GUIDELINES FOR

CANADIAN RECREATIONAL WATER QUALITY

MICROBIOLOGICAL SAMPLING AND ANALYSIS

Guideline Technical Document

Health Canada Santé Canada

Canada

Health Canada is the federal department responsible for helping the people of Canada maintain and improve their health. Health Canada is committed to improving the lives of all of Canada's people and to making this country's population among the healthiest in the world as measured by longevity, lifestyle and effective use of the public health care system.

Également disponible en français sous le titre :
Recommandations au sujet de la qualité des eaux utilisées à des fins récréatives au Canada : Échantillonnage et analyse microbiologiques – Document technique

To obtain additional information, please contact:

Health Canada
Address Locator 0900C2
Ottawa, ON K1A 0K9
Tel.: 613-957-2991
Toll free: 1-866-225-0709
Fax: 613-941-5366
TTY: 1-800-465-7735
E-mail: publications-publications@hc-sc.gc.ca

Publication date: March 2024

Cat.: H144-122/2024E-PDF
ISBN: 978-0-660-70525-5
Pub.: 230817

TABLE OF CONTENTS

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 website (Health Canada, 2024).

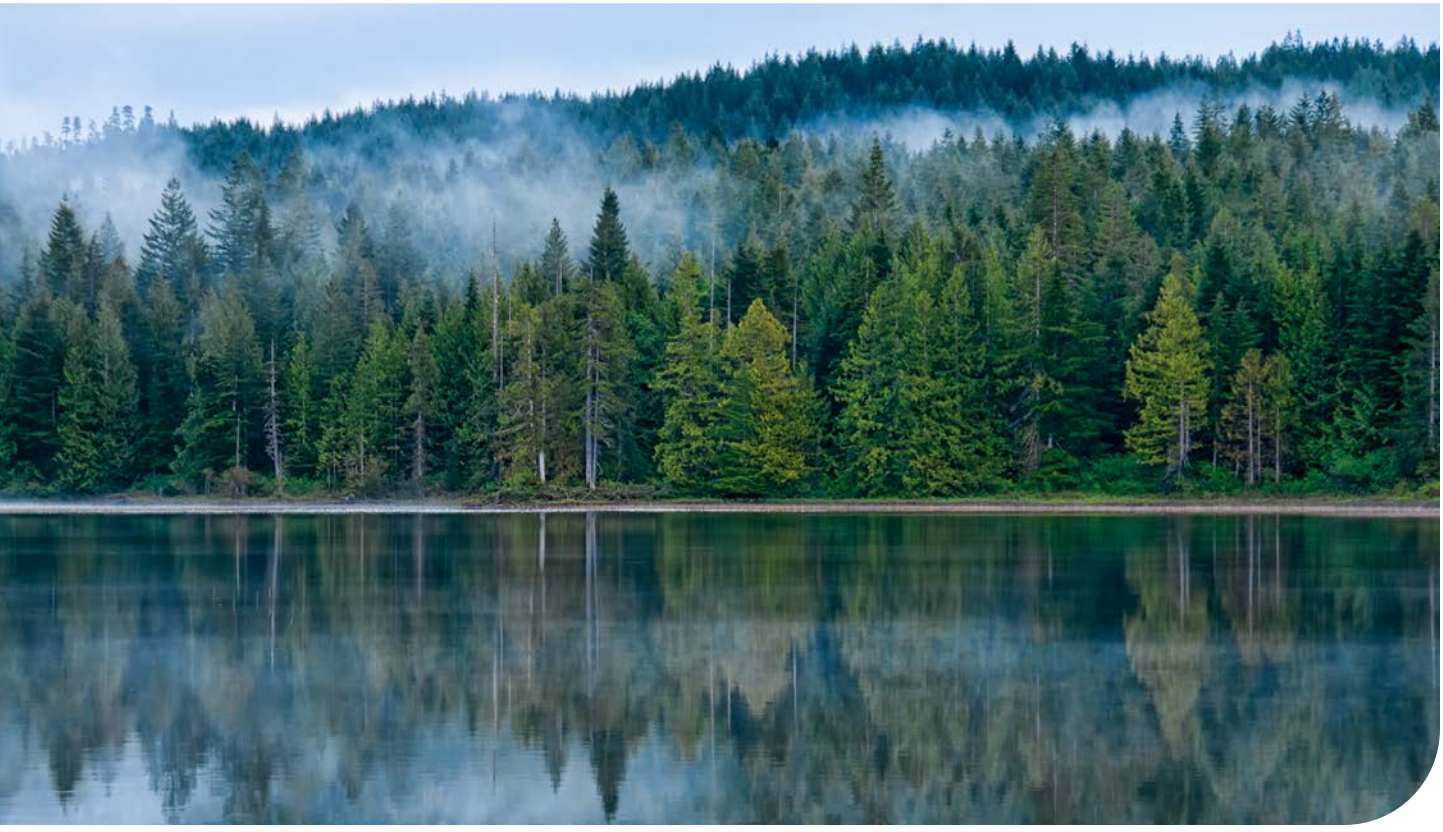
1.0 OVERVIEW OF MICROBIOLOGICAL SAMPLING AND ANALYSIS

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 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]
E. coli				
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]	mEI 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; [b]U.S. EPA, 2021; [c]ISO, 2021a; [d]Approved for freshwater only; [e]Confirmed 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.	*E. coli*: Dufour, 1984; U.S. EPA, 1986; Wiedenmann et al., 2006; Marion et al., 2010 Enterococci: 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 *E. coli* 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 specificmicroorganisms 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 qPCRbased 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 *Bacteroides* spp.	
ISO 10704–4:2001[a]	Enumeration of bacteriophages infecting *Bacteroides fragilis*
Clostridium perfringens	
ISO 14189:2013[a]	Enumeration of *Clostridium perfringens*—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

[b] ISO, 2021a; [b]APHA et al., 2023; [c]U.S. EPA, 2001b; [d]U.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).

REFERENCES

Alberta Health (2022). Alberta safe beach protocol. Alberta Health, Government of Alberta. May 2022. Accessed July 2022 from https://open.alberta.ca/publications/9781460145395

Angelescu, D.E., Huynh, V., Hausot, A., Yalkin, G., Plet, V., Mouchel, J.-M., Guérin-Rechdaoui, S., Azimi, S. and Rocher, V. (2019). Autonomous system for rapid field quantification of *Escherichia coli* in surface waters. J. Appl. Microbiol., 126(1): 332–343.

APHA, AWWA and WEF. (2023). Standard methods for the examination of water and wastewater. 23rd edition. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC.

ASTM (2004). ASTM D244–92: Standard practice for recovery of enteroviruses from waters. American Society for Testing and Materials, Philadelphia, PA.

Aw, T.G., Sivaganesan, M., Briggs, S., Dreelin, E., Aslan, A., Dorevitch, S., Shrestha, A., Isaacs, N., Kinzelman, J., Kleinheinz, G., Noble, R., Rediske, R., Scull, B., Rosenberg, S., Weberman, B., Sivy, T., Southwell, B., Siefring, S., Oshima, K. and Haugland, R. (2019). Evaluation of multiple laboratory performance and variability in analysis of recreational freshwaters by a rapid *Escherichia coli* qPCR method (draft method C). Water Res., 156: 465–474.

Badgley, B. and Hagedorn, C. (2015). Microbial source tracking: Advances in research and a guide to application. In: Advances in Watershed Science and Assessment. Younos, T and Parece, T.E. eds. The Handbook of Environmental Chemistry. Springer International Publishing, Switzerland. Pp. 267–288.

Ballesté, E., Demeter, K., Masterson, B., Timoneda, N., Sala-Comorera, L. and Meijer, W.G. (2020). Implementation and integration of microbial source tracking in a river watershed monitoring plan. Sci. Tot. Environ., 736:139573.

Bertke, E.E. (2007). Composite analysis for *Escherichia coli* at coastal beaches. J. Great Lakes Res., 33(2): 335–341.

Blanch, A.R., Lucena, F., Muniesa, M. and Jofre, J. (2020). Fast and easy methods for the detection of coliphages. J. Microbiol. Methods, 173.

Boehm, A.B. (2007). Enterococci concentrations in diverse coastal environments exhibit extreme variability. Environ. Sci. Technol., 41(24): 8227–8232.

Boehm, A.B., Griffith, J., McGee, C., Edge, T.A., Solo-Gabriele, H.M., Whitman, R., Cao, Y., Getrich, M., Jay, J.A., Ferguson, D., Goodwin, K.D., Lee, C.M., Madison, M. and Weisberg, S.B. (2009). Faecal indicator bacteria enumeration in beach sand: A comparison study of extraction methods in medium to coarse sands. J. Appl. Microbiol., 107(5): 1740–1750.

Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D. and Weisberg, S.B. (2013). Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. Water Res., 47(18): 6812–6828.

Bramburger, A.J., Stephen Brown, R., Haley, J. and Ridal, J.J. (2015). A new, automated rapid fluorometric method for the detection of *Escherichia coli* in recreational waters. J. Great Lakes Res., 41(1): 298–302.

Brandão, J. (2019). Microorganisms in beach sands: what do we still not know? In: Encyclopedia of Environmental Health. Nriagu, J. (ed.). Elsevier, 390–2.

Briciu-Burghina, C. and Regan, F. (2023). Sensors for monitoring faecal indicator bacteria in bathing waters. In: Biosensors for the Marine Environment: Present and Future Challenges, Regan, F., Hansen, P-D and Barceló, D. (eds.), Hdb Env Chem (2023) 122: 201–228, Springer Nature, Switzerland. DOI 10.1007/698_2022_946,

Burnet, J.-., Dinh, Q.T., Imbeault, S., Servais, P., Dorner, S. and Prévost, M. (2019a). Autonomous online measurement of β-D-glucuronidase activity in surface water: Is it suitable for rapid E. coli monitoring? Water Res., 152: 241–250.

Burnet, J.-., Sylvestre, É, Jalbert, J., Imbeault, S., Servais, P., Prévost, M. and Dorner, S. (2019b). Tracking the contribution of multiple raw and treated wastewater discharges at an urban drinking water supply using near real-time monitoring of β-D-glucuronidase activity. Water Res., 164.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J. and Wittwer, C.T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin. Chem., 55(4): 611–622.

Byappanahalli, M.N., Nevers, M.B., Shively, D.A., Spoljaric, A. and Otto, C. (2018). Real-time water quality monitoring at a Great Lakes national park. J. Environ. Qual., 47(5): 1086–1093.

Cabelli, V.J. (1983). Health effects criteria for marine recreational waters. United States Environmental Protection Agency, Document No.: EPA-600/1-80-031, Cincinnati, OH.

Campbell, A. and Kleinheinz, G. (2020). Comparison of qPCR and traditional beach monitoring methods at select Great Lakes beaches and the impact on beach management. Lake Reserv. Manage., 36(2): 155–168.

Cao, Y., Griffith, J.F., Dorevitch, S. and Weisberg, S.B. (2012). Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring *Enterococcus* in environmental waters. J. Appl. Microbiol., 113(1): 66–75.

Cao, Y., Raith, M.R. and Griffith, J.F. (2015). Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. Water Res., 70: 337–349.

Cao, Y., Raith, M.R. and Griffith, J.F. (2016a). A duplex digital PCR assay for simultaneous quantification of the *Enterococcus* spp. And the human fecal-associated HF183 marker in waters. J. Visualized Exp., 2016(109).

Cao, Y., Griffith, J.F. and Weisberg, S.B. (2016b). The next-generation PCR-based quantification method for ambient waters: Digital PCR. P. 18.

Cazals, M., Stott, R., Fleury, C., Proulx, F., Prévost, M., Servais, P., Dorner, S. and Burnet, J.-B. (2020). Near realtime notification of water quality impairments in recreational freshwaters using rapid online detection of β-D-glucuronidase activity as a surrogate for *Escherichia coli* monitoring. Sci. Total Environ., 720.

CEAEQ (2023). Centre d'Expertise en Analyse Environnementale du Québec. Méthodes d'analyses

(analyses biologie, microbiologie et toxicologie). Québec, Ministère de l'Environnement et de la Lutte contre les changements climatiques de la Faune et des Parcs. Government du Québec. Accessed June 2023 from : https://www.ceaeq.gouv.qc.ca/methodes/bio_toxico_micro.htm

Crain, C., Kezer, K., Steele, S., Owiti, J., Rao, S., Victorio, M., Austin, B., Volner, A., Draper, W., Griffith, J., Steele, J. and Seifert, M. (2021). Application of ddPCR for detection of *Enterococcus* spp. In coastal water quality monitoring. J. Microbiol. Methods, 184.

Del Mar Lleó, M., Tafi, M.C. and Canepari, P. (1998). Nonculturable *Enterococcus faecalis* cells are metabolically active and capable of resuming active growth. Syst. Appl. Microbiol., 21(3): 333–339.

Demeter, K., Burnet, J.-., Stadler, P., Kirschner, A., Zessner, M. and Farnleitner, A.H. (2020). Automated online monitoring of fecal pollution in water by enzymatic methods. Curr. Opin. Environ. Sci. Health, 16: 82–91.

The dMIQE Group (2020). The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020. Clin. Chem. 66(8): 1012–1029.

Dorevitch, S., Doi, M., Hsu, F.-C., Lin, K.-T., Roberts, J.D., Liu, L.C., Gladding, R., Vannoy, E., Li, H., Javor, M. and Scheff, P.A. (2011). A comparison of rapid and conventional measures of indicator bacteria as predictors of waterborne protozoan pathogen presence and density. J. Environ. Monit., 13(9): 2427–2435.

Dorevitch, S., Shrestha, A., DeFlorio-Barker, S., Breitenbach, C. and Heimler, I. (2017). Monitoring urban beaches with qPCR vs. culture measures of fecal indicator bacteria: Implications for public notification. Environ. Health, 16(1): 45.

Dufour, A.P. (1984). Health effects criteria for fresh water recreational waters. United States Environmental Protection Agency, Document Number: EPA 600/1-84-004, Cincinnati, OH.

Edge, T.A. and Hill, S. (2007). Multiple lines of evidence to identify the sources of fecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario. Water Res., 41: 3585–3594.

Edge, T.A., Hill, S., Seto, P. and Marsalek, J. (2010). Library-dependent and library-independent microbial source tracking to identify spatial variation in faecal contamination sources along a Lake Ontario beach (Ontario, Canada). Water Sci. Technol., 62: 719–727.

Edge, T.A., Boyd, R.J., Shum, P. and Thomas, J.L. (2021). Microbial source tracking to identify fecal sources contaminating the Toronto harbour and Don River watershed in wet and dry weather. J. Great Lakes Res., 47(2): 366–377.

EGLE (2019). EGLE is creating safer beaches with rapid-testing technology. Michigan Department of Environment, Great Lakes and Energy (EGLE). Michigan. Accessed March 2022 from: https://www.michigan.gov/mienvironment/0,9349,7-385-90161-499038--,00.html

Ferretti, J.A., Tran, H.V., Peterson, S.J. and Loftin, V. (2013). Rapid method demonstration project at four New Jersey marine beaches using real time quantitative polymerase chain reaction (qPCR). Mar. Pollut. Bull., 71(1–2): 51–63.

Frahm, E., Heiber, I., Hoffmann, S., Koob, C., Meier, H., Ludwig, W., Amann, R., Schleifer, K.H. and Obst, U. (1998). Application of 235 rDNA-targeted oligonucleotide probes specific for enterococci to water hygiene control. Syst. Appl. Microbiol., 21(3): 450–453.

Frahm, E. and Obst, U. (2003). Application of the fluorogenic probe technique (TaqMan PCR) to the detection of *Enterococcus* spp. and *Escherichia coli* in water samples. J. Microbiol. Methods, 52(1): 123–131.

Francy, D.S., Brady, A.M.G., Carvin, R.B., Corsi, S.R., Fuller, L.M., Harrison, J.H., Hayhurst, B.A., Lant, J., Nevers, M.B., Terrio, P.J. and Zimmerman, T.M. (2013a). Developing and implementing predictive models for estimating recreational water quality at Great Lakes beaches: United States Geological Survey Scientific Investigations Report 2013–5166, 68 p., http://dx.doi.org/10.3133/sir20135166/

Francy, D.S., Bushon, R.N., Brady, A.M.G. and Kephart, C.M. (2013b). Comparing rapid and culture indicator bacteria methods at inland lake beaches. Lake Reserv. Manage., 29(2): 99–102.

Gonzalez, R.A., Conn, K.E., Crosswell, J.R. and Noble, R.T. (2012). Application of empirical predictive modeling using conventional and alternative fecal indicator bacteria in eastern North Carolina waters. Water Res., 46(18): 5871–5882.

Goodwin, K.D., Gruber, S., Vondrak, M. and Crumpacker, A. (2016). Watershed assessment with beach microbial source tracking and outcomes of resulting gull management. Environ. Sci. Technol., 50(18): 9900–9906.

Griffith, J.F. and Weisberg, S.B. (2011). Challenges in implementing new technology for beach water quality monitoring: Lessons from a California demonstration project. Mar. Technol. Soc. J., 45(2): 65–73.

Griffith, J.F., Weisberg, S.B., Arnold, B.F., Cao, Y., Schiff, K.C. and Colford, J.M., Jr. (2016). Epidemiologic evaluation of multiple alternate microbial water quality monitoring indicators at three California beaches. Water Res., 94: 371–381.

Harwood, V.J., Staley, C., Badgley, B.D., Borges, K. and Korajkic, A. (2014). Microbial source tracking markers for detection of fecal contamination in environmental waters: Relationships between pathogens and human health outcomes. FEMS Microbiol. Rev., 38(1): 1–40.

Haugland, R.A., Siefring, S.C., Wymer, L.J., Brenner, K.P. and Dufour, A.P. (2005). Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Res., 39(4): 559–568.

Haugland, R.A., Siefring, S., Lavender, J. and Varma, M. (2012). Influences of sample interference and interference controls on quantification of enterococci fecal indicator bacteria in surface water samples by the qPCR method. Water Res., 46(18): 5989–6001.

Haugland, R.A., Siefring, S., Varma, M., Oshima, K.H., Sivaganesan, M., Cao, Y., Raith, M., Griffith, J., Weisberg, S.B., Noble, R.T., Blackwood, A.D., Kinzelman, J., Anan'eva, T., Bushon, R.N., Stelzer, E.A., Harwood, V.J., Gordon, K.V. and Sinigalliano, C. (2016). Multi-laboratory survey of qPCR enterococci analysis method performance in US coastal and inland surface waters. J. Microbiol. Methods, 123: 114–125.

Haugland, R., Oshima, K., Sivaganesan, M., Dufour, A., Varma, M., Siefring, S., Nappier, S., Schnitker, B. and Briggs, S. (2021). Large-scale comparison of *E. coli* levels determined by culture and a qPCR method (EPA draft method C) in Michigan towards the implementation of rapid, multi-site beach testing. J. Microbiol. Methods, 184.

Health Canada (2017). Guidelines for Canadian drinking water quality—cyanobacterial toxins. Prepared by the Federal-Provincial-Territorial Committee on Drinking Water of the Federal-Provincial-Territorial Committee on Health and the Environment, Ottawa, ON.

Health Canada (2022). Guidelines for Canadian recreational water quality—cyanobacteria and their toxins. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON.

Health Canada (2023a). Guidelines for Canadian recreational water quality—microbiological pathogens and biological hazards. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON.

Health Canada (2023b). Guidelines for Canadian recreational water quality—understanding and managing risks in recreational waters. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON.

Health Canada (2024). Guidelines for Canadian recreational water quality—summary document. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON.

Higgins, M.J., Chen, Y-C., Murthy, S.N., Hendrickson, D., Farrel, J. and Schafer, P. (2007). Reactivation and growth of non-culturable indicator bacteria in anaerobically digested biosolids after centrifuge dewatering. Water Res., 41(3): 665–673.

Hussain, Q. (2019). Chapter 10: Bacteria—the natural indicator of environmental pollution. In: Freshwater Microbiology Perspectives of Bacterial Dynamics in Lake Ecosystems, Bandh, S.A., Shafi, S. and Shameem, N. (eds.), Academic Press.

ISO (2021a). ISO ICS 07.100.20—Microbiology of Water. International Organization for Standardization. Geneva, Switzerland. Accessed from www.iso.org

ISO (2021b). ISO ICS 13.060.45—Examination of Water in General. International Organization for Standardization. Geneva, Switzerland. Accessed from: www.iso.org

Kinzelman, J., Ng, C., Jackson, E., Gradus, S. and Bagley, R. (2003). Enterococci as indicators of Lake Michigan recreational water quality: Comparison of two methodologies and their impacts on public health regulatory events. Appl. Environ. Microbiol., 69(1): 92–96.

Kinzelman, J.L., Pond, K.R., Longmaid, K.D. and Bagley, R.C. (2004). The effect of two mechanical beach grooming strategies on *Escherichia coli* density in beach sand at a southwestern Lake Michigan beach. Aquatic Ecosyst. Health Manage., 7(3): 425–432.

Kinzelman, J.L., Dufour, A.P., Wymer, L.J., Rees, G., Pond, K.R. and Bagley, R.C. (2006). Comparison of multiple point and composite sampling for monitoring bathing water quality. Lake Reserv. Manage., 22(2): 95–102.

Kinzelman, J.L., Bushon, R.N., Dorevitch, S. and Noble, R.T. (2011). Comparative evaluation of molecular and culture methods for fecal indicator bacteria for use in inland recreational waters. Report #1569. Water Research Foundation, Denver, CO.

Kinzelman, J.L., Field, K.G., Green, H.C., Harwood, V.J. and McPhail, C. (2012). Chapter 9: Indicators, sanitary surveys and source attribution techniques. In: Animal Waste, Water Quality and Human Health. Dufour, A.P., Bartram, J., Bos, R., and Gannon, V. IWA, London, UK and World Health Organization. Geneva, Switzerland.

Kinzelman, J., Byappanahalli, M.N., Nevers, M.B., Shively, D., Kurdas, S. and Nakatsu, C. (2020). Utilization of multiple microbial tools to evaluate efficacy of restoration strategies to improve recreational water quality at a Lake Michigan beach (Racine, WI). J. Microbiol. Methods, 178.

Köster, W., Egli, T., Ashbolt, N., Botzenhart, K., Burlion, N., Endo, T., Grimont, P., Guillot, E., Mabilat, C., Newport, L., Niemi, M., Payment, P., Prescott, A., Renaud, P. and Rust, A. (2003). Chapter 8: Analytical methods for microbiological water quality testing. In: Assessing Microbial Safety of Drinking Water—Improving approaches and methods. World Health Organization and Organisation for Economic Co-operation and Development. IWA Publishing, London, UK. pp. 237–282.

Lane, M.J., Rediske, R.R., McNair, J.N., Briggs, S., Rhodes, G., Dreelin, E., Sivy, T., Flood, M., Scull, B., Szlag, D., Southwell, B., Isaacs, N.M. and Pike, S. (2020). A comparison of *E. coli* concentration estimates quantified by the EPA and a Michigan laboratory network using EPA draft method C. J. Microbiol. Methods, 179.

Lavender, J.S. and Kinzelman, J.L. (2009). A cross comparison of QPCR to agar-based or defined substrate test methods for the determination of *Escherichia coli* and enterococci in municipal water quality monitoring programs. Water Res., 43(19): 4967–4979.

Maheux, A.F., Bouchard, S., Bérubé, E. and Bergeron, M.G. (2017). Rapid molecular identification of fecal origincolonies growing on *Enterococcus* spp.-specific culture methods. J. Water Health, 15(2): 239–250.

Marion, J.W., Lee, J., Lemeshow, S. and Buckley, T.J. (2010). Association of gastrointestinal illness and recreational water exposure at an inland U.S. beach. Water Res., 44(16): 4796–4804.

McKee, A., Molina, M., Cyterski, M. and Couch, A. (2020). Microbial source tracking (MST) in Chattahoochee River National Recreation Area: Seasonal and precipitation trends in MST marker concentrations, and associations with *E. coli* levels, pathogenic marker presence, and land use. Wat. Research, 171(2020):115435.

Mocé-Llivina, L., Lucena, F. and Jofre, J. (2005). Enteroviruses and bacteriophages in bathing waters. Appl. Environ. Microbiol., 71(11): 6838–6844.

Nappier, S.P., Ichida, A., Jaglo, K., Haugland, R. and Jones, K.R. (2019). Advancements in mitigating interference in quantitative polymerase chain reaction (qPCR) for microbial water quality monitoring. Sci. Total Environ., 671: 732–740.

Nevers, M.B. and Whitman, R.L. (2010). Policies and practices of beach monitoring in the Great Lakes, USA: A critical review. J. Environ. Monit., 12(3): 581–590.

Nevers, M.B., Byappanahalli, M.N. and Whitman, R.L. (2013). Choices in recreational water quality monitoring: New opportunities and health risk trade-offs. Environ. Sci. Technol., 47(7): 3073–3081.

Nevers, M.B., Byappanahalli, M.N., Edge, T.A. and Whitman, R.L. (2014). Beach science in the Great Lakes. J. Great Lakes Res., 40(1): 1–14.

Nijak, G.M., Geary, J.R., Larson, S.L. and Talley, J.W. (2012). Autonomous, wireless in-situ sensor (AWISS) for rapid warning of *Escherichia coli* outbreaks in recreational and source waters. Environ. Eng. Sci., 29(1): 64–69.

Payment, P., Waite, M. and Dufour, A. (2003). Chapter 2: Introducing parameters for the assessment of drinking water quality. In: Assessing Microbial Safety of Drinking Water—Improving approaches and methods. World Health Organization and Organisation for Economic Co-operation and Development. IWA Publishing, London, UK. pp. 47–77.

Pope M.L., Bussen M., Feige M.A., Shadix, L., Gonder, S., Rodgers, C., Chambers, Y., Pulz, J., Miller, K., Connell, K. and Standridge, J. (2003). Assessment of the effects of holding time and temperature on *Escherichia coli* densities in surface water samples. Appl Environ Microbiol., 69(10): 6201–6207.

Public Health Ontario (2022). Public beach water. Laboratory services, Public Health Ontario, Toronto, Ontario. Updated June 2022. Accessed August 2023 from: https://www.publichealthontario.ca/en/Laboratory-Services/Test-Information-Index/Public-Beach-Water

Raith, M.R., Ebentier, D.L., Cao, Y., Griffith, J.F. and Weisberg, S.B. (2014). Factors affecting the relationship between quantitative polymerase chain reaction (qPCR) and culture-based enumeration of *Enterococcus* in environmental waters. J. Appl. Microbiol., 116(3): 737–746.

Reicherts, J.D. and Emerson, C.W. (2010). Monitoring bathing beach water quality using composite sampling. Environ. Monit. Assess., 168: 33–43.

Robertson, W., Stanfield, G., Howard, G. and Bartram, J. (2003). Chapter 6: Monitoring the quality of drinking water during storage and distribution. Assessing Microbial Safety of Drinking Water—Improving approaches and methods. World Health Organization and Organisation for Economic Co-operation and Development. IWA Publishing, London, UK. pp. 179–204.

Saleem, F., Edge, T.A. and Schellhorn, H.E. (2022). Validation of qPCR method for enterococci quantification at Toronto beaches: Application for rapid recreational water monitoring. J. Great Lakes Res., 48(3): 707–716.

Saleem, F., Schellhorn, H.E., Simhon, A., Edge, T.A. (2023). Same-day *Enterococcus* qPCR results of recreational water quality at two Toronto beaches provide added public health protection and reduced beach days lost. Can. J. Public Health. 114(4): 676–687.

Schang, C., Henry, R., Kolotelo, P.A., Prosser, T., Crosbie, N., Grant, T., Cottam, D., O'Brien, P., Coutts, S., Deletic, A. and McCarthy, D.T. (2016). Evaluation of techniques for measuring microbial hazards in bathing waters: A comparative study. PLoS ONE, 11(5).

Schoen, M.E., Soller, J.A. and Ashbolt, N.J. (2011). Evaluating the importance of faecal sources in human-impacted waters. Water Res., 45(8): 2670–2680.

Sercu, B., Van De Werfhorst, L. C., Murray, J.L.S. and Holden, P.A. (2011). Cultivation-independent analysis of bacteria in IDEXX quanti-tray/2000 fecal indicator assays. Appl. Environ. Microbiol., 77(2): 627–633.

Shanks, O.C., Sivaganesan, M., Peed, L., Kelty, C.A., Blackwood, A.D., Greene, M.R., Noble, R.T., Bushon, R.N., Stelzer, E.A., Kinzelman, J., Anan'Eva, T., Sinigalliano, C., Wanless, D., Griffith, J., Cao, Y., Weisberg, S., Harwood, V.J., Staley, C., Oshima, K.H., Varma, M. and Haugland, R.A. (2012). Interlaboratory comparison of real-time pcr protocols for quantification of general fecal indicator bacteria. Environ. Sci. Technol., 46(2): 945–953.

Shanks, O. and Mattioli, M. (2018). Use of microbial source tracking tools in waterborne disease outbreak response. SSWR Webinar, Cincinnati, OH, June 27, 2018.

Sheth, N., McDermott, C., Busse, K. and Kleinheinz, G. (2016). Evaluation of *Enterococcus* concentrations at beaches in Door County, WI (Lake Michigan, USA) by qPCR and defined substrate culture analysis. J. Great Lakes Res., 42(4): 768–774.

Shrestha, A. and Dorevitch, S. (2019). Evaluation of rapid qPCR method for quantification of *E. coli* at non-point source impacted Lake Michigan beaches. Water Res., 156: 395–403.

Shrestha, A. and Dorevitch, S. (2020). Slow adoption of rapid testing: Beach monitoring and notification using qPCR. J. Microbiol. Methods, 174.

Sivaganesan, M., Aw, T.G., Briggs, S., Dreelin, E., Aslan, A., Dorevitch, S., Shrestha, A., Isaacs, N., Kinzelman, J., Kleinheinz, G., Noble, R., Rediske, R., Scull, B., Rosenberg, S., Weberman, B., Sivy, T., Southwell, B., Siefring, S., Oshima, K. and Haugland, R. (2019). Standardized data quality acceptance criteria for a rapid *Escherichia coli* qPCR method (draft method C) for water quality monitoring at recreational beaches. Water Res., 156: 456–464.

Stachler, E., Akyon, B., De Carvalho, N.A., Ference, C. and Bibby, K. (2018). Correlation of crAssphage qPCR markers with culturable and molecular indicators of human fecal pollution in an impacted urban watershed. Environ. Sci. Technol., 52(13): 7505–7512.

Stoeckel, D.M. (2005). Selection and application of microbial source tracking tools for water-quality investigations. United States Geological Survey, United States Department of the Interior. 43 pp. Techniques and Methods 2-A3. Available at http://pubs.usgs.gov/tm/2005/tm2a3/pdf/Book2_Collection%20of%20Environmental%20Data.pdf

Staley, Z.R., Boyd, R.J., Shum, P. and Edge, T.A. (2018). Microbial source tracking using quantitative and digital PCR to identify sources of fecal contamination in stormwater, river water, and beach water in a Great Lakes Area of Concern. Appl. Environ. Microbiol. 84(20): e01634–18.

Thapa, S., Stoodley, S., Farnsworth-Hobeck, K. and Dzialowski, A. (2020). Review on effects of holding time exceedances on ambient water quality. Proc. Okla. Acad. Sci. 100:102–111.

Tiwari, A., Ahmed, W., Oikarinen, S., Sherchan, S.P., Heikinheimo, A., Jiang, G., Simpson, S.L., Greaves, J. and Bivins, A. (2022). Application of digital PCR for public health-related water quality monitoring. Sci. Total Environ., 837.

U.S. EPA (1986). Ambient water quality criteria for bacteria—1986. United States Environmental Protection Agency, Washington, DC, January (EPA 440/5-84-02).

U.S. EPA (1996). ICR microbial laboratory manual. Office of Research and Development, United States Environmental Protection Agency, Washington, DC (Report No. EPA/600/R-95/178).

U.S. EPA (1997). Chapter 5: Water quality conditions. In: Volunteer stream monitoring—a methods manual. United States Environmental Protection Agency, Office of Water, Washington, DC. EPA 841-B-97-003. Accessed April 2022 from: https://archive.epa.gov/water/archive/web/html/vms50.html

U.S. EPA (2001a). Manual of methods for virology. Office of Research and Development, United States Environmental Protection Agency, Washington, DC (Report No. EPA/600/4-84/013).

U.S. EPA (2001b). Method 1602: Male-specific (F+) and somatic coliphage in water by single agar layer (SAL) procedure. Office of Water, United States Environmental Protection Agency, Washington, DC (EPA 821-R-01-029).

U.S. EPA (2005a). Method 1623: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. Office of Water, Office of Science and Technology, Engineering and Analysis Division, United States Environmental Protection Agency, Washington, DC (EPA 821-R-01-025).

U.S. EPA (2005b). Microbial source tracking guide. Office of Research and Development, United States Environmental Protection Agency, Cincinnati, OH. 133 pp. (EPA/600-R-05-064).

U.S. EPA (2005c). The EMPACT beaches project. Results from a study on microbiological monitoring in recreational waters. United States Environmental Protection Agency. Office of Research and Development. Cincinnati, OH.

U.S. EPA (2006). National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment; final rule. Fed. Regist., 71(3):678–671; 782–783.

U.S. EPA (2008). Great Lakes beach sanitary survey user manual. Office of Water, United States Environmental Protection Agency, Washington, DC. 81 pp. (EPA/823-B06-001).

U.S. EPA (2010). Sampling and consideration of variability (temporal and spatial) for monitoring of recreational waters. EPA-823-R-10-005. United States Environmental Protection Agency. Office of Water. December 2010. Accessed August 2021 from: https://www.epa.gov/sites/default/files/2015-11/documents/sampling-consideration-recreational-waters.pdf

U.S. EPA (2012a). Method 1615.1: Measurement of enterovirus and norovirus occurrence in water by culture and RT-qPCR. Office of Research and Development, National Exposure Research Laboratory, United States Environmental Protection Agency, Cincinnati, OH (Report No. EPA 600/R-10/181).

U.S. EPA (2012b). Method 1623.1: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. Office of Water, Office of Science and Technology, Engineering and Analysis Division, United States Environmental Protection Agency, Washington, DC (EPA 821-R-01-025).

U.S. EPA (2012c). Recreational water quality criteria. United States Environmental Protection Agency. Office of Water, 820-F-12-058.

U.S. EPA (2013). Acceptability of the EPA qPCR Test at Your Beach. United States Environmental Protection Agency. Office of Water, Washington, DC. EPA-820-R-13-012.

U.S. EPA (2015a). Method 1609.1: Enterococci in water by quantitative polymerase chain reaction (qPCR) with internal amplification control (IAC) assay. United States Environmental Protection Agency. Office of Water, Washington, DC. EPA-820-R-15-099.

U.S. EPA (2015b). Method 1611.1: Enterococci in water by TaqMan® quantitative polymerase chain reaction (qPCR). United States Environmental Protection Agency. Office of Water, Washington, DC. EPA-820-R-15-008.

U.S. EPA (2016). Six key steps for developing and using predictive tools at your beach. United States Environmental Protection Agency. Office of Water, Washington, DC. EPA-820-R-16-001.

U.S. EPA (2018). Method 1642: Male-specific (F+) and somatic coliphage in recreational waters and wastewater by ultrafiltration (UF) and single agar layer (SAL) procedure. United States Environmental Protection Agency. Office of Water, Washington, DC. EPA-820-R-18-001.

U.S. EPA (2019a). Method 1696: Characterization of human fecal pollution in water by HF183/BacR287 TaqMan® quantitative polymerase chain reaction (qPCR) assay. United States Environmental Protection Agency. Office of Water, Washington, DC. EPA-821-R-19-002.

U.S. EPA (2019b). Method 1697: Method 1697: Characterization of human fecal pollution in water by HumM2 TaqMan® quantitative polymerase chain reaction (qPCR) assay®. United States Environmental Protection Agency. Office of Water, Washington, DC. EPA-821-R-19-003.

U.S. EPA (2019c). Other Clean Water Act test methods: Microbiological. United States Environmental Protection Agency. Office of Water, Washington, DC. Accessed November 2021 from: https://www.epa.gov/cwa-methods/other-clean-water-act-test-methods-microbiological

U.S. EPA (2021). Code of Federal Regulations 40 CFR 136. Guidelines establishing test procedures for the analysis of pollutants. Accessed August 18, 2021, from: https://www.ecfr.gov/

U.S. EPA (2022). Environmental modeling community of practice: Virtual Beach (VB). United States Environmental Protection Agency. Center for Exposure Assessment Modeling (CEAM). Athens, GA. Accessed September 8, 2022, from: https://www.epa.gov/ceam/virtual-beach-vb

Viau, E. and Peccia, J. (2009). Evaluation of the enterococci indicator in biosolids using culture-based and quantitative PCR assays. Water Res., 43(19): 4878–4887.

Vogel, L.J., Edge, T.A., O'Carroll, D.M., Solo-Gabriele, H.M., Kushnir, C.S.E. and Robinson, C.E. (2017). Evaluation of methods to sample fecal indicator bacteria in foreshore sand and pore water at freshwater beaches. Water Res., 121: 204–212.

Wade, T.J., Calderon, R.L., Sams, E., Beach, M., Brenner, K.P., Williams, A.H. and Dufour, A.P. (2006). Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness. Environ. Health Perspect., 114(1): 24–28.

Wade, T.J., Calderon, R.L., Brenner, K.P., Sams, E., Beach, M., Haugland, R., Wymer, L. and Dufour, A.P. (2008). High sensitivity of children to swimming-associated gastrointestinal illness: Results using a rapid assay of recreational water quality. Epidemiology, 19(3): 375–383.

Wade, T.J., Sams, E., Brenner, K.P., Haugland, R., Chern, E., Beach, M., Wymer, L., Rankin, C.C., Love, D., Li, Q., Noble, R. and Dufour, A.P. (2010). Rapidly measured indicators of recreational water quality and swimming associated illness at marine beaches: A prospective cohort study. Environ. Health Global Access Sci. Sour., 9(1).

Wade, T.J., Arnold, B.F., Schiff, K., Colford, J.M., Jr., Weisberg, S.B., Griffith, J.F. and Dufour, A.P. (2022). Health risks to children from exposure to fecally-contaminated recreational water. PLoS ONE, 17(4 April).

Wang, D., Yamahara, K.M., Cao, Y. and Boehm, A.B. (2016). Absolute quantification of enterococcal 23S rRNA gene using digital PCR. Environ. Sci. Technol., 50(7): 3399–3408.

Water Quality Research Australia (2009). Chapter 3. Development and implementation of a monitoring program. In: International Guidance Manual for the Management of Toxic Cyanobacteria. Newcombe, G (ed.). Global Water Research Coalition, London, UK. pp. 18–37.

Welker, M. and Raymond, H. (2021). Chapter 12. Fieldwork: Site inspection and sampling. In: Toxic Cyanobacteria in Water (2nd ed.). Chorus, I. and Welker, M. (eds.). World Health Organization. Geneva, Switzerland. pp. 669–688.

Whitman, R. L., Nevers, M. B. and Gerovac, P.J. (1999). Interaction of ambient conditions and fecal coliform bacteria in southern Lake Michigan waters: Monitoring program implications. Nat. Areas J. 19:166–171.

Whitman, R.L. and Nevers, M.B. (2003). Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. Appl Environ Microbiol., 69(9): 5555–5562.

WHO (2000a). Chapter 8: Sanitary inspection and microbiological water quality. In: Monitoring Bathing Waters. Bartram, J. and Rees, G. (eds.) World Health Organization. Geneva, Switzerland.

WHO (2000b). Chapter 9: Approaches to microbiological monitoring. In: Monitoring Bathing Waters. Bartram, J. and Rees, G. (eds.) World Health Organization. Geneva, Switzerland.

WHO (2021). Chapter 7: Beach sand. In: Guidelines on Recreational Water Quality. Volume 1, Coastal and Fresh Waters. World Health Organization. Geneva, Switzerland.

Wiedenmann, A., Krüger, P., Dietz, K., López-Pila, J.M., Szewzyk, R. and Botzenhart, K. (2006). A randomized controlled trial assessing infectious disease risks from bathing in fresh recreational waters in relation to the concentration of *Escherichia coli*, intestinal enterococci, *Clostridium perfringens*, and somatic coliphages. Environ. Health Perspect. 114(2): 228–236.

Wollum, A.G. (2018). Soil sampling for microbiological analysis. In: Methods of soil analysis, part 2: Microbiological and biochemical properties. Soil sampling for microbiological analysis. pp. 1–14.

Zhang, Y., Hong, P-Y., LeChevallier, M.W. and Liu, W.-T. (2015). Phenotypic and phylogenetic identification of coliform bacteria obtained using 12 coliform methods approved by the US Environmental Protection Agency. Appl. Environ. Microbiol., 81(17): 6012–6023.

Zimmerman, A.M., Rebarchik, D.M., Flowers, A.R., Williams, J.L. and Grimes, D.J. (2009). *Escherichia coli* detection using mTEC agar and fluorescent antibody direct viable counting on coastal recreational water samples. Lett. Appl. Microbiol., 49(4): 478–483.

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	*Escherichia coli*
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