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Preliminary Interpretation of Deer "Quantification" ID™ Results

Detection and Quantification of the Fecal Enterococcus Deer Gene Biomarker for Deer Fecal Contamination by Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: ABC Company
Date Received: October 3, 2011
Date Reported: October 11, 2011

SM #	Client #	Approximate Contribution of Deer Fecal Pollution in Water Sample	Comment
SM 16298	01012011E	**Major Contributor**	High levels of deer biomarker detected
SM 16300	01012011A	Negative	Negative for the deer biomarker

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Deer Enterococcus Quantification ID™

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Submitter: ABC Company
Date Received: October 3, 2011
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SM #	Client #	Analysis Requested	General Marker Quantified*	Deer Specific Marker Quantified*	DNA Analytical Results
SM 16298	01012011E	Deer Enterococcus	5440	344	**Positive**
SM 16300	01012011A	Deer Enterococcus			Negative

*Numbers reported as copy numbers per 100 mL of water

Laboratory Comments
Submitter: ABC Company
Report Date: October 11, 2011

Each submitted water sample was filtered for Enterococci and the DNA was extracted and purified for DNA analysis. All reagents, chemicals and apparatus were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regard, positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminates, including PCR inhibitors.

--Samples 276 (Our Ref: SM 0226) and 278 (Our Ref: SM 0228) tested negative for the fecal Enterococcus deer gene biomarker. It is important to note that a negative result does not mean that the sample definitely has no deer contamination. The biomarkers serve as an indicator of the targeted fecal pollution, but the absence of the biomarker does not mean that you, conclusively, have no form of fecal pollution. Only repeated sampling events (both during wet and dry events) will you be able you to draw more definitive conclusions.

--On the other hand, one can infer the presence of animal sources of fecal pollution since generic forms of fecal Enterococci were present in the negative samples.

--Samples 275 (Our Ref: SM 0225) and 277 (Our Ref: SM 0227) tested positive for the fecal Enterococcus deer gene biomarker suggesting that deer fecal contamination is present in these water samples. Nonetheless only repeated sampling events (both during wet and dry events) will you be able you to draw more definitive conclusions.

DNA Analytical Method Explanation

Each submitted water sample was filtered through 0.45 micron membrane filters. Membranes were placed on mEI agar and incubated at 37ºC for 24-48 hours. Each filter was then placed in a separate, sterile 5ml disposable tube containing a unique mix of beads and lysis buffer. It was then bead beated for 5min. DNA extraction was prepared using the MoBio PowerWater DNA Isolation kit (MoBio, Carlsbad, CA), as per manufacturer's protocol.

Amplifications were run on an Applied Biosystems StepOne real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing sample extract, forward primer, reverse primer, master mix 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. Absolute quantification was 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 containing the deer-specific marker and a negative control containing 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 Deer Enterococcus "Quantification" ID™

Enterococci are a subgroup of Fecal *Streptococci* and are characterized by their ability to grow in 6.5% sodium chloride, at low and elevated temperatures (10°C and 45°C), and at elevated pH (9.5). These microorganisms have been used as indicators of fecal pollution for many years and have been especially valuable in the marine and recreational waters as indicators of potential health risks and swimming-related gastroenteritis.[1] *Enterococci* are benign bacteria when they reside in their normal habitat such as the gastrointestinal tracts of human or animals.

The Deer Enterococcus "Quantification" ID™ service is designed around the principle that certain strains of the *Enterococcus* genus are specific to Deers.[2] These *Enterococci* can be used as indicators of deer fecal contamination. The Deer Enterococcus "Quantification" ID™ service targets the deer-specific Enterococcus biomarker.[2]

One of the advantages of the Deer Enterococcus ID™ service is that the entire cultured population of *Enterococci* in the selected portion of the water sample is screened. This method avoids the randomness effect of selecting isolates. This is a particular advantage for highly contaminated water systems with 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 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 for analysis.

Real-time quantitative PCR (qPCR) adds a variant to the PCR step by including a fluorescent probe or a fluorescent dye to the reaction. 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. The Deer Enterococcus "Quantification" ID™ service uses real-time quantification PCR to simultaneously confirm and quantify the deer-specific enterococcus biomarker.

References

[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] Soule, M., Kuhn, E., Loge, F., Gay, J., Call, D.R. **Using DNA Microarrays to Identify Library-Independent Markers for Bacterial Source Tracking**. Appl. Environ. Microbiol. (2006) 72: 1843-1851.