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ISO 17025 Accredited Testing Laboratory

Ruminant Fecal Quantification ID

Detection of the fecal associated Ruminant 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	Ruminant Fecal ID: Target 1	**Detected**
SM-6A00002	Sample 2	Ruminant Fecal ID: Target 1	Not Detected
SM-6A00003	Sample 3	Ruminant Fecal ID: Target 1	Not Detected
SM-6A00004	Sample 4	Ruminant Fecal ID: Target 1	**Detected**
SM-6A00005	Sample 5	Ruminant Fecal ID: Target 1	**Detected**
SM-6A00006	Sample 6	Ruminant Fecal ID: Target 1	Not Detected

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Revision 1.2
Effective Date 11/2/17

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.

Theory Explanation of Ruminant Fecal ID™ Quantification

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 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 Ruminant Fecal Quantification 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 ruminants. Within these *Bacteroidetes*, certain genetic sequences in the *Bacteroides* and *Prevotella* genus have been found in ruminants.[7] As such, these bacterial strains can be used as indicators of ruminant fecal contamination.

One of the advantages of the Ruminant Fecal Quantification 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.

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. Quantitative PCR (qPCR) adds a variant to the PCR process by inserting of a fluorescent probe within the primer set. This fluorescent probe serves as a molecular beacon for the quantification step. During each PCR cycle, quantitative PCR monitors the fluorescence emitted during the reaction. This is done in real-time during the first PCR cycles as a way to quantify the targeted gene. Absolute quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of plasmid DNA containing a known amount of the ruminant-specific biomarker. The Ruminant Fecal Quantification ID™ service uses qPCR to simultaneously confirm and quantify the ruminant- specific fecal *Bacteroidetes* genetic biomarker. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus.

References

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