
| U.S. EPA 1642 ^d | Method 1642: Male-specific (F+) and somatic coliphage in recreational waters and wastewater by ultrafiltration and single agar layer) procedure |

^a ISO, 2021a; ^bAPHA et al., 2023; ^cU.S. EPA, 2001b; ^dU.S. EPA, 2018

Details on the use of methods in research settings can be found in the scientific literature (Mocé-Llivina et al., 2005; Weidenmann et al., 2006; Wade et al., 2010; Gonzalez et al., 2012; Griffith et al., 2016; Stachler et al., 2018; Blanch et al., 2020).



3.3 Pathogenic microorganisms

Routine monitoring of pathogenic microorganisms (that is, pathogenic bacteria, viruses and protozoa) is not recommended due to the associated complexity and costs. Using primary indicators of fecal contamination to show the potential presence of fecal pathogens is an accepted practice (WHO, 2000b; Hussain, 2019). However, certain circumstances may warrant testing for the presence of specific microorganisms, such as during waterborne illness outbreak investigations. Information on pathogenic microorganisms of potential concern can be found in the recreational water quality guideline technical document *Microbiological pathogens and biological hazards* (Health Canada, 2023a).

Bacterial pathogen testing can be conducted by qualified staff in laboratories with proper biosafety level, design, equipment and procedures. Authorities should consult with laboratories on their analytical capabilities when testing is necessary. Standard methods are available for bacterial pathogens that may exist in Canadian recreational water settings (APHA et al., 2023; ISO, 2021a).

Testing for pathogenic protozoa and viruses (for example, *Giardia*, *Cryptosporidium*, enteric viruses such as noroviruses) is outside the scope of services provided by most water testing laboratories. Analysis requires specialized equipment and highly trained personnel. Standard methods are available for use by laboratories with the capacity for testing (ASTM, 2004; APHA et al., 2023; ISO, 2021a; U.S. EPA, 1996, 2001a, 2005a, 2006, 2012a, 2012b).

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APPENDIX A: LIST OF ABBREVIATIONS

| | |
|-----------------|---|
| APHA | American Public Health Association |
| BAV | Beach action value |
| DNA | Deoxyribonucleic acid |
| dPCR | Digital polymerase chain reaction |
| DST | Defined substrate technology |
| E. coli | <i>Escherichia coli</i> |
| EHSS | Environmental Health and Safety Surveys |
| EMPACT | Environmental monitoring for public access and community tracking |
| FST | Fecal source tracking |
| MF | Membrane filtration |
| MPN | Most probable number |
| MST | Microbial source tracking |
| MTF | Multiple tube fermentation |
| PCR | Polymerase chain reaction |
| qPCR | Quantitative polymerase chain reaction |
| rRNA | Ribosomal ribonucleic acid |
| SM | Standard methods (for the Examination of Water and Wastewater) |
| U.S. EPA | United States Environmental Protection Agency |
| WHO | World Health Organization |