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Cow Fecal Quantification ID

**Detection and quantification of the fecal associated Cow 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

ND: Not Detected
DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-6A00001	Sample 1	Cow Bacteroidetes ID: EPA 1	6.74E+03	Detected
SM-6A00002	Sample 2	Cow Bacteroidetes ID: EPA 1	ND	Not Detected
SM-6A00003	Sample 3	Cow Bacteroidetes ID: EPA 1	ND	Not Detected
SM-6A00004	Sample 4	Cow Bacteroidetes ID: EPA 1	1.06E+04	Detected
SM-6A00005	Sample 5	Cow Bacteroidetes ID: EPA 1	DNQ	Detected
SM-6A00006	Sample 6	Cow Bacteroidetes ID: EPA 1	ND	Not Detected

Limitation of Damages – Repayment of Service Price

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Preliminary Interpretation of Cow Fecal “Quantification” ID Results

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

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Sample ID	INTERPRETATION	
	Concentration of Cow Fecal Pollution in Sample	Comment
Sample 1	Low Concentration	Low levels of Cow fecal biomarker(s)
Sample 2	Not Detected	Cow fecal biomarker not detected
Sample 3	Not Detected	Cow fecal biomarker not detected
Sample 4	Moderate Concentration	Moderate levels of Cow fecal biomarker(s)
Sample 5	Low Concentration	Low levels of Cow fecal biomarker(s)
Sample 6	Not Detected	Cow fecal biomarker not detected

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: Company A

Report Generated: January 8, 2017

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. Deviations 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 Cow Bacteroidetes “Quantification” ID™

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. Fecal Bacteroidetes are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and Enterococci. Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems.

The Cow Bacteroidetes “Quantification” ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.^{1,2,3,4,5,6} Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately found in cows. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found to be specific to cows.^{2,3} As such, these bacterial strains can be used as indicators of cow 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, real-time quantification 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 cow-specific biomarker. The Cow Bacteroidetes “Quantification” ID™ service uses qPCR to simultaneously confirm and quantify the cattle-specific fecal Bacteroidetes genetic biomarker. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. This data should serve only as a preliminary indicator of cow pollution in the water sample. To strengthen the validity of the results, the Cow Bacteroidetes “Quantification” ID™ service should also be combined with other DNA analytical services such as the Cow Enterococcus “Quantification” ID™.

References

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- ³ Shanks, Orin C., Atikovic, Emma., Blackwood, Denene., Lu, Jingrang., Noble, Rachel T., *et al* (2007). **Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution.** Appl. Environ. Microbiol., 74: 3,745-752.
- ⁴ Kreader, C.A. (1998). **Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water.** Appl. Environ. Microbiol., 64: 4,103-4,105.
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- ⁶ Layton, Alice., McKay, Larry., Williams, Dan., Garrett, Victoria., Gentry, Randall., *et al* (2006). **Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water.** Appl. Environ. Microbiol. 72:6, 4,214-4,224