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Modifications and findings of the Township of Georgian Bay Coastal Monitoring Program, Bacteriological Testing.

An assessment of Microbial Source Tracking to identify the presence and source of microbial contamination in recreational waters.

February 9th, 2016

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Modifications and findings of the Township of Georgian Bay Coastal Monitoring Program, Bacteriological Testing.

The Coastal Water Monitoring program has been modified over the past 5 years to more effectively deliver the monitoring data needed by the Township of Georgian Bay. As part of that ongoing transition, a number of specific questions have been raised relating to the potential of septic contamination in developed areas of Honey Harbour. Microbial Source Tracking was proposed to investigate these concerns.

What is Microbial Source Tracking?

Quantitative Polymerase Chain Reaction (qPCR) is a technique that has evolved rapidly since its discovery a decade ago and now provides a rapid, cost effective tool to analyse the genetic material of subject organisms. Not only can organisms be detected, but their abundance in the environment can be determined. Microbial Source Tracking (MST) is a technique using qPCR to determine the host animal that contributes fecal pollution to an environment.

In the Township of Georgian Bay program, our interest is in examining sources of potential fecal contamination to a water body. Fecal pollution is a problem that occurs in many water bodies where human populations are concentrated or where animal sources such as dogs, cows, deer, geese, seagulls, and other birds are present.

Serious health risks can arise when fecal contamination is present if it is also associated with human pathogens. To detect this pathogenic contamination directly would be very expensive based upon existing technology and lab test procedures. Currently, common fecal bacteria (those present in the gut and expelled in feces) are used as to indicate the potential presence of these human pathogens. In Ontario, total coliforms (TC) and e.Coli (EC) are used as surrogate measures of fecal contamination. Their presence is used as an indicator of fecal contamination but is not directly a measure of the presence of human pathogens or a direct indicator of health issues. These surrogates exist because the indicator species were easier to collect and culture than trying to isolate the actual pathogens.

The limitation of previous techniques was that the origin of the host species was unknown and so any indicator bacteria found had to be assumed to have human health implications or result from human fecal contamination. However, these indicator bacteria are present in other animals common to the natural environment and, as facultative anaerobes, they can also be found naturally occurring in the sediments and beaches along the shore. Such species then, if found in sampling programs, would give rise to false positive test results. The testing for indicator species requires a 24 hour incubation period post collection. Therefore, in locations where recreational users are present, bathers or beach users may be exposed to possible contaminants or may be excluded from using an uncontaminated location unnecessarily because management actions lag the potential presence or absence of these indicators by a day.

Environmental parameters can be accumulated to better anticipate the likelihood of contamination but are specific to the location and must be incorporated into a predictive computer model in order to provide more direct human health protection and minimize detrimental economic impacts. These programs require

extensive data collection for a couple of years to ensure the predictive model is accurate. This is not economically practical in many areas of the Township.

Application of MST to Management Decisions

The application of MST to risk assessment, total maximum daily load (TMDL) assessment or beach monitoring provides a tool for municipalities and health authorities to identify the source of the pollution. This provides better management decision making and appropriate remediation actions and preventative measures to be implemented.

For example, if it is determined that the major contributor of the fecal pollution of a certain water system is of human origin, then compromised sewage or septic systems in the area would be a likely starting point for remedial action. If the major contributor is cattle then nearby farms should be suspected. If geese are the major source, a municipality may take steps to discourage lawns along shorelines. In some cases it may be determined that sources are part of the natural background and pose no human threat in which case no management actions may be needed.

Sources

Fecal pollution can originate from point and non-point sources. Point sources may include sewage, effluent from wastewater treatment plants and storm water. Nonpoint sources may include leaking septic systems, agriculture or wildlife runoffs where the entry point of contamination to surface waters is not obvious. Nonpoint sources are most apparent after storm events and are the cause of many water system impairments.

There are currently no source tracking tests for Coliforms and EC directly. Coliforms and EC do not have sufficient diversity to allow for this. Other classes of fecal bacteria (typically bacterides) that are not culturable but are present in much higher concentrations within fecal pollution are used due to the specificity of genetic markers present.

TC and EC counts at a variety of sites in Church Bay in Honey Harbour were monitored in 2002 and 2012 by the Ontario Ministry of the Environment and Georgian Bay Forever respectively (Figure 1). These results were published in the previous GBF report to ToGB entitled “Coastal Monitoring Program – 2012 Results” prepared by Hutchinson Environmental.

In both previous studies the recreational water quality was found to be excellent in the study area.

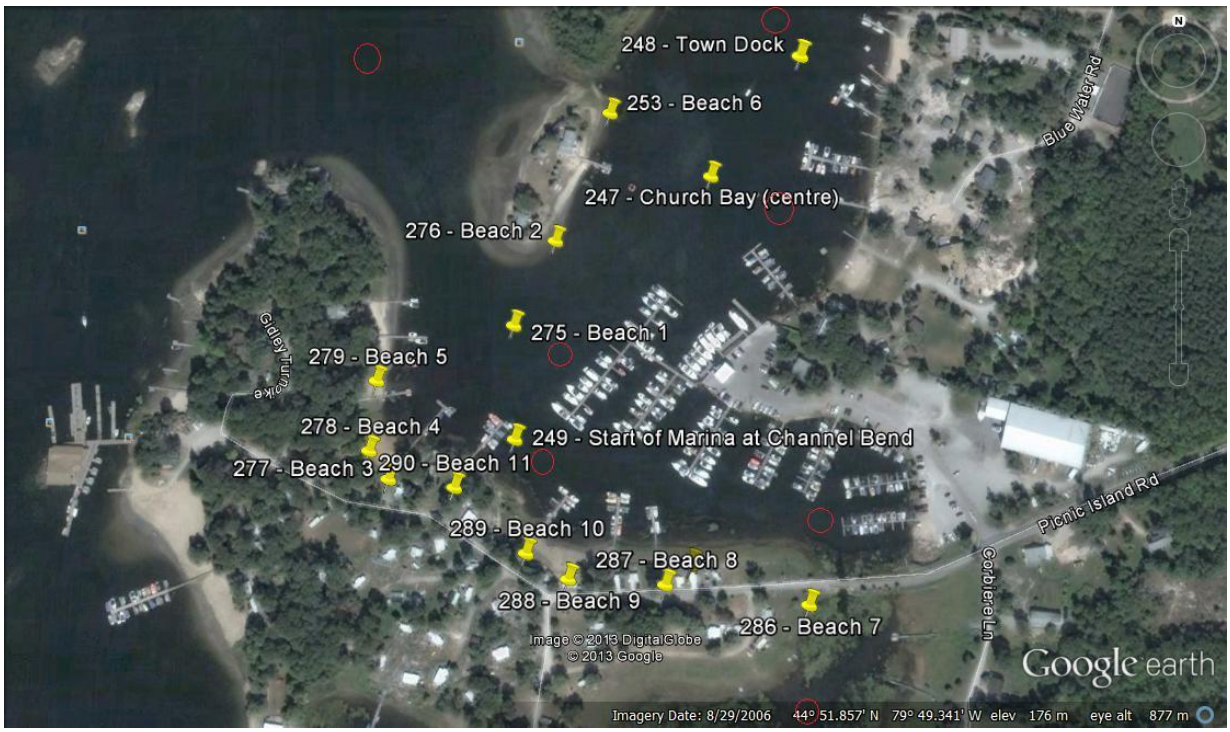

Figure 1- Church Bay Bacterial Monitoring Sites

Sampling

Duplicate samples were collected in Honey Harbour's Church Bay to coincide with previous testing programs by MOE (2002) and GBF (2012). Site 275 (GBF HH1-1,-2) and site 249 (GBF HH2-1,-2) above used representative mid-bay location and a disturbed sediment beach site respectively. Aggregate samples were also taken from several points in Go Home Lake (GBF GH-Ag) including a local community beach site.

One other location (GBF HH3) was sampled at a beach near the Delawana Inn on Delawana Road at GPS coordinates 44.869539N, 79.822935W.

Conditions were light wind and sunny.

Sample Results

The detailed methodology of the testing is provided in the attached lab reports in Appendix A.

No Human, Dog or Bird DNA was detectable in any of the samples collected. According to the lab technician, this means that even if EC or TC were present below the health criteria concentrations, they are likely from a naturally occurring source and not of human, bird or dog origin.

Next Steps

No direct management actions are suggested from the results obtained.

The waters are pristine and did not contain detectable bacterial contamination from the sources suspected, however, MST is a useful qualitative and quantitative new tool that may be applied in future testing to aid in the identification and source of potential microbial contamination. This technique is evolving rapidly and costs are declining for laboratory services as well as in field equipment.

Suggestions for next steps are contained in the detailed lab analysis included in Appendix A.

One option suggested is to run a DNA based test for general fecal bacteria (this will be for fecal bacteridetes, rather than TC or EC). This would indicate whether there is a lot or a little fecal bacteria DNA in the environment.

If there is a lot that would indicate some source other than human, dog or bird sources that might be the contributing load. If there is little that would mean there may not enough fecal DNA to ID sources or that the TC and EC might be naturalized in the environment and not coming from a recent point pollution source. TC is pretty persistent and could potentially re-grow in the environment.

The cost would be $215 ($175 +$40 for quantification) per sample to perform this analysis. Fifty (50) samples would provide adequate special and temporal coverage.

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Appendix A – Lab Results

Human Fecal Toolbox ID™

Detection of the fecal Human gene biomarker for Human fecal contamination by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Georgian Bay Forever
Date Received: August 13, 2014
Date Reported: September 2, 2014

SM #	Client #	Analysis Requested	Species	DNA Analytical Results
SM-4H13001	GBF HH 1-1	Human Bacteroidetes ID	Dorei	Absent
SM-4H13002	GBF HH 1-2	Human Bacteroidetes ID	Dorei	Absent
SM-4H13003	GBF HH 2-1	Human Bacteroidetes ID	Dorei	Absent
SM-4H13004	GBF HH 2-2	Human Bacteroidetes ID	Dorei	Absent
SM-4H13005	GBF GH-Ag	Human Bacteroidetes ID	Dorei	Absent
SM-4H13006	GBF-HH3	Human Bacteroidetes ID	Dorei	Absent

Laboratory Comments

Negative Results
In sample(s) classified as negative, the human-associated *Bacteroidetes* gene biomarker was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis. It is important to note that a negative result does not mean that the sample does not definitely have human fecal contamination. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

In order to strengthen the result, a negative sample should be analyzed further for human fecal contamination with other DNA analytical tests. A list of human fecal ID tests can be found at **www.sourcemolecular.com/human**.

Human Fecal Reference Samples
The client is encouraged to submit samples from the surrounding wastewater facilities and/or septic systems in order to gain a better understanding of the concentration of the human-associated fecal Bacteroidetes genetic marker as well as the concentration of the general fecal Bacteroidetes genetic marker in the geographic region of interest. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **www.sourcemolecular.com/tests**

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DNA Analytical Method Explanation

Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample was homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol.

Amplifications were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing the sample extract, forward primer, reverse primer, probe and an optimized buffer. The following thermal cycling parameters were used: 50°C for 2 min, 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. '

All assays were run in duplicate.

For quality control purposes, a positive control consisting of appropriate genomic DNA and a negative control consisting of PCR-grade water were run alongside the sample(s) to ensure a properly functioning reaction and to reveal any false negatives or false positives. The accumulation of PCR product is detected and graphed in an amplification plot. If the fecal indicator organism is absent in the sample, this accumulation is not detected and the sample is considered negative. If accumulation of PCR product is detected, the sample is considered positive.

Human Bacteroidetes ID™ Species*: B. dorei*

The Human Bacteroidetes IDTM Species: *B. dorei* service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.[1],[2]

The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.[3] The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world[1,3,4] makes the Human Bacteroidetes ID™ Species: *B. dorei* service the primary service for identifying human fecal pollution at Source Molecular.

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[1] Boehm, A., Fuhrman, J., Mrse, R., Grant, S. **Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California**. Environ Sci Technol. 2003 37**:** 673–680.

[2] [2]Bakir, M., Sakamoto, M., Kitahara, M., Matsumoto, M., Benno, Y. **Bacteroides dorei sp. nov., isolated from human faeces**. Int. J. Syst. Evol. Microbiol. 2006 56: 1639–1641.

[3] Bernhard, A., Field, K. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Appl. Environ. Microbiol. 2000b 66: 4571-4574.

[4] [4]Ahmed, w., Masters, N., Toze, S. **Consistency in the host specificity and host sensitivity of the Bacteroides HF183 marker for sewage pollution tracking. Lett. Appl. Microbiol. 2012 55: 283-289.**

Fecal Bacteroidetes are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[5] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID ™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[3,5,6,7,8] Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.[3,6] As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as:
Human Bacteroidetes ID ™ Species: *B. stercoris*,
Human Bacteroidetes ID™ Species: *B. fragilis*, and
Human Bacteroidetes ID™ Species: *B. thetaiotaomicron*.

[5] Scott, T., Rose, J., Jenkins, T., Farrah, S., Lukasik, J. **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. 2002 68: 5796-5803.
[6] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Appl. Environ. Microbiol. 2000a 66: 1587-1594.
[7] Fogarty, L., Voytek, M. **A Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species.** Appl. Environ. Microbiol. 2005 71: 5999-6007.
[8] Dick, L., Bernhard, A., Brodeur, T., Santo Domingo, J., *et al*. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic**

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Bird Fecal ID™

Detection of Bird-associated fecal indicator bacteria by real-time quantitative Polymerase Chain Reaction (qPCR)

Submitter: Georgian Bay Forever
Date Received: August 13, 2014
Date Reported: September 2, 2014

SM #	Client #	Analysis Requested	DNA Analytical Results
SM-4H13007	GBF HH 1-1	Bird Fecal ID	Absent
SM-4H13008	GBF HH 1-2	Bird Fecal ID	Absent
SM-4H13009	GBF HH 2-1	Bird Fecal ID	Absent
SM-4H13010	GBF HH 2-2	Bird Fecal ID	Absent
SM-4H13011	GBF GH-Ag	Bird Fecal ID	Absent
SM-4H13012	GBF-HH3	Bird Fecal ID	Absent

Laboratory Comments

Negative Results
In sample(s) classified as negative, the bird-associated fecal gene biomarker was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis. It is important to note that a negative result does not mean that the sample does not definitely have bird fecal contamination. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Bird Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the bird-associated fecal genetic in the geographic region of interest. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at www.**sourcemolecular.com/tests**

DNA Analytical Method Explanation
Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample was homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol.

Amplifications to detect the target gene biomarker were run in duplicate on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing sample extract, forward primer, reverse primer and an optimized buffer.

For quality control purposes, a positive control consisting of bird fecal DNA and a negative control consisting of PCR-grade water were run alongside the sample(s) to ensure a properly functioning reaction and to reveal any false negatives or false positives. The accumulation of PCR product was detected and graphed in an amplification plot. If the Helicobacter strain was absent in the sample, this accumulation was not detected and the sample was considered negative. If accumulation of PCR product was detected, the sample was considered positive.

Bird Fecal ID™ Theory Explanation

The genus *Helicobacter* is a group of gram-negative, microaerophilic bacteria that were initially classified under the *Campylobacter* genus prior to 1989. Since then, they have been reclassified into the genus *Helicobacter* after 16S rRNA sequencing differentiated them from other *Campylobacter* species. This group of bacteria typically have a spiral, curved or fusiform morphology with multiple flagella allowing them to rapidly maneuver in the intestinal mucous lining of their hosts. *Helicobacter* species colonize the gastrointestinal tract of mammals and birds and are shed in feces. There are approximately 20 strains of *Helicobacter*[1]. Certain strains, such as *Helicobacter pylori*, are pathogenic to humans causing chronic gastritis, peptic ulcers and stomach cancer.

The Bird Fecal ID™ service is designed around the principle that certain DNA sequences contained within strains of the *Helicobacter* genus are specific to wild birds. These *Helicobacter* sequences can be used as indicators of bird fecal contamination. Several species have been isolated from specific animal hosts such as *H. fennelliae* from humans, *H. hepaticus* from mice and *H. felis* from cats and dogs.[1] The Bird Fecal ID™ service targets a bird-associated gene biomarker in *Helicobacter pametensis.*[2] The biomarker is present at different degrees in the feces of various birds including but not limited to gull, goose, chicken, pigeon and duck.

One of the advantages of the Bird Fecal ID™ service is that the entire population of *Helicobacter* of the selected portion of the water sample is screened. As such, this method avoids the randomness effect of selecting isolates off a petri dish.

Accuracy of the results is possible because the method uses real-time (quantitative) PCR DNA technology. Real-time (quantitative) PCR allows small DNA sequences to be amplified exponentially and detected in real-time. DNA amplification is accomplished with small pieces of DNA called primers that are specific to the genomes of interest. Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers. The DNA is replicated to create exact copies of the desired DNA fragment (i.e. the gene biomarker). This process is repeated rapidly many times ensuring an exponential growth in the number of copied DNA.

If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available for detection. With real-time (quantitative) PCR, the desired DNA fragments also bind to a fluorescent dye. Consequently, the more copies of the desired DNA fragments that are made, the stronger the fluorescent signal, thus allowing for a straightforward detection of the targeted gene in real-time.

[1] Goldman, E. and Green, L. H. (2009). *Practical Handbook of Microbiology* (2nd ed) . Boca Raton, FL: CRC Press.

[2] Seymour, C., Lewis, R.G., Kim, M., Gagnon, D.F., Fox, J.G., Dewhirst, F.E., and Paster, B.J. Isolation of *Helicobacter* Strains from Wild Bird and Swine Feces. Appl. Environ. Microbiol. (1994) 60:3, 1025-1028.

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Dog Bacteroidetes ID™

Detection of the fecal Dog gene biomarker for Dog fecal contamination by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Georgian Bay Forever
Date Received: August 13, 2014
Date Reported: September 2, 2014

SM #	Client #	Analysis Requested	DNA Analytical Results
SM-4H13013	GBF HH 1-1	Dog Bacteroidetes ID	Absent
SM-4H13014	GBF HH 1-2	Dog Bacteroidetes ID	Absent
SM-4H13015	GBF HH 2-1	Dog Bacteroidetes ID	Absent
SM-4H13016	GBF HH 2-2	Dog Bacteroidetes ID	Absent
SM-4H13017	GBF GH-Ag	Dog Bacteroidetes ID	Absent
SM-4H13018	GBF-HH3	Dog Bacteroidetes ID	Absent

Laboratory Comments

Negative Results
In sample(s) classified as negative, the dog-associated fecal gene biomarker was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis. It is important to note that a negative result does not mean that the sample does not definitely have dog fecal contamination. Only repeated sampling (both during wet and dry sampling events) will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Dog Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the dog-associated fecal genetic marker in the geographic region of interest. A more precise interpretation would be available to the client if baseline samples are provided.

Additional Testing
A portion of all sample(s) has been frozen and will be archived for 3 months. The client is encouraged to arrange for additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at www.**sourcemolecular.com/tests**

DNA Analytical Method Explanation
Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample was homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing sample extract, forward primer, reverse primer, probe and an optimized buffer. The following thermal cycling parameters were used: 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. All assays were run in duplicate.

For quality control purposes, a positive control consisting of dog fecal DNA and a negative control consisting of PCR-grade water, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives. The accumulation of PCR product is detected and graphed in an amplification plot. If the fecal indicator organism is absent in the sample, this accumulation is not detected and the sample is considered negative. If accumulation of PCR product is detected, the sample is considered positive.

Dog Bacteroidetes ID Theory Explanation

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Dog Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in dog. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in dog[2,3,5,6] As such, these bacterial strains can be used as indicators of dog fecal contamination.

One of the advantages of the Dog Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

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[1] Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

[2] Bernhard, A.E., and K.G. Field (2000a). **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Applied and Environmental Microbiology, 66: 1,587-1,594.

[3] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[4] Kreader, C.A. (1995). **Design and evaluation of Bacteroides DNA probes for the specific detection of human fecal pollution.** Applied and Environmental Microbiology, 61: 1,171-1,179.

[5] Fogarty, Lisa R., Voytek, Mary **A.Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

[6] Dick, Linda K., Bernhard, Anne E., Brodeur, Timothy J., Santo Domingo, Jorge W., Simpson, Joyce M., Walters, Sarah P., Field, Katharine G. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification** Appl. Environ.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the dog *Bacteroidetes* gene biomarker is not present.

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