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Human Enterococcus “Quantification” ID™

Detection and Quantification of the *Enterococcus faecium* esp Human Gene Biomarker for Human Fecal Contamination by Real-Time Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: Raintree Lake Property Owners Association

Submitter #: Ward North

Source Molecular #: SM 10867

Samples Received: September 27, 2005

Date Reported: October 04, 2005

SM #	Client #	Total <i>E. faecium</i> Quantified*	DNA Analytical Results (with relative percentage)**
SM 10867	W. North	BDL ***	Negative (No Human Gene Biomarker Detected) ***

* After 24 hours of incubation at 41°C. Total is copy no./ml of extract. See laboratory comments.

** Percentage is *E. faecium* containing the esp human gene biomarker relative to the total *E. faecium* population in the sample.

*** Below Detection Limit. Detection limit is <1000 copy no./ml of DNA extract.

Laboratory Comments

The submitted water sample was filtered and incubated at 41°C for 24 hours. **Please note that the total *E. faecium* number given in the table above is after cultivation.** Afterwards, the filter was eluted in a buffer. The buffer was centrifuged and DNA was extracted from the resultant pellet. qPCR (i.e.: real-time quantitative PCR) targeting the *E. faecium* esp human gene biomarker was performed on the DNA extract.

All reagents, chemicals and apparatuses were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regards, positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminants, including PCR inhibitors.

Sample Ward North (Our Ref: SM 10867) tested negative for both the *Enterococcus faecium* generic marker and the *Enterococcus faecium* human gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have human contamination. In order to strengthen the result, a negative sample should be analyzed further for human fecal contamination with other DNA analytical tests such as the Human Bacteroidetes ID™ and Human Fecal Virus ID™ services.

DNA Analytical Method Explanation

100 ml of water was filtered through a 0.45micron membrane filter and placed on mEI agar. The sample was incubated for 24 hours at 41°C. The filter was removed, placed in buffer and vortexed vigorously. The filter was removed, the buffer spun to pellet the bacteria, the supernatant removed and the pellet resuspended in a small volume of water. DNA extraction was prepared using the Qiagen DNA extraction kit, as per manufacturers instructions.

Five micro-liter aliquots of purified DNA extraction were used directly as template for subsequent qPCR reactions. All assays were run on an ABI 7700 under the following thermal cycling conditions: 50°C for 10 minutes followed by 40 cycles of 95°C for 10 seconds, 57°C for 10 seconds and 72°C for 1 minute. Default data collection parameters were employed. The Taqman master mix supplied by Applied Biosystems was used with the forward and reverse primers added to a final concentration of 900nM and the probe added to a final concentration of 5uM.

DNA Analytical 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 (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 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 Human Enterococcus “Quantification” ID™ service is designed around the principle that certain strains of the *Enterococcus* genus are specific to humans.^{2,3,4} These *Enterococci* can be used as indicators of human fecal contamination. Strains of *Enterococcus faecium*, *Enterococcus faecalis* and yellow-pigmented *Enterococci* have been shown to be from human sources.^{2,3,4} Within these *Enterococcus spp.* are genes associated with *Enterococci* that are specific to humans.⁵ The Human Enterococcus “Quantification” ID™ service targets the esp human gene biomarker in *Enterococcus faecium*.⁶

One of the advantages of the Human Enterococcus “Quantification” ID™ service is that the entire population of *Enterococci* of the selected portion of the water sample is screened. As such, this method avoids the randomness effect of selecting isolates off a petri dish.

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 for analysis.

Real-time quantitative PCR (qPCR) adds a variant to the PCR step 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.

The Human Enterococcus “Quantification” ID™ service uses real-time quantification PCR to simultaneously confirm and quantify total *Enterococcus faecium* and the esp human gene biomarker in *E. faecium*. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. The results are presented on a computer screen and printout thus avoiding ambiguities in interpretation.

Once each targeted gene is quantified, a relative percentage can be calculated. As such, it has been hypothesized that relative levels of human pollution can be interpreted by the proportion of the esp human gene biomarker found in *E. faecium* relative to the total population of *E. faecium* in the water sample.⁶ Nonetheless this data should serve only as a preliminary indicator of relative human pollution in the water sample. Furthermore, the context of the sample should be taken into account when interpreting the relative percentage provided. To strengthen the validity of the results, the Human Enterococcus “Quantification” ID™ service should also be combined with other DNA analytical services such as the Human Bacteroidetes ID™ and Human Fecal Virus ID™ services.

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 Wheeler, A.L., P.G. Hartel, D.G. Godfrey, J.L. Hill, and Segars W.I. 2002. **Potential of *Enterococcus faecalis* as a human fecal indicator for microbial source tracking.** J Environ Qual. 31(4):1286-93.

³ Bahirathan ML, Puente L, Seyfried P. 1998. **Use of yellow-pigmented enterococci as a specific indicator of human and nonhuman sources of faecal pollution.** Can J Microbiol 44:1066-1071.

⁴ Quednau, M., Ahrne, S., Molin, G. **Genomic Relationships between *Enterococcus faecium* Strains from Different Sources and with Different Antibiotic Resistance Profiles Evaluated by Restriction Endonuclease Analysis of Total Chromosomal DNA Using EcoRI and PvuII.** Appl. Environ. Microbiol. 1999 65: 1777-1780.

⁵ Hammerum, A.M., and L.B. Jensen. 2002. **Prevalence of esp, encoding the enterococcal surface protein, in *Enterococcus faecalis* and *Enterococcus faecium* isolates from hospital patients, poultry, and pigs in Denmark.** J. Clin. Microbiol. 40: 4396.

⁶ Scott, T.M., T.M. Jenkins, J. Lukasik, and J.B. Rose. 2005. **Potential Use of a Host Associated Molecular Marker in *Enterococcus faecium* as an Index of Human Fecal Pollution.** Environ. Sci. Technol. 39: 283-287.

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