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ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory

Human Fecal Quantification ID

Detection of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Company A
Date Received: January 2, 2017
Report Generated: January 8, 2017

SM #	Sample ID	Analysis Requested	DNA Analytical Results
SM-6A00001	Sample 1	Human Bacteroidetes ID: Dorei	**Detected**
SM-6A00002	Sample 2	Human Bacteroidetes ID: Dorei	Not Detected
SM-6A00003	Sample 3	Human Bacteroidetes ID: Dorei	Not Detected
SM-6A00004	Sample 4	Human Bacteroidetes ID: Dorei	**Detected**
SM-6A00005	Sample 5	Human Bacteroidetes ID: Dorei	**Detected**
SM-6A00006	Sample 6	Human Bacteroidetes ID: Dorei	Not Detected

Revision 1.2
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Laboratory Comments

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Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) 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.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

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 host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

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 **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

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 sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Human Bacteroidetes ID™ Species*: B. dorei*

The **Human Bacteroidetes ID™ 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.

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*.

[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]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]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.**
[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 Markers for Fecal Source Identification.** Appl. Environ. Microbiol. 2005 71: 3184-3191.