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Pig Bacteroidetes ID™

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

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SM #	Client #	Analysis Requested	DNA Analytical Results
SM-4G01042	Sample 1	Pig Bacteroidetes ID	Negative
SM-4G01043	Sample 2	Pig Bacteroidetes ID	**Positive**
SM-4G01044	Sample 3	Pig Bacteroidetes ID	**Positive**
SM-4G01045	Sample 4	Pig Bacteroidetes ID	**Positive**

Laboratory Comments

Negative Results
In sample(s) classified as negative, the pig-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 pig 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.

Positive Results
In sample(s) classified as positive, the pig-associated fecal gene biomarker(s) was detected in both test replicates suggesting that pig fecal contamination is present in the water sample(s). All detected concentration levels are classified as “Present”, including trace levels. For more insight on the concentration of the biomarker, quantification is required. The biomarker(s) serve as an indicator of the targeted fecal pollution, but the absence of the biomarker does not signify conclusively the absence of that form of fecal pollution. 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.

Pig 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 pig-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 samples 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 **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 Pig 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.

Theory Explanation of Pig Bacteroidetes 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. 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 Pig Bacteroidetes 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 strains of Bacteroidetes have been shown to be predominately detected in pigs. As such, these bacterial strains can be used as indicators of pig fecal contamination.

One of the advantages of the Pig Bacteroidetes 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. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the pig Bacteroidetes gene biomarker is not present.

References

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[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. Microbiol. 2005 71: 3,184-3,191.