SOURCE MOLECULAR CORPORATION
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-268-8363, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

Deer (Elk) Enterococcus ID™

Detection of the *Enterococcus casseliflavus* Deer (Elk) Gene Biomarker for Deer (Elk) Fecal Contamination by Polymerase Chain Reaction (PCR) DNA Analytical Technology

Submitter: ABC Beach Park
Submitter #'s: 775, 776, 777 and 778
Source Molecular #'s: SM 0125, SM 0126, SM 0127 and SM 0128
Samples Received: January 3rd, 2011
Date Reported: January 10th, 2011

SAMPLE

SM #	Client #	Enterococci (CFU/100mL)[7]	DNA Analytical Results
SM 0125	775	45	Deer (Elk) Gene Biomarker Detected
SM 0126	776	150	Negative
SM 0127	777	255	Deer (Elk) Gene Biomarker Detected
SM 0128	778	15	Negative

Laboratory Comments

The submitted water samples were filtered for *Enterococcus spp.* and the *Enterococci* were enumerated on petri plates. Afterwards, the *Enterococci* were eluted and centrifuged directly from the filter 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.

Samples 776 (Our Ref: SM 0126) and 778 (Our Ref: SM 0128) tested negative for the *Enterococcus casseliflavus* deer (elk) gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have deer (elk) contamination. In order to strengthen the result, a negative sample should be analyzed further for deer (elk) fecal contamination with other DNA analytical tests such as the E. coli ID™ service.

Samples 775 (Our Ref: SM 0125) and 777 (Our Ref: SM 0127) tested positive for the *Enterococcus casseliflavus* deer (elk) gene biomarker suggesting that deer (elk) fecal contamination is present in these water samples. The client is nonetheless encouraged to conduct other DNA analytical tests such as the service mentioned above to further confirm the results.

DNA Analytical Method Explanation

For each sample, 100 ml of water was filtered through a 0.45 micron membrane filter and placed on mEI agar. The samples were incubated for 24 hours. Each filter was removed, placed in a buffer and vortexed vigorously. Once the buffer was spun to pellet the bacteria, the supernatant was removed and the pellet was resuspended in a small volume of water. DNA extraction was prepared using the Qiagen DNA extraction kit, as per manufacturer's instructions.

Five micro-liter aliquots of purified DNA extraction were used directly as template for subsequent PCR reactions. Amplification of PCR primers were carried out using HotStarTaq polymerase (Qiagen, Inc.) and master mix, which contained a final concentration of 1.5 mM MgCl2, 150 mM dNTP, and 0.3 mM of each primer.

An Eppendorf Gradient Thermocycler was used with the following cycling parameters: 95 ^{o}C for 15 minutes (to lyse cells and activate polymerase), followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72 ^{o}C for 1 minute and a final extension at 72 ^{o}C for 5 minutes. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (Cambrex, Inc.) and visualized under UV light.

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 ^{o}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.[1,2,3]

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.[4,5] 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 (Elk) Enterococcus ID™ service is designed around the principle that certain DNA sequences contained within strains of the *Enterococcus* genus are specific to deer (elk). These *Enterococci* sequences can be used as indicators of deer (elk) fecal contamination.[6] Strains of *Enterococcus casseliflavus* and *Enterococcus mundtii* have been shown to be from deer (elk) and other ruminant sources.[6] The Deer (Elk) Enterococcus ID™ service targets the deer (elk) gene biomarker in *Enterococcus casseliflavus*.

One of the advantages of the Deer (Elk) Enterococcus 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 detection by gel electrophoresis.

The gel electrophoresis apparatus uses an electrical field to distinguish different DNA fragments according to their molecular weights. Lighter DNA fragments will move farther along the gel than their heavier counterparts. At the end of the procedure different bands of accumulated DNA fragments will aggregate at different parts of the gel. It is this accumulation of DNA fragments that creates a band on the gel. Researchers use these bands to distinguish certain genomes such as the deer (elk) gene biomarker from *Enterococcus casseliflavus.*

These banding patterns confirm or negate the presence of the *Enterococci* deer (elk) gene biomarker. As such, the banding patterns provide a reliable indicator of deer (elk) fecal contamination. To strengthen the validity of the results, the Deer (Elk) Enterococcus ID™ service should be combined with other DNA analytical services such as the E. coli ID™ service.

[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] 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.

[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] Soule, Marilyn, Kuhn, Edward, Loge, Frank, Gay, John, Call, Douglas R. **Using DNA Microarrays To Identify Library-Independent Markers for Bacterial Source Tracking** Appl. Environ. Microbiol. 2006 72: 1843-1851.

[7] **EPA Method 1600: Membrane Filter Test Method for Enterococci In Water (1997).**

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