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Deer Enterococcus ID™

Detection 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 #	Analysis Requested	DNA Analytical Results
SM 16298	01012011E	Deer Enterococcus	Positive
SM 16300	01012011A	Deer Enterococcus	Negative

Laboratory Comments

Each submitted water sample was filtered and the DNA from captured microorganisms was extracted and purified for DNA analysis. All reagents, chemicals and apparatuses 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.

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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 Power Water 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 the sample extract, forward primer, reverse primer, probe 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.

For quality control purposes, a positive control consisting of appropriate genomic DNA and a negative control consisting of PCR-grade water were run alongside the sample(s) to ensure a properly functioning reaction and to 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.

Deer Enterococcus Theory Explanation

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 (10ºC and 45ºC), 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 environment 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. Outside of their normal habitat, *Enterococci* are pathogenic causing urinary tract and wound infections, and life-threatening diseases such as bacteraemia, endocarditis, and meningitis. *Enterococci* easily colonize open wounds and skin ulcers.

Compounding their pathogenesis, *Enterococci* are also some of the most antibiotic resistant bacteria, particularly from human sources. Studies have shown that certain strains of *Enterococci* are resistant to expensive and potent antibiotics such as vancomycin. This is particularly worrisome for the medical community since these antibiotics are given as a last resort to fight severe bacterial infections. Several intrinsic features of the *Enterococcus* genus allow it to survive for extended periods of time, leading to its extended survivability and diffusion. For example, *Enterococci* have been shown to survive for 30 minutes at 60°C and persist in the presence of detergents. As such, the inherent ruggedness of *Enterococcus* confers it a strong tolerance to many classes of antibiotics.

The Deer Enterococcus 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 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* of 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 quantitative PCR (qPCR) DNA technology. qPCR allows 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 and biomarkers 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 detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve indicates that the deer-specific biomarker is not present. To strengthen the validity of the results, repeated sampling events (both during wet and dry events) are recommended.

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.