Source Molecular Corporation
Leader in Genetic Microbial Source Tracking
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-268-8363, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

Preliminary Interpretation of Human "Quantification" ID™ Results

Detection and Quantification of the Fecal Human Gene Biomarker for Human Fecal Contamination by Real-Time 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 Human Fecal Pollution in Water Sample	Comment
SM 16297	01012011D	Major Contributor	High levels of human biomarker detected
SM 16298	01012011E	Major Contributor	High levels of human biomarker detected
SM 16300	01012011A	Negative	Negative for human biomarker
SM 16301	01012011B	Negative	Negative for human biomarker
SM 16302	01012011F	Major Contributor	High levels of human biomarker detected
SM 16302	01012011C	Major Contributor	High levels of human biomarker detected

Source Molecular Corporation
Leader in Genetic Microbial Source Tracking
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) (786) 220-0379, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

Human Fecal Pollution Quantification ID™

Detection and Quantification of the Fecal Human Gene Biomarker for Human Fecal Contamination by Real-Time 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	General Marker Quantified*	Human Specific Marker Quantified*	DNA Analytical Results
SM 16297	01012011D	Human Bacteroidetes ID	4.57E+03	9.66E+01	**Positive**
SM 16298	01012011E	Human Bacteroidetes ID	5.44E+03	3.44E+02	**Positive**
SM 16300	01012011A	Human Urine Virus ID			Negative
SM 16301	01012011B	Human Urine Virus ID			Negative
SM 16302	01012011F	Human Bacteroidetes ID	5.65E+03	1.29E+02	**Positive**
SM 16302	01012011C	Human Urine Virus ID	5.97E+02	1.58E+02	**Positive**

*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 and the DNA from captured microorganisms was extracted and purified for DNA analysis. qPCR (i.e.: real-time quantitative PCR) targeting the generic and human specific biomarkers were 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 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.

"

-Summary and Evaluation of Client Results

"

The client is encouraged to submit additional samples from this site both during wet and dry events to get a better understanding of the human fecal pollution contribution. Furthermore, a baseline of raw sewage samples from the surrounding wastewater facilities and/or septic systems would help gain a better understanding of the percentage of the human marker present within the local population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

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 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, 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. Absolute quantification was achieved by extrapolating genome copy numbers from standard curves generated from serial dilutions of Human specific and generic genomic DNA.

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 reveal any false negatives or false positives.

Theory Explanation of Human Bacteroidetes "Quantification" ID™

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 because of new chemical and biochemical findings. Fecal Bacteroidetes are considered for several reasons an interesting alternative to more traditional indicator organisms such as E. coli and Enterococci. Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems.

The Human Bacteroidetes "Quantification" ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[1,2,3,4,5] 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.[1,2] As such, these bacterial strains can be used as indicators of human 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 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". Quantitative PCR (qPCR) adds a variant to the PCR process 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 Bacteroidetes "Quantification" ID™ service uses qPCR to simultaneously confirm and quantify total fecal *Bacteroidetes* and the human-specific fecal Bacteroidetes genetic biomarkers.[5,6] This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus.

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 human gene biomaker found in fecal *Bacteroidetes* relative to the total population of fecal *Bacteroidetes* in the water sample.[5,6] Nonetheless this data should serve only as a preliminary indicator of relative human pollution in the water sample. To strengthen the validity of the results, the Human Bacteroidetes "Quantification" ID™ service should also be combined with other DNA analytical services such as the Human Enterococcus ID™ and Human Urine Virus ID™ services.

References

[1] Bernhard, A.E., and K.G. Field (2000a). **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Applied and Environmental Microbiology, 66: 1,587-1,594.

[2] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[3] Kreader, C.A. (1995). **Design and evaluation of Bacteroides DNA probes for the specific detection of human fecal pollution.** Applied and Environmental Microbiology, 61: 1,171-1,179.

[4] Kreader, C.A. (1998). **Persistence of PCR-detectable Bacteroides distasonis from human feces in river water.** Applied and Environmental Microbiology, 64: 4,103-4,105.

[5] Dick, Linda K., Field, Katharine G.**Rapid Estimation of Numbers of Fecal Bacteroidetes by Use of a Quantitative PCR Assay for 16S rRNA Genes.** Appl. Environ. Microbiol. 2004 70: 5695-5697.

[6] Seurinck, S., T. Defoirdt, W. Verstraete, and S. D. Siciliano. **Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human fecal pollution in freshwater.** Environmental Microbiology 2005 7:2 p. 249.

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 $X10^9$ 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 polymaviruses 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

[1] Bofill-Mas, Silvia, Pina, Sonia, Girones, Rosina **Documenting the Epidemiologic Patterns of Polyomaviruses in Human Populations by Studying Their Presence in Urban Sewage** Appl. Environ. Microbiol. 2000 66: 238-245.

[2] Bofill-Mas, Silvia, Albinana-Gimenez, Nestor, Clemente-Casares, Pilar, Hundesa, Ayalkibet, Rodriguez-Manzano, Jesus, Allard, Annika, Calvo, Miquel, Girones, Rosina **Quantification and Stability of Human Adenoviruses and Polyomavirus JCPyV in Wastewater Matrices** Appl. Environ. Microbiol. 2006 72: 7894-7896.

[3] Chapron, Christopher D., Ballester, Nicola A., Fontaine, Justin H., Frades, Christine N., Margolin, Aaron B. **Detection of Astroviruses, Enteroviruses, and Adenovirus Types 40 and 41 in Surface Waters Collected and Evaluated by the Information Collection Rule and an Integrated Cell Culture-Nested PCR Procedure** Appl. Environ. Microbiol. 2000 66: 2520-2525.

[4] McQuaig, Shannon M., Scott, Troy M., Harwood, Va**l**erie J., Farrah, Samuel R., Lukasik, Jerzy O. **Detection of Human-Derived Fecal Pollution in Environmental Waters by Use of a PCR-Based Human Polyomavirus Assay** Appl. Environ. Microbiol. 2006 72: 7567-7574.

[5] McQuaig, Shannon M., Scott, Troy M., Lukasik, Jerzy O., Paul, John H., Harwood, Valerie J. **Quantification of Human Polyomaviruses JC Virus and BK Virus by TaqMan Quantitative PCR and Comparison to Other Water Quality Indicators in Water and Fecal Samples** Appl. Environ. Microbiol. 2009 75: 3379-3388.