Memorandum

Date: April 21, 2025

To: Honorable Chairman Anthony Rodriguez
and Members, Board of County Commissioners

Agenda Item No. 2(B)(3)
May 20, 2025

From: Daniella Levine Cava
Mayor

Subject: Report on Testing Methodologies for Fecal Contamination - Directive No. 193124

Background

The Board of County Commissioners (Board) adopted Resolution No. R-145-20, sponsored by then Commissioner, Daniella Levine Cava, directing the County Mayor or County Mayor's designee to research alternative testing methodologies for fecal indicator bacteria in County waters that provide faster test method results for fecal indicator bacteria than a methodology that does not provide results until a full day later, and provide recommendations on the potential use of alternative methodologies and ways to improve public awareness regarding surface water quality in recreational waters and beaches. Resolution No. R-145-20 further directs the County Mayor or County Mayor's designee to prepare a report summarizing County staff's ongoing efforts to use DNA analysis to determine the source of fecal indicator bacteria in County waters (Attachment 1). The resolution also established County policy that publishes historic and current water quality data, collected as part of the County's ambient groundwater and surface water quality monitoring program, online for public access.

The Department of Regulatory and Economic Resources, Division of Environmental Resources Management (RER-DERM) is currently monitoring Florida House and Senate companion bills HB 73 and SB 156, filed for consideration during the Florida Legislature's 2025 regular session, which may affect how the State conducts bacteriological sampling and influence the recommendations provided in the attached report. The proposed bills transfer duties related to bacteriological sampling of beach waters from the Florida Department of Health (FDOH) to the Florida Department of Environmental Protection (FDEP); establish mandatory reporting by municipalities, counties, and related entities on incidents potentially affecting water quality; create new health advisory signage requirements; direct FDEP to adopt and enforce rules pertaining to bacteriological sampling and the mandatory issuance of health advisories; and provide for a public interagency database for reporting of fecal indicator bacteria data.

Testing Methodology Analysis

Based on an extensive literature research, interviews with local and national experts and laboratory staff, comparing the advantages and limitations of both traditional and molecular methods, sampling time and frequency, water residence time and other environmental conditions, RER-DERM concluded that the current culture-based methods for measuring fecal indicator bacteria remain the most suitable for ambient monitoring programs, while molecular methods, such as DNA analysis are recommended for specific research studies with defined objectives performed in a particular area known to have high fecal indicator bacteria. This is due in part to substantial increases in cost, complex laboratory instrumentation and processing requirements, compared to the traditional methods.

Community Monitoring and Public Awareness Efforts
In addition to FDOH's monitoring of bacteria levels on select County beaches and reporting results online, there are two organizations who have implemented monitoring programs- Surfrider Foundation and Miami Waterkeeper. Both employ outreach and education initiatives to alert or inform the public about the levels of enterococci at their respective sampling locations. Based on staff review of current outreach initiatives, as well as discussion with beachgoers and ocean rescue staff, RER-DERM's recommendations to improve public awareness of water quality on County beaches include the use of social media and other platforms to provide more information about the factors contributing to fecal bacteria pollution that beachgoers can directly contribute to minimizing (for example: presence of dogs and properly disposing of trash). RER-DERM also recommends enhancing signage at beach entry points that note, in part, prohibition of pets, if applicable, with the goal to reduce potential bacterial pollution. RER-DERM will coordinate with the County's Department of Parks, Recreation, and Open Spaces (PROS) on enhanced compliance efforts and beach management activities as needed, and on proposed educational signage, along with potential signage requirements from the State should bills HB 73 and SB 156 be signed into law. These efforts would be applied at beach parks managed by the County, which include Haulover Beach Park and Crandon Beach Park. The County encourages these efforts amongst all jurisdictions managing beaches within Miami-Dade County, such as Sunny Isles, Bal Harbour, Surfside, Miami Beach, and the State.

Public Access and Transparency
To facilitate the public's access to the County's surface water and groundwater quality data, on January 12, 2021 a portal using geographic information systems (GIS) technology was launched at the following link: Surface and Groundwater Quality Viewer. RER-DERM's water quality programs are intended to characterize county-wide, larger-scale patterns and temporal trends in water quality, and help determine background conditions and ranges of normal variability in water quality. The data is refreshed on a weekly basis and will reflect the latest information depending on the occurrence and frequency of sampling events.

RER-DERM worked with the Department's Communications Office to create messaging that was, and continues to be, promoted via social media and in RER's website. Upon the launch of the Surface and Groundwater Quality Viewer RER published a news item promoting it on their homepage. Information about the Viewer was also promoted in the "Weekly News" newsletter and on the Department's social media. Since then, general messaging regarding water quality has continued to be shared on social media, with posts educating the public about the importance of groundwater and how the public can help protect water resources, including cleaning up after their pets and not allowing fecal matter to enter local waterways. These posts are shared on an ongoing basis and can be further tailored to more specifically address activities on local beaches.

Moving forward, RER staff will use already available County resources to implement a public awareness campaign that focuses more specifically on the impact of dog waste, proper trash disposal, and other human contributing factors on water quality. This includes creation of a news item for RER's homepage that offers tips and information, promotion in County newsletters, including Weekly News, Office of Resilience newsletter and PROS newsletter and promotion on social media. Staff will also procure contact information for condo buildings located close to

County beaches and will send targeted messaging to those buildings. This information can also be incorporated into the annual Biscayne Bay Report Card viewer, available online through the County's Biscayne Bay webpage.

Ongoing Research Efforts

Ongoing efforts to use DNA analysis to determine the source of fecal indicator bacteria in County waters have included a stormwater system study in the Fall of 2019 by RER-DERM's Restoration and Enhancement Section. The study utilized microbial source tracking methods to better identify potential sources of fecal pollution (using human and dog markers). The Section also completed a fecal indicator bacteria study on County beaches, from April – August 2020, which was developed following a series of beach advisories issued by FDOH related to high levels of *Enterococcus spp*. The goal of this study was to characterize potential fecal pollution sources on County beaches, as well as select beaches also sampled by FDOH for comparison. Analysis included quantification of *Enterococcus spp*. as well as use of bacteria source tracking. More recently, in response to FDEP establishing Total Maximum Daily Loads (TMDL) for Fecal Coliform for the Little River Canal (C-7) water body, Miami-Dade County hired a consultant to prepare a Bacteria Pollution Control Plan, which was approved by FDEP on April 2, 2024. The County has prioritized the Little River TMDL for bacteria reduction projects during the current NPDES Permit Cycle. Tasks included installation of stormwater treatment devices at fifty (50) locations across three areas of the Little River Basin. The County's contractor will conduct post-installation water quality sampling and analyses to evaluate nutrient reduction efficiency of the devices, scheduled to be completed in June 2025. Based on any results showing higher concentration of bacteria parameters, additional sampling activities will be conducted to identify possible source(s) of pollution. RER-DERM will continue to identify and implement research studies as needed, where the use of DNA analysis to determine the source of fecal indicator bacteria in County waters is most beneficial.

In accordance with Ordinance No. 14-65, this report will be placed on the next available Board meeting agenda. If you have any questions or require additional information, please contact Lisa Spadafina, Assistant Director, Division of Environmental Resources Management in the Department of Regulatory and Economic Resources, at Lisa.Spadafina@miamidade.gov.

Attachment

c: Geri Bonzon-Keenan, County Attorney
Gerald Sanchez, First Assistant County Attorney
Jess McCarty, Executive Assistant County Attorney
Office of the Mayor Senior Staff
Lourdes M. Gomez, Director, Department of Regulatory and Economic Resources
Lisa Spadafina, Assistant Director, Department of Regulatory and Economic Resources
Office of Policy and Budgetary Affairs
Yinka Majekodunmi, Commission Auditor
Basia Pruna, Director, Clerk of the Board
Eugene Love, Agenda Coordinator

Attachment 1

"Testing Methodologies for Fecal Contamination (Resolution No. R-145-20)"

Restoration and Enhancement Section

Division of Environmental Resources Management

Department of Regulatory and Economic Resources

Report

March 2025

TESTING METHODOLOGIES FOR FECAL CONTAMINATION

Executive Summary

Escherichia coli and enterococci are fecal indicator bacteria (FIB) used to assess surface water quality around the world. *Escherichia coli (E.coli)* is generally used to determine the presence of fecal indicator bacteria in freshwater whereas enterococci is used in both fresh and marine waters. Traditional beach water quality monitoring is based on culture-dependent methods using fecal indicator bacteria (enterococci). These methods require culturing enterococci for 24 hours. In accordance with Resolution R-145-20, staff researched potential alternative FIB testing methodologies that employ molecular methods that could potentially replace current culture-dependent methods and may provide faster results.

In the summer of 2000, the State of Florida began its Florida Healthy Beaches Program, which is a monitoring program implemented in over 30 coastal counties managed by the Florida Department of Health (FDOH). FDOH collects weekly seawater samples along the beaches of Miami-Dade County and tests for enterococci using the culture-based membrane filtration method, which requires a 24-hour incubation period. These data are collected every Monday with a follow-up on Tuesday; if results remain out of compliance, an advisory is issued for that beach for the week. Miami-Dade County's surface water quality monitoring program was established in 1979 and collects a variety of physical and chemical parameters, including bacteria, in Biscayne Bay and major canals on a monthly basis. While these data are made part of the state's water quality database, advisories that warn against contact with the water are generally only issued in the instance of a sanitary sewer overflow that leads to surface water impacts.

This report is submitted in partial fulfillment of directives outlined in resolution R-145-20 with the following objectives:

Section 1:

1- **Summarize research findings with respect to alternative testing methodologies** (i.e., alternative testing methodologies being those methodologies for fecal indicator bacteria that provide results in less than one full day).
2- **Provide recommendations on potential use of these methodologies in the County's efforts to monitor and respond to fecal contamination.**
3- **Provide recommendations on ways to improve public awareness regarding surface water quality in recreational waters, including at County beaches.**

Section 2:

4- **Summarize County staff's ongoing efforts to use DNA analysis to determine the source of fecal indicator bacteria in County waters.**

Staff performed an extensive literature review, interviews with renowned experts specialized in the field of molecular biology from the U.S. Environmental Protection Agency (EPA), the National Oceanic and Atmospheric Administration (NOAA), academia, and molecular laboratories. Additionally, interviews with ocean rescue staff were performed to gain their perspective as observers of beach-related activities, as well as interviews with beachgoers to understand their perspective.

Per the Department of Regulatory and Economic Resources, Division of Environmental Resources Management (DERM) staff's research, there are currently two traditional culture-based methods approved by EPA: Membrane Filtration (EPA Method 1600) and Quanti-tray IDEXX method, and both measure live enterococci bacteria. Currently, there are two molecular-based methods also approved and developed by EPA: 1611 & 1609, which employ a newer analytical technique called "quantitative Polymerase Chain Reaction (qPCR)". These molecular methods count DNA which corresponds to both live and dead bacteria, therefore enterococci by culture are not the same as enterococci by qPCR. Regulatory guidelines are set for culture methods, but not set for levels as determined via qPCR.

The main differences between methods are cost, laboratory equipment, simplicity in terms of lab procedures and time consumed, as well as existing regulatory guidelines. Culture-based methods are run with basic lab equipment, they are accurate, simple, not lab intensive and inexpensive (approximately $15 per sample); therefore, it has been used around the world for many years in monitoring programs. In contrast, molecular methods are run in sophisticated laboratories with complex, specialized, and expensive instrumentation, therefore the cost of analysis is higher (approximately $300 per sample or more). These methods require intensive laboratory work and more complex data analysis that require more time than the traditional methods. Their main advantage is their source tracking potential; therefore, the most efficient use and cost justification for microbial source tracking may be for specific studies of areas known to have high fecal indicator bacteria and other specific research studies meant to identify potential sources.

Based on an extensive literature research, interviews with local and national experts and laboratory staff, comparing the advantages and limitations of both traditional and molecular methods, sampling time and frequency, water residence time and other environmental conditions, staff concluded that culture-based method are the most suitable for ambient monitoring programs, while molecular methods and microbial source tracking are recommended for specific research studies with defined objectives performed in a particular area.

In addition to the Florida Department of Health's directive to monitor bacteria levels on select County beaches and report results online, there are two organizations who have implemented monitoring programs- Surfrider Foundation and Miami Waterkeeper. Both employ outreach and education initiatives to alert or inform the public about the levels of enterococci at their respective sampling locations on County beaches. Based on staff review of current outreach initiatives, as well as discussion with beachgoers and ocean rescue staff, staff recommendations on how to improve public awareness of water quality on County beaches includes the use of social media and other platforms to provide more information about the factors contributing to fecal bacteria pollution that beachgoers can directly contribute to minimizing (for example: presence of dogs and properly disposing of trash). RER-DERM also recommends enhancing signage at beach entry points that note, in part, prohibition of pets, if applicable, with the goal to reduce potential bacterial pollution. RER-DERM will coordinate with the County's Department of Parks, Recreation, and Open Spaces (PROS) on enhanced compliance efforts and beach management activities as needed, and on proposed educational signage. These signs will help educate pet owners and provide rationale for the prohibition. These efforts would be applied at beach parks managed by the County, which include Haulover Beach Park and Crandon Beach Park. The County encourages these efforts amongst all jurisdictions managing beaches within Miami-Dade County, such as Sunny Isles, Bal Harbour, Surfside, Miami Beach, and the State.

Regarding ongoing efforts to employ source tracking technology, the Restoration and Enhancement Section of DERM conducted a stormwater system study in the fall of 2019 that included microbial source tracking methods to better identify potential sources of fecal pollution

(using human and dog markers). The Section also completed a fecal indicator bacteria study on County beaches, from April 2020 – August 2020, to avail DERM of the unique opportunity to collect samples during COVID-related beach closures and build upon efforts to date undertaken by university partners. The goal of the study was to characterize potential fecal pollution sources on County beaches as well as select beaches sampled as part of FDOH's program for comparison, under beach-closure conditions. Analysis included quantification of *Enterococcus spp.,* the fecal indicator bacteria typically used in such studies, as well as use of bacterial source tracking technology available through the County's contractor. The impetus for the study was in largely DERM's determination to better understand cause of and/or exacerbation of bacteria values on County beaches at levels that had been regularly out of compliance with established human health and safety standards developed by USEPA and adopted by Florida Department of Environmental Protection (FDEP). This effort followed a series of beach advisories issued by FDOH related to high levels of *Enterococcus spp.* previous summers. Additionally, in response to FDEP establishing a Total Maximum Daily Load (TMDL) for Fecal Coliform for the Little River Canal (C-7), the County hired a consultant to prepare a Bacteria Pollution Control Plan, which was approved by FDEP on April 2, 2024. The County has prioritized the Little River TMDL for bacteria reduction projects during the current NPDES Permit Cycle. Tasks included installation of stormwater treatment devices at fifty (50) locations across three areas of the Little River Basin as part of funding received through FDEP's Biscayne Bay Water Quality Improvement Grant. The County contractor will conduct post-installation water quality sampling and analyses to evaluate nutrient reduction efficiency of the devices, scheduled to be completed in June 2025. Based on any results showing higher concentration of bacteria parameters, additional sampling activities will be conducted to identify possible source(s) of pollution.

Introduction

Growing public awareness of and concern for controlling water pollution nationwide led to enactment of the Federal Water Pollution Control Act in 1948; and together with the Clean Water Act of 1977, and the Water Quality Act of 1987, provide the foundation for protecting the nation's surface waters. Collectively, they are referred to as the "Clean Water Act", which is a comprehensive statute intended to restore and maintain the chemical, physical, and biological integrity of the waters of the United States.

In October 2000, the U.S. Environmental Protection Agency (EPA) established the Beaches Environmental Assessment and Coastal Health (BEACH) Act (U.S. EPA, 2000). This amendment to the Clean Water Act was made in response to potential beachgoer risks from waterborne bacterial pathogens and gastrointestinal illnesses associated with unsafe water quality. The BEACH Act required EPA to develop performance criteria for testing, monitoring, and notifying public users of possible coastal recreation water problems related to fecal indicator bacteria (FIB). This act provided funding for the creation of 35 statewide (including the U.S. territories and Great Lakes) recreational water quality monitoring programs that test fecal indicator bacteria (E. coli or enterococci). As a result, more than 3100 beaches nationwide have been monitored and millions of data points have been generated over the past 15 years (Donahue et al., 2017; https://www.epa.gov/beach-tech/about-beach-act).

In the summer of 2000, the State of Florida began the Florida Healthy Beaches Program (FHBP) as authorized by state legislation (Senate Bill 1412 and House Bill 2145). From 2000 to date, the Florida Department of Health (FDOH) has run a surveillance program across 30 coastal counties, at varying frequencies and times of year. In Miami-Dade County, water samples from select County beaches are collected each Monday and are analyzed for confirmed enterococci by membrane filtration method, which requires a 24-hour incubation period. Resampling occurs the

following day should Monday's results exceed either the single sample standard of 70 colony forming units (CFU) per 100 ml or the 30-day geometric mean of 35 CFU/100 ml (FDOH; Aranda et al., 2016).

Traditional beach water quality monitoring is based on culture-dependent methods using fecal indicator bacteria (FIB) such as E. coli (freshwater) or enterococci (fresh and marine water), but it has been proposed to replace current traditional marine water quality testing methods, which require culturing enterococci for 24 hours, with alternative FIB rapid molecular methods, which may provide faster results (such as the Bacteroidales group) (Sinigalliano et al., 2010). However, most of the new techniques are expensive and require sophisticated detection instruments which may remain a limitation for smaller laboratories and unattractive for field use and early warning systems application. While these alternative methods can contribute greatly to project design and decision-making regarding fecal contamination presence and mitigation, they still need validation before they can be accepted in routine monitoring by regulatory and accreditation bodies.

Fecal pollution can originate from many sources related to human activities, domestic animals, wildlife, agricultural activities, among others. However, general fecal indicator methods using *Escherichia coli* (E. coli) and enterococci for routine water quality monitoring do not discriminate between pollution sources making it difficult to manage sites impacted by more than one source. In an effort to successfully determine potential sources of fecal contamination, resource managers are employing fecal source identification technologies such as molecular source tracking, or MST, to compliment general fecal indicator approaches (Staley et al., 2018). These host-associated methods are deliberately designed to characterize levels of fecal pollution in water samples from a specific animal group. Technologies that target human fecal pollution are of particular interest because exposure to human waste may represent a higher public health risk compared to exposure from most other animal feces such as seagull, dogs, chicken, and swine (Soller et al., 2010). As a result, there is a growing interest in the application of human-associated fecal source identification quantitative real-time PCR (qPCR) technologies for water quality research and management. However, there are currently no standardized approaches for field implementation and interpretation of qPCR data (Cao et al., 2017).

Exceedances of FIB on beaches is a very complex issue to pinpoint and manage because, aside from the various potential sources of pollution mentioned above, there are several environmental factors and beach management activities that may affect presence and abundance of FIB such as beach geomorphology, ocean currents, water residence time, temperature, seaweed, content of organic matter, beach management and grooming practices (availability of restrooms, trash receptacles, solid waste management, seaweed collection, burial, wrack disturbances, etc.), rivers and canals in close proximity, and development. Therefore, to better understand the impact of a of a particular set of conditions in a certain location, a combination of different methodologies are suggested for a research study. It is important to note the difference between the goals of an environmental monitoring program with a research study that specifically seeks to address specific objectives and hypotheses such as identifying potential sources of and activities that can exacerbate exceedances of FIB on beaches. FDOH manages an ongoing monitoring program that does not seek to identify or eliminate sources of FIB but rather reports weekly on exceedances via an online resource. The current study being undertaken by DERM specifically seeks to identify potential sources of and activities that can exacerbate conditions that foster exceedances of FIB on beaches.

This report is submitted in partial fulfillment of directives outlined in resolution R-145-20 with the following objectives:

1. **Summarize research findings with respect to alternative testing methodologies** (i.e., alternative testing methodologies being those methodologies for fecal indicator bacteria that provide results in less than one full day).
2. **Provide recommendations on potential use of these methodologies in the County's efforts to monitor and respond to fecal contamination.**
3. **Provide recommendations on ways to improve public awareness regarding surface water quality in recreational waters, including at County beaches.**
4. **Summarize County staff's ongoing efforts to use DNA analysis to determine the source of fecal indicator bacteria in County waters.**

1. Summarize research findings with respect to alternative testing methodologies.

Culture Methods:

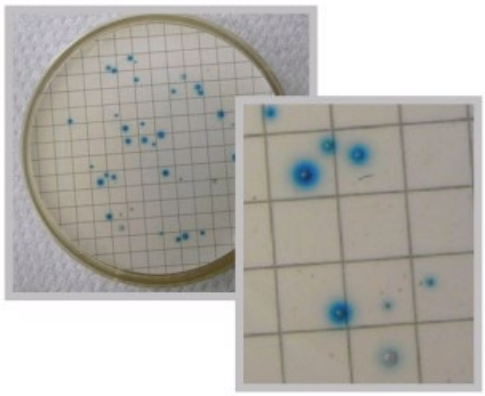
A) Enterococci in Water by Membrane Filtration Using Membrane-Enterococcus Indoxyl-ß-D-Glucoside Agar (mEI) - **EPA Method 1600:**

This method was established by EPA for the Beaches Environmental Assessment Closure and Health (BEACH) Program to prevent the public health risks of infectious diseases caused by microbial organisms in the nation's beaches. It has been used since 1985 in ambient water quality monitoring for the bacteriological quality of recreational waters.

The Membrane Filter method provides a direct count of enterococci bacteria in water based on the development of colonies on the surface of the membrane filter. Enterococci include *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus avium*, and their variants. A water sample is filtered through the membrane, which retains the bacteria. Following filtration, the membrane containing the bacterial cells is placed on petri dishes with a selective medium (mEI Agar) and incubated for 24 hours at 41°C. All colonies (regardless of color) with a blue halo are recorded as enterococci colonies. Magnification and a small fluorescent lamp are used for counting to give maximum visibility of colonies (Figure 1).

Pros:
- Most common and historically used.
- Provide a good correlation with swimming-associated gastrointestinal illness.
- Relatively cheap.
- Can be used to isolate different pathogens.

Cons:
- Require 24-hour incubation time.
- Does not give source tracking.
- Lab intensive.



Colonies of any color that have a blue halo under magnification with a small fluorescent lamp are counted as enterococci.

Fig 1. Enterococci in Water by Membrane Filtration Method.

B) Quanti-tray IDEXX method:

This method is used to quantify Enterococci, Coliforms, *E. coli*, and *Pseudomonas aeruginosa*, depending on the cultured medium selected. For Enterococci analysis, Enterolert (cultured medium) is dissolved in 100 mL (10 ml seawater sample + 90 ml MilliQ water) and poured into a tray divided into 97 wells of two different sizes. The tray is incubated at 41°C for 24 hours and checked with a fluorescent lamp to count the blue wells representing the enterococci colonies (Figure 2). The Standard Methods' Most Probable Number (MPN) approach is then used to determine the number of bacteria in the original sample.

Pros:

- Procedurally simple and fast.
- Simple calculations by using a table.
- Cost effective (~$15 per sample).
- Accurate: detects down to one organism per 100 mL.
- 95% confidence limits better than or comparable to membrane filtration (MF).

Cons:

- 24-hour incubation time.
- Results time is limited by sample submission.

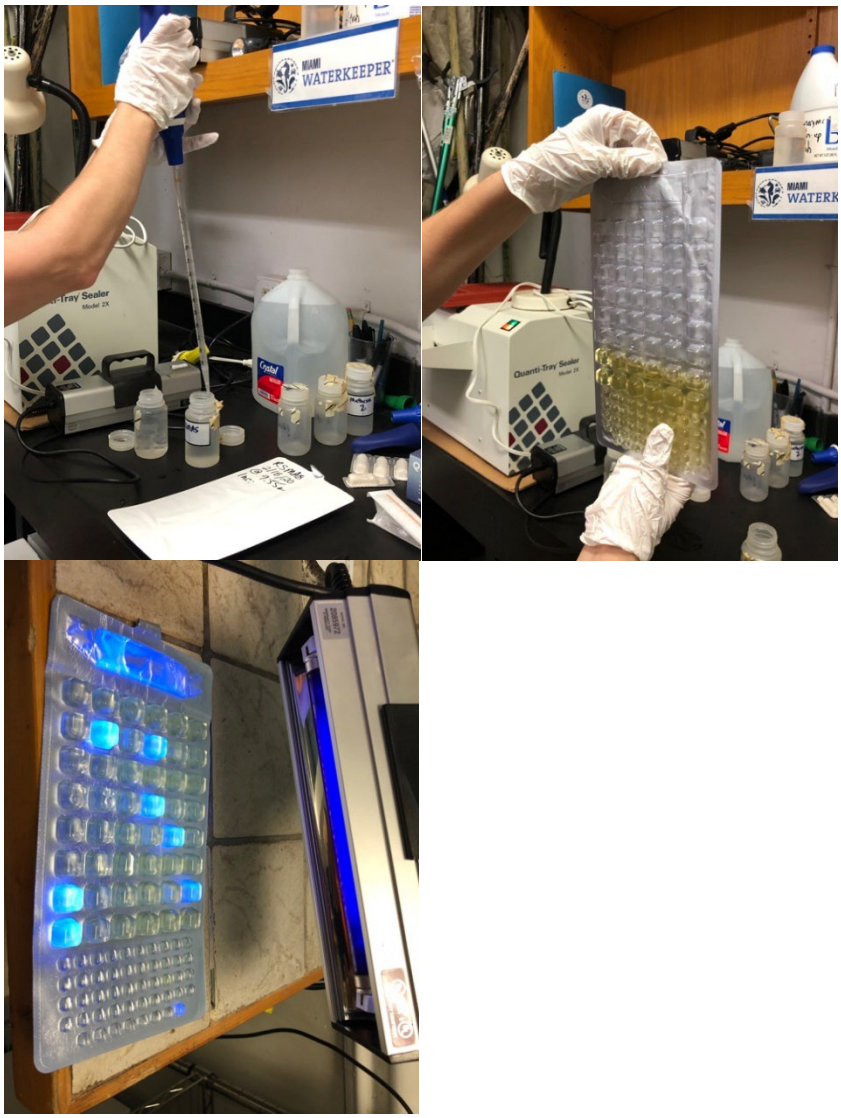


Fig. 2 Quanti-tray - IDEXX method procedure.

Molecular Methods:

Significant amounts of a sample of DNA are necessary for molecular and genetic analyses, and studies of isolated pieces of DNA are nearly impossible without PCR amplification (replication or copy). The analytical technique used for molecular methods is the **Polymerase Chain Reaction (PCR),** which detects, characterizes and quantifies nucleic acids (DNA & RNA) for numerous applications, including the detection of bacteria and viruses. This sophisticated laboratory technique is used to replicate or amplify a segment of DNA by the enzyme "Taq polymerase", which synthetizes two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on. The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one billion exact copies of the original DNA (https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet). (Figures 3 - 6).

The entire cycling process of PCR is automated and can be completed in just a few hours. However, the laboratory work requires an expert analyst for the precision required, sophisticated software, and is time consuming.

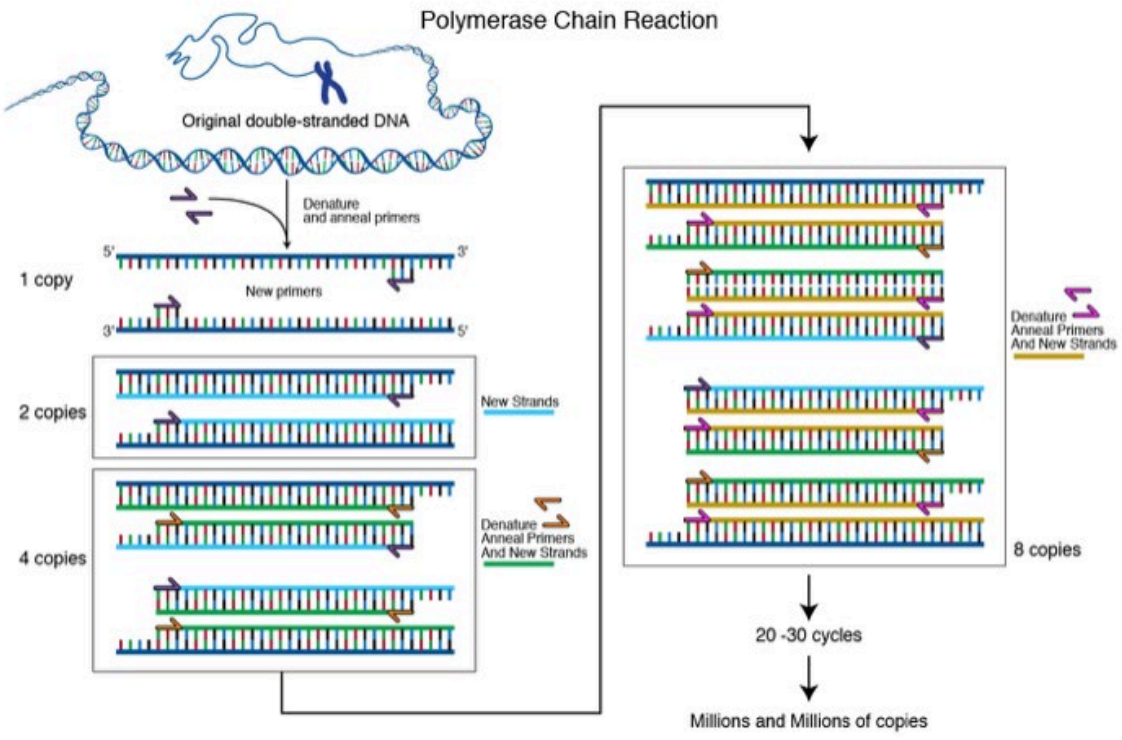


Fig. 3 Polymerase Chain Reaction (PCR).

Fig. 4 Molecular Laboratory with sophisticated equipment.

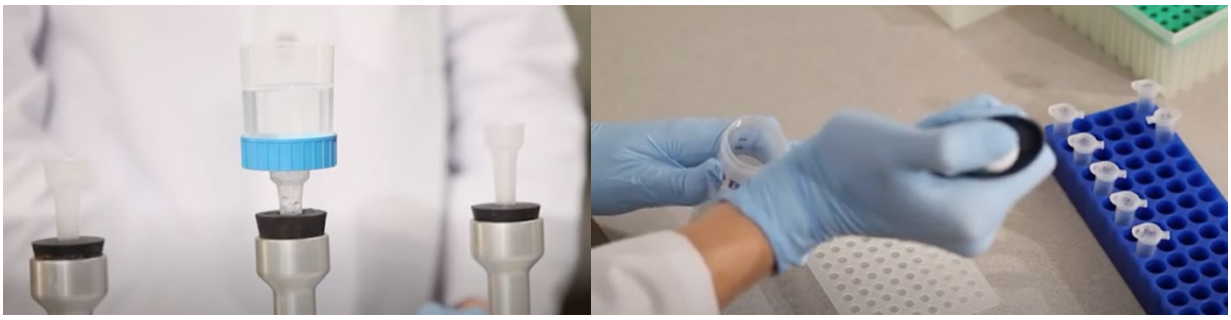


Fig. 5 Sample filtration and DNA extraction.

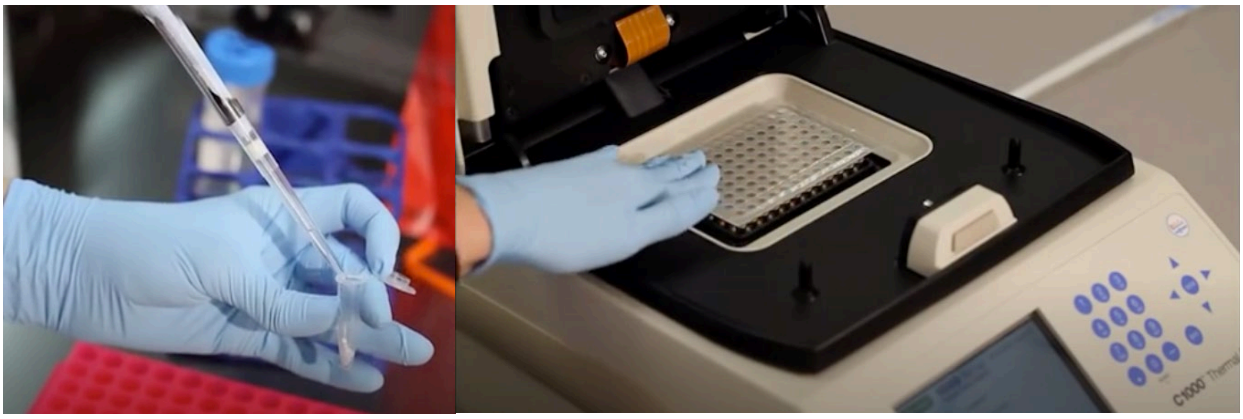


Fig. 6 Thermocycler with qPCR plate.

Real-time PCR or Quantitative Polymerase Chain Reaction (qPCR) is a technique able to simultaneously amplify and detect changes in amplicon concentration. It collects data during PCR amplification by using fluorescence signals emitted by either special probes or DNA binding dyes. This technique has many applications, it is mainly used for qualitative analysis of gene expression, validating DNA microarray results and quantifying virus, bacteria, and fungus (Figures 7 - 9).

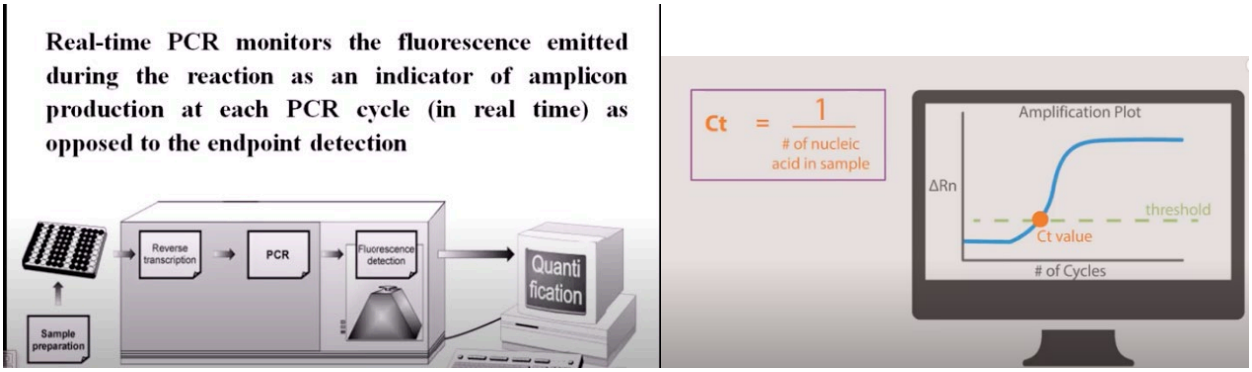


Fig. 7 Instrumentation, software and amplification plot.

Commonly, in PCR, DNA is amplified by 3 repeating steps: denaturation, annealing and elongation. However, in qPCR (**quantitative Polymerase Chain Reaction)**, fluorescent labeling enables the collection of data as PCR progresses. This technique has many benefits due to a range of methods and chemistries available. There are two types of qPCR: Green based method and Taqman probe method.

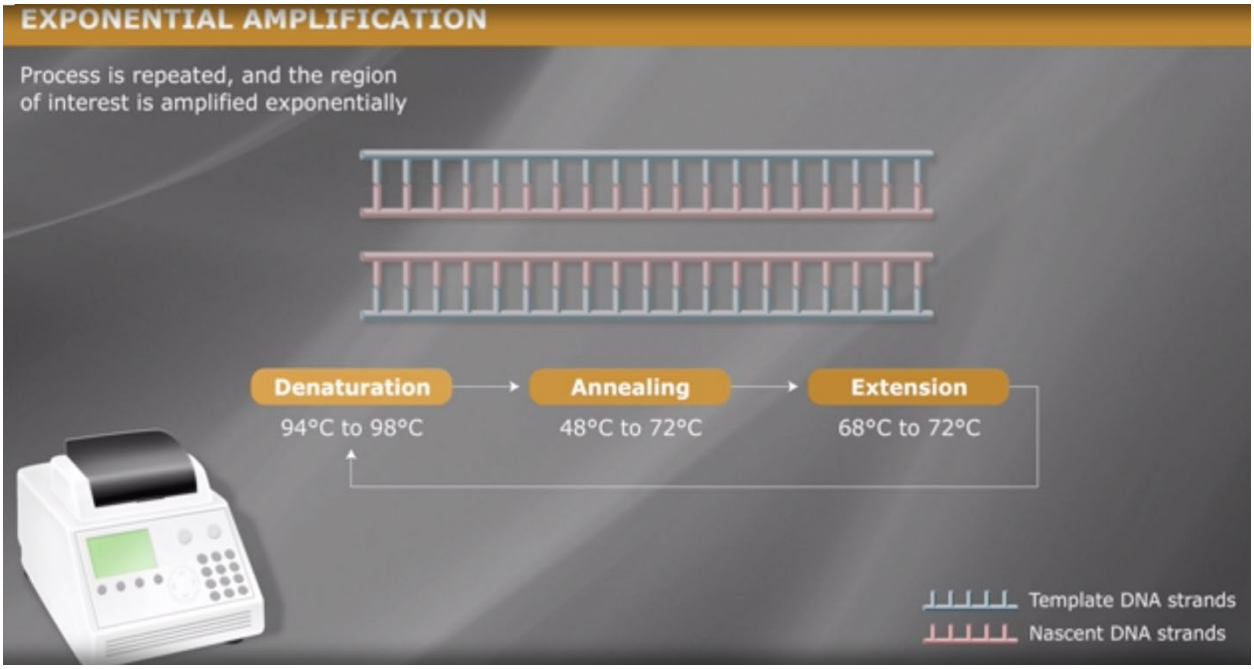


Fig. 8 PCR steps.

a) In **dye-based qPCR** (typically green), fluorescent labeling allows the quantification of the amplified DNA molecules by employing the use of a double stranded DNA binding dye. During each cycle, the fluorescence is measured. The fluorescence signal increases proportionally to the amount of replicated DNA and hence the DNA is quantified in "real time". The disadvantages to dye-based qPCR are that only one target can be examined at a time and that the dye will bind to any double stranded DNA present in the sample.

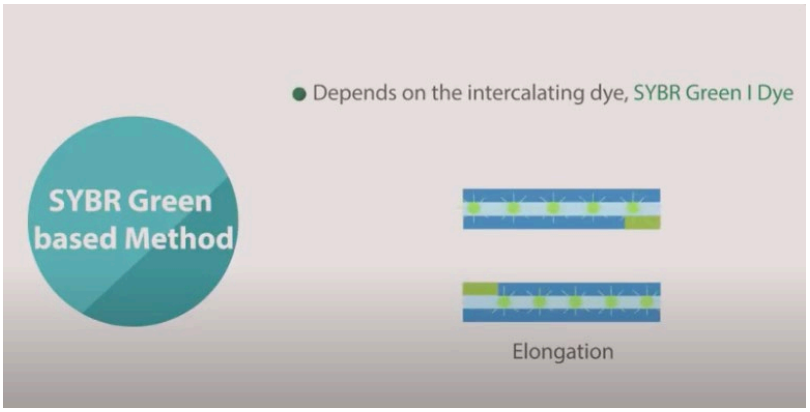


Fig.9 dye-based qPCR.

b) In **probe-based qPCR**, many targets can be detected simultaneously in each sample, but this requires optimization and design of a target specific probe(s), used in addition to primers. There are several types of probe designs available, but the most common type is a hydrolysis probe, which incorporates the use of a fluorophore and quencher. Fluorescence resonance energy transfer (FRET) prevents the emission of the fluorophore via the quencher while the probe is intact. However, during the PCR reaction, the probe is hydrolyzed during primer extension and amplification of the specific sequence it is bound. The cleavage of the probe separates the fluorophore from the quencher and results in an amplification-dependent increase in fluorescence. Thus, the fluorescence signal from a probe-based qPCR reaction is proportional to the amount of the probe target sequence present in the sample. Because probe-based qPCR is more specific than dye-based qPCR, it is often the technology used in qPCR diagnostic assays. The signal is compared to a known quantity of desired genetic material and a calculation yields the amount of starting DNA.

EPA methods 1611 & 1609

EPA has published two analysis methods for enterococci, **EPA Method 1611** (U.S. EPA, 2012b) and **EPA Method 1609** (U.S. EPA, 2013a), that differ primarily in the type of PCR mastermix reagents used. Method 1611 calls for the use of a long-available reagent, TaqMan® Universal Master Mix (UMM), whereas Method 1609 specifies the use of a more recently introduced reagent, TaqMan® Environmental Master Mix (EMM).

Both methods 1611 & 1609 describe a quantitative polymerase chain reaction (qPCR) procedure for the detection of DNA from enterococci bacteria in ambient water matrices based on the amplification and detection of a specific region of the large subunit ribosomal RNA gene (lsrRNA, 23S rRNA) from these organisms. The advantage of this method over currently accepted culture methods that require 24 − 48 hours to obtain results is its relative rapidity. Results can be obtained by this method in 3 − 4 hours (U.S. EPA, 2012).

EPA method 1611:

In Method 1611, water samples are filtered to collect enterococci on polycarbonate membrane filters. Following filtration, total DNA is solubilized from the filter retentate using a bead beater. Enterococci target DNA sequences present in the clarified homogenate are detected by the real time polymerase chain reaction (PCR) technique using TaqMan® Universal Master Mix PCR reagent and the TaqMan® probe system.

The TaqMan® system signals the formation of PCR products by a process involving enzymatic hydrolysis of a fluorogenically-labeled oligonucleotide probe when it hybridizes to the target sequence.

This method is recommended as a measure of ambient marine and fresh recreational water quality. The significance of finding *Enterococcus* DNA target sequences in recreational water samples stems from the association between the density of these sequences and the reported occurrence of gastrointestinal illness associated with swimming observed in recent epidemiological studies (Haugland et al., 2005; Wade et al., 2006).

Pros:

- Source tracking potential.
- Multiple applications per sample.
- Speed of results within 4 hours.

Cons:

- Lab intensive.
- Requires multiple instruments, reagents, calibration curves, spike samples, etc.
- Highly variable recoveries.
- Prone to contamination between multiple steps and pipetting errors.
- Complex data analysis and calculations.
- Greater assay failure rate than EPA method 1609.
- Expensive (~$300 per sample).
- Results time is limited by sample submission.

EPA method 1609:

The method is initiated by filtering a water sample through a membrane filter. Following filtration, the membrane containing the bacterial cells and DNA is placed in a microcentrifuge tube with glass beads and buffer, and then shaken at high speed to extract the DNA into solution. The supernatant is used for PCR amplification and detection of target sequences using the TaqMan® Environmental Master Mix PCR reagent and probe system (U.S. EPA, 2013).

Pros:

- Method 1609, which is based on an environmental mastermix, was found to be superior to Method 1611, which is based on a universal mastermix.
- Source tracking potential.
- Multiple applications per sample.
- Speed of results within 4 hours.

Cons:

- Lab intensive.
- Require sophisticated instrumentation.
- Prone to contamination between multiple steps and pipetting errors.
- Complex data analysis and calculations.

- Highly variable recoveries.
- Expensive (~$300 per sample).
- Results time is limited by sample submission.

Microbial Source Tracking (MST) and DNA Analysis

MST methods are a valuable approach and tool in water quality management to identify and characterize fecal pollution sources, measure fecal pollution levels, clean-up contaminated beaches, and prevent future water contamination events (USEPA, 2018).

Approximately $2.2- $3.7 billion are spent in medical bills each year in the U.S. due to exposure to microbial contaminants in U.S. recreational waters, which have caused an estimated 90 million illnesses per year. Therefore, academia and EPA researchers are using host-associated qPCR methods to identify microbial contamination from key animal groups (human, canine, ruminant (e.g., cattle, deer), avian (i.e., birds), etc.) potentially contaminating recreational waters. Researchers use qPCR methods to make millions of copies of highly diluted fecal bacterial host-associated target genes found in a contaminated water sample to determine if a particular pollution source is present and at what level (USEPA, 2018).

Examples of comprehensive studies using MST methods include the Great Lakes Region and Southern California. In the Great Lakes Region, beaches are sometimes unsafe for recreational use due to elevated fecal contamination from combined sewer overflows, leaking septic systems, contaminated beach sand, pets, local wildlife, or nearby agricultural practices. Having a better understanding of the contamination sources helped resource managers mitigate the effects of the pollution or stop it from entering the water in the first place. This decreased the risk of people being exposed to fecal pollution and minimized the community costs associated with closing and reopening beaches (USEPA, 2018).

Human Markers

Researchers have developed more than a dozen human markers over the last decade (BacH, BacHum-UCD, Bacteroides thetaiotaomicron (BtH), BsteriF1, gyrB, HF183 endpoint, HF183 SYBR, HF183 Taqman®, HumM2, and Methanobrevibacter smithii nifH (Mnif)) (Layton et al., 2013), but one of these, HF183 Taqman®, performed best overall in method evaluation studies. A slightly less sensitive marker, HumM2, also performed well. Both of these markers target *Bacteroides* bacteria in human fecal material. This group of bacteria consistently exhibits host-associated gene sequences. In evaluating human-associated markers, studies have evaluated method sensitivity (i.e., does the method detect human material when it is present in the sample?) and specificity (i.e., does it also detect other fecal sources?) (Griffith et al., 2013).

The HF183 Taqman marker is the best starting point for detecting human fecal material because it provides the best combination of sensitivity and specificity. However, although it performs highest among other markers on sensitivity, it has been shown to occasionally detect ("cross-react" with) chicken or dog feces. If those sources pose a concern in the watershed, or if managers simply desire to add certainty about the results, HF183 can be paired with HumM2 (Griffith et al., 2013).

EPA methods for human markers:

Method 1696: Characterization of Human Fecal Pollution in Water by **HF183/BacR287 TaqMan®** Quantitative Polymerase Chain Reaction (qPCR) Assay **- (2019)**

Method 1697: Characterization of Human Fecal Pollution in Water by **HumM2 TaqMan®** Quantitative Polymerase Chain Reaction (qPCR) Assay® **- (2019)**

Methods 1696 & 1697 are based on the isolation of Bacteroidales on membrane filters, extraction of total DNA, and detection of human-associated target sequences in purified DNA extracts by real time qPCR using TaqMan® Environmental master mix PCR reagent and the TaqMan® probe chemistry. TaqMan® chemistry signals the formation of PCR products by a process involving the enzymatic hydrolysis of a labeled fluorogenic probe that hybridizes to the target sequence (USEPA, 2019).

The difference between the HF183 assay and the HumM2 assay, both target the same bacterial cells of the genus Bacteroides that have co-evolved with human hosts as intestinal normal flora; however, these two assays target different independent genes of these human-host associated Bacteroides cells (Dr. Sinigalliano (NOAA), personal communication 2020).

HF183 and HumM2 target the same human-specific Bacteroides cells, but by amplifying two completely independent genes in those same cells, one a structural gene (the HF183 assay) and one a functional protein-encoding gene (the HumM2). Thus, when both of these independent human Bacteroides targets come from the same sample, it gives a very high level of confidence that human-specific fecal Bacteroidales group cells are present in the sample (Dr. Sinigalliano (NOAA), personal communication 2020).

Selecting appropriate Markers

Reliable source-associated fecal markers are currently available for cattle, dogs, pigs, horses, and gulls. Selecting which non-human source-associated marker to use depends on the hypothesized sources identified during the information-gathering phase of the study. Researchers can choose one or multiple markers as appropriate to answer the study questions. To save cost, stored DNA extracts used to conduct analysis for human-associated markers may be re-analyzed with additional markers. Additional samples may need to be collected to ensure adequate spatial and temporal coverage (Griffith et al., 2013).

Given a limited budget, a balance must be struck between the need to collect an adequate number of samples with the expense of gaining information about an additional source. Take care to run multiple markers only when host sources are numerous enough in the watershed to warrant the added effort and expense. For example, combining non-human markers would be helpful at an off-leash dog beach that also has a resident gull population. In this case, managers might hypothesize that both sources are contributing FIB to the water and seek to better isolate the most significant source (Griffith et al., 2013).

Case Study: ***Use of multiple nonhuman markers to identify significant FIB sources***

Sampling at Arroyo Burro Beach in Santa Barbara, CA revealed an intermittently breaching lagoon as the dominant source of dry weather FIB to the surf zone. Human sources in the lagoon and downstream creeks feeding the lagoon were ruled out using the HF183 and HumM2 markers. The DogBact and Gull2 markers implicated dogs and gulls as the likely nonhuman sources at this beach. While gull markers were detected at low levels in the surf zone and lagoon, dog markers correlated highly with FIB in the surf zone. Dog markers were also present in the lagoon and at a downstream creek location. Load calculations revealed that dog markers measured in the surf zone and lagoon came primarily from the upstream creek. Follow-up actions taken by the City of Santa Barbara helped to limit the input of dog feces from homeowners living along this creek.

2. Recommendations on potential use of molecular methods versus traditional methods for Fecal indicator bacteria monitoring programs.

Florida Department of Health (FDOH) is charged with FIB beach monitoring through its **Florida Healthy Beaches Program**. As part of this program, FDOH collects one seawater sample per week (Monday) at 16 recreational beaches within Miami-Dade County. Sampling time requires six to seven hours to complete. This program uses the traditional method of **Membrane Filtration (EPA Method 1600)** established by EPA in 1985 for monitoring the bacteriological quality of recreational waters to prevent the public health risks of infectious diseases. This method is accurate, easy and inexpensive; therefore, it has been used many years and across the country. It has established standards and measures the concentration of live bacteria that can form colonies and reproduce (i.e., colony forming units or CFU). Samples collected by Miami-Dade County-based FDOH staff are run at the Bureau of Public Health Labs in Miami, Florida.

EPA also approved the **Quanti-tray IDEXX method** for bacteria monitoring in recreational waters for the same reasons listed above in the membrane filtration method (accurate, easy, simple, inexpensive ($15 per sample) and approved standards). It also measures live bacteria, but the quantification is different than the membrane filtration method using a table for providing the Most Probable Number.

Both methods require 24 hours of incubation, but the results are obtained immediately after the incubation without having to perform a complicated quantification using calibration curves or other type of data analysis as in the case of molecular methods, which is one of the biggest advantages for a monitoring program.

In contrast, **molecular methods** are more expensive (~$300 per sample, depending on the contract and the time to provide the results, next day is more expensive than within 5 or 10 days), because they require specialized and sophisticated equipment. Lab requirements are intensive, and calculations and data analysis are complex and consume more time than the traditional methods. These methods count DNA, which corresponds to both live and dead bacteria, therefore enterococci by culture is not the same as enterococci by qPCR. Regulatory guidelines are not set for levels as determined via qPCR.

Due to these reasons, molecular methods are not used in monitoring programs, even if the results may be obtained sooner than incubation methods. However, due to the source tracking potential molecular methods are ideal for use in acute research studies when it is imperative to determine the specific sources of contamination.

Taking into account the sampling time and frequency, the time of laboratory analysis and the residence time of the water, obtaining results in 24 hours is a reasonable time for a monitoring program. Additional studies of ocean currents, residence time, more frequent sampling and additional funding would be required for molecular methods that promise results in four hours to be worthwhile and effective.

To protect human health, some regulations regarding behavior on beaches are in place to varying degrees within certain jurisdictions. Enforcement of these regulations can be difficult, given the volume of beachgoers, environmental conditions, and competing priorities of those conducting enforcement. Regulations include but are not limited to the prohibition of pets and litter management. Based on staff evaluation of case studies, implementation of an effective education program to reduce dog waste, which has been cited in literature as an increasing problem in many coastal cities across the country, may be helpful. This could include plainly worded signage in

several languages at the entrance of the beaches. During staff's research, lifeguards shared their estimation of number of dogs on County beaches that, along Miami Beach, ranged from 20-50 dogs per day on weekdays and 50-80 dogs on weekends along 3 block distance or 0.2 miles (South Point to Nikki Beach). In addition, review and implementation of the most appropriate methods for beach maintenance that are demonstrated to not create or exacerbate the problem is recommended.

Based on staff's research, staff recommends the Quanti-tray IDEXX method or membrane filtration (EPA 1600), currently used, for regular monitoring programs and rapid monitoring in the event of an SSO (Sewage Spill) or any bacteriological water quality event. Both are proven methods for quantification of enterococcus and E.coli, and give results timely enough to be effective, and are cost effective.

3. Provide recommendations on ways to improve public awareness regarding surface water quality in recreational waters, including at County beaches.

Miami-Dade County's surface water quality data, that includes bacteria parameters, are collected monthly and are entered into the state's water quality database. To facilitate the public's access to both the County's surface water and groundwater quality data, a portal using geographic information systems technology was established via the viewer available at the following link: Surface and Groundwater Quality Viewer (arcgis.com). Additional information about this deliverable per the Miami-Dade County Board of County Commissioners Resolution R-145-20, was provided under separate cover.

RER-DERM worked with the Department's Communications Office to create messaging that was, and continues to be, promoted via social media and in RER's website. Upon the launch of the Surface and Groundwater Quality Viewer in January 2021, RER published a news item promoting it on their homepage. Information about the Viewer was also promoted in the "Weekly News" newsletter and on the Department's social media. Since then, general messaging regarding water quality has continued to be shared on social media, with posts educating the public about the importance of groundwater and how the public can help protect water resources, including cleaning up after their pets and not allowing fecal matter to enter local waterways.

Besides Florida Department of Health (FDOH) monitoring bacteria levels on 16 beaches across Miami Dade County, there are two environmental groups (non-governmental organizations) monitoring bacteria on the beaches one day per week using IDEXX method: Surfrider Foundation (19 sampling locations) and Waterkeeper (seven locations). The public has access to these platforms should they wish to stay abreast of the data.

To improve public awareness of water quality on County beaches, staff makes the following recommendations. To reach people who actively use County beaches, larger, clear and informative signs should be placed at the beach entrance, prohibiting dogs, as applicable, proper trash disposal, and restrictions on feeding wildlife, stating the purpose is to reduce potential bacterial pollution, which directly affects the health of beachgoers. These signs will educate beach users and provide rationale for such rules. The presence of dogs on the beaches, despite being banned, is an ongoing problem on the County's beaches. Personal observations and interviews with lifeguards (Ocean Rescue) working on Miami beaches for 10-16 years revealed an increasing number of dogs on the beaches in recent years (up to 80 on the weekends in 0.2 miles) since

people claim dogs as service animals. Lifeguards patrol the beach to constantly advise all owners to keep dogs on the leash, which is a heavy duty for them without achieving the proper order. Both dog owners and lifeguards are not aware of the high fecal bacteria concentrations that dogs have naturally (Figure 15). Dog owners state they didn't see the sign prohibiting dogs on the beaches, the few signs posted are very small and don't explain the real bacteria issue. Therefore, improving signage is recommended. RER-DERM will coordinate with the County's Department of Parks, Recreation, and Open Spaces (PROS) on enhanced compliance efforts and beach management activities as needed, and on proposed educational signage and will investigate funding options. These efforts would be applied at beach parks managed by the County, which include Haulover Beach Park and Crandon Beach Park. The County encourages these efforts amongst all jurisdictions managing beaches within Miami-Dade County, such as Sunny Isles, Bal Harbour, Surfside, Miami Beach, and the State. Education programs have been very effective in many coastal cities in the USA, which have been suffering from this pollution problem (Ervin et al., 2014).

Two examples of an educational approach to inform the public about bacteria concentrations in California beaches due to dogs density on the beaches are these websites:
http://cynthiacudaback.org/Bootkext/Regions/California/Hbeach/Calculation.html
http://www.beachapedia.org/Pet_Waste

Table 1. Numbers of Viable Bacteria Found Per Gram of Feces of Adult Animals[3]
(Median values from 10 animals)

Animal	*E. coli*	*C. perfringens*	Enterococci	*Bacteriodes*	*Lactobacilli*
Cow	20,000	200	200,000	No Data	250
Horse	13,000	No Data	6,300,000	No Data	10,000,000
Pig	3,200,000	4,000	2,500,000	500,000	250,000,000
Sheep	3,200,000	20,000	1,300,000	No Data	7,900
Chicken	4,000,000	250	32,000,000	No Data	320,000,000
Dog	32,000,000	250,000,000	40,000,000	500,000,000	40,000
Cat	40,000,000	25,000,000	200,000,000	790,000,000	1,300,000,000
Human	5,000,000	1,600	160,000	5,000,000,000	630,000,000

Fig. 15 Dogs at the beaches in Miami, and table with bacteria levels in diverse animals and human.

RER staff will use already available County resources to implement a public awareness campaign that focuses more specifically on the impact of dog waste and other human contributing factors on water quality. This includes creation of a news item for RER's homepage that offers tips and information, promotion in County newsletters, including Weekly News, Office of Resilience newsletter and PROS newsletter and promotion on social media. Staff will also procure contact information for condo buildings located close to County beaches and will send targeted messaging to those buildings.

4. Summary of staff's ongoing efforts to use DNA analysis to determine the source of fecal indicator bacteria in County waters.

A) Municipal stormwater system study (2019):

In October 2019, the Restoration & Enhancement Section of DERM implemented a study to better understand the various stormwater systems employed on Miami Beach and, if possible, identify potential causes of exceedances of bacteriological and nutrient standards. From 2015 to the present, chronic bacteria exceedances at several stormwater structures were documented due to potential issues in stormwater and/or sanitary sewer infrastructure and known connections between newer and aging infrastructure that could result in exfiltration/infiltration, as well as potential illicit connections. In this study, enterococci and E. coli concentrations were measured in numerous pumps, as well as human and dog markers using the Microbial Source Tracking (MST) methods for characterizing the fecal pollution sources. Results showed that 50% of the pumps sampled for MST had detected human and dog Bacteroidetes.

B) Fecal Indicator Bacteria (FIB) Study on Miami-Dade County Beaches (2020):

DERM's Restoration & Enhancement Section completed a Fecal Indicator Bacteria (FIB) Beach Study from April 4th 2020 to August 7th, 2020 along 14 County beaches, located in Key Biscayne and Miami Beach. The study was developed following a series of beach advisories issued by FDOH related to high levels of Enterococcus spp. This study was developed during the pandemic (COVID-19), to avail County scientists of the opportunity to evaluate potential presence of bacteria on beaches at a time when beachgoers were prohibited from accessing beaches due to Covid restrictions. This provided an opportunity to evaluate potential sources and factors suggested by previous studies. There were constraints on sampling design frequency, staffing, and lab availability were also limiting factors due to COVID-19. The objectives of this study were to determine the concentrations of *Enterococci* and sources of FIB during the closure of the beaches as a result of COVID-19, and immediately after re-opening, to better understand and identify potential causes of increasing bacteria concentrations, as well as characterize the fecal pollution sources on County beaches, comparatively with and without the presence of human activity on the beach.

Many peer-reviewed studies suggest that the presence of dogs, humans (beachgoers, tourists and homeless) and birds, causes an increase in Fecal Indicator Bacteria (FIB) at recreational beaches. Additionally, selected beach management practices (availability of restrooms, trash receptacles, solid waste management, etc.) and beach grooming practices like seaweed collection, burial, wrack disturbances and disposal, influence the level of bacteria at the beaches. Beach geomorphology, water residence time and currents also play an important role in the level of FIB in the environment.

This study considered the unique opportunity of beaches closed due to the pandemic (COVID-19) when anthropogenic activity was significantly reduced. Sampling beaches during the COVID-19 related closures provided an opportunity to develop a baseline wherein impacts from beachgoers is largely eliminated, allowing multiple potential factors to be tested as potential sources or conditions that exacerbate exceedances of FIB. Sample and data collection during and after the closures of beaches are instrumental to evaluate these factors and sources.

The initial five-month study was relatively inconclusive on the factors and sources influencing the *Enterococci* exceedances as the data were highly variable and fluctuated across time and beach location. It is also not possible to compare the results between the two methods (IDEXX and Microbial Source Tracking) because the results do not directly correlate. It should also be noted that some beaches, Crandon Beach on Key Biscayne for example, still regularly had people and

dogs observed walking on the beach during the absence of police to enforce the beach's closure during the pandemic.

Measurable results from this project include: 1) The determination of FIB reference condition at Miami-Dade County beaches during closures related to COVID-19; 2) A characterization and quantification of FIB concentrations to determine their principal sources and factors that affect them; 3) This unique dataset will foster a greater understanding and more rigorous evaluation of beach management and grooming practices, as they may relate to FIB concentrations on County beaches.

Additionally, RER-DERM collaborated with NOAA's Atlantic Oceanographic & Meteorological Laboratory (AOML) to achieve and expand the goals of this study. Thank you to Dr. Sinigalliano and Dr. Gidley for their contributions, as two top researchers with expertise in the development and deployment of Molecular Microbial Source Tracking.

C) Little River Canal WBID 3287 Bacteria Pollution Control Plan (BPCP) (2024):

A Bacteria Pollution Control Plan (BPCP) is required by the Florida Department of Environmental Protection (FDEP) for any surface water body that has been identified as being fecally / bacterialogically impaired and has an FDEP established Total Maximum Daily Loads (TMDL). The BPCP summarizes preliminary internal potential point source identification activities of bacteria in surface water bodies and provides a source identification sampling plan and a series of reduction strategies to address the impairment and improve overall water quality.

The Little River Canal (C-7) is a primary canal under the jurisdiction of the South Florida Water Management District, and its FDEP established Water Body Identification (WBID) is 3287. FDEP has established TMDL for Fecal Coliform for the Little River Canal (C-7). Miami-Dade County hired a consultant to prepare a BPCP for the Little River water body, which was approved by FDEP on April 2, 2024.

National Pollutant Discharge Elimination System (NPDES) permittees are required by FDEP to select one TMDL water body during each permit cycle and carry out tasks to improve the water quality. Miami-Dade County, as a permittee under NPDES Permit No. FLS000003-004, has prioritized the Little River TMDL for bacteria reduction projects during the current NPDES Permit Cycle. Activities performed included the collection and review of pertinent Geographic information System (GIS) mapping layers, an in-depth review of aerial photographs, an internal preliminary windshield survey of a portion of the WBID, stakeholder Maps on Table (MOT) exercise, and Walk the WBID (WTW) event. The Maps on Table and Walk the WBID exercises offered an efficient and quick starting point to identify possible sources of bacteria contamination, conditions of stormwater and wastewater infrastructure, and hydrological conditions of the water body.

Tasks included installation of stormwater treatment devices at fifty (50) locations across three areas of the Little River Basin. The County's consultant is contracted to conduct post-installation water quality sampling and analyses to evaluate nutrient reduction efficiency of the devices, scheduled to be completed in June 2025. Based on any results showing higher concentration of bacteria parameters, additional sampling activities will be conducted to identify possible source(s) of pollution.

RER-DERM will continue to identify and implement research studies as needed, where the use of DNA analysis to determine the source of fecal indicator bacteria in County waters is most beneficial.

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