



Source Molecular Corporation

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Human Fecal Toolbox ID™

Detection of the Fecal Human Gene Biomarker for Human Fecal Contamination by Polymerase Chain Reaction (PCR) DNA Analytical Technology

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

SM #	Client #	Analysis Requested	DNA Analytical Results
SM 16294	01012011A	Human Bacteroidetes ID	Negative
SM 16295	01012011B	Human Bacteroidetes ID	Negative
SM 16296	01012011C	Human Bacteroidetes ID	Positive
SM 16297	01012011D	Human Enterococcus ID	Positive
SM 16298	01012011E	Human Enterococcus ID	Positive
SM 16302	01012011F	Human Enterococcus ID	Positive
SM 16300	01012011A	Human Urine Virus ID	Negative
SM 16301	01012011B	Human Urine Virus ID	Negative
SM 16302	01012011C	Human Urine Virus ID	Positive
SM 16303	01012011D	Human Urine Virus ID	Positive
SM 16304	01012011E	Human Urine Virus ID	Positive
SM 16305	01012011F	Human Urine Virus ID	Positive

Limitation of Damages – Repayment of Service Price

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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 contaminants, including PCR inhibitors.

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-Summary and Evaluation of Client Results

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DNA Analytical Method Explanation

Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 5ml disposable tube containing a unique mix of beads and lysis buffer. It was then bead beaten 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.

Human Bacteroidetes Theory Explanation

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. *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 since it has been shown that they are able to proliferate in soil, sand and sediments.

The Human Bacteroidetes 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 categories of *Bacteroidetes* have been shown to be predominately found in humans. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found to be specific to humans.^{2,3} As such, these bacterial strains can be used as indicators of human fecal contamination.

An advantage of the Human Bacteroidetes ID™ service is that the entire portion of water sampled is filtered for *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates. This is an advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses quantitative PCR (qPCR) DNA technology. qPCR allows quantities of DNA to be amplified into a 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 *Bacteroidetes* genome.

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 indicates that the *Bacteroidetes* Human gene biomarker is not present. To strengthen the validity of the results, the Human Bacteroidetes ID™ service should be combined with other DNA analytical services such as the Human Enterococcus ID™ and Human Urine Virus ID™ services.

References

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Human 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 (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 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 ID™ service targets the esp human gene biomarker in *Enterococcus faecium*.⁶

One of the advantages of the Human 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 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 esp Human gene biomarker is not present. To strengthen the validity of the results, the service should be combined with other DNA analytical services such as the Human Bacteroidetes ID™ and Human Urine Virus ID™ services.

References

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Human Urine Virus Theory Explanation

Polyomavirus is the sole genus in the family Polyomaviridae. These viruses have a 5-kbp double-stranded DNA genome surrounded by a 40- to 50-nm icosahedral capsid.⁵ Human polyomaviruses (HPyVs) (JC virus [JCV] and BK virus [BKV]) are unique to humans and are widespread throughout the population. High titers of these viruses have been documented in municipal sewage. Both viruses are excreted in the urine either simultaneously or individually. Consequently, human polyomaviruses can be used as an indicator of human fecal pollution. JC virus (JCV) and BK virus (BKV) are human viruses classified in the genus *Polyomavirus* of the family *Papovaviridae*.^{1,2,4}

Asymptomatic primary infection usually occurs during childhood, followed by establishment of latent infections in the renal tissue, which can allow the viruses to persist indefinitely. Asymptomatic viruria can occur occasionally or continuously in infected individuals, and high titers of viral particles can be shed in urine from a healthy individual.⁴

HPyVs are shed by more than 50% of immunocompetent individuals. It has been documented that individuals experiencing asymptomatic viruria can shed up to 1.5×10^9 virus particles in a day, and therefore, high titers of HPyVs are generally found in urban sewage.⁴

Polyomaviruses can be detected through a DNA analytical technique called quantitative polymerase chain reaction (qPCR). qPCR allows quantities of DNA from the polyomavirus to be amplified into large number of small copies of DNA sequences.³ This is accomplished with small pieces of DNA called primers that are complementary and specific to the polyomaviruses 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 virus or 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 indicates that the Polyomavirus is not present.

To strengthen the validity of the results, the Human Urine Virus ID™ service should be combined with other DNA analytical services such as the Human Bacteroidetes ID™ and Human Enterococcus ID™ services. Negative results should also be analyzed further for the presence of other human enteric viruses such as enteroviruses and adenoviruses.

References

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