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Preliminary Interpretation of Cow "Quantification" ID™ Results

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

Submitter: ABC Company
Submitter Reference: 01012011A, 01012011B, 01012011C, 01012011D, 01012011E, 01012011F
Source Molecular #'s: SM 16294, SM 16295, SM 16296, SM 16297, SM 16298, SM 16302
Date Received: October 3, 2011
Date Reported: October 11, 2011

SM #	Client #	Approximate Contribution of Cow Fecal Pollution in Water Sample	Comment
SM 16294	01012011A	Negative	Negative for the cow biomarker
SM 16295	01012011B	Negative	Negative for the cow biomarker
SM 16296	01012011C	**Major Contributor**	High levels of cow biomarker detected
SM 16297	01012011D	**Major Contributor**	High levels of cow biomarker detected
SM 16298	01012011E	**Major Contributor**	High levels of cow biomarker detected
SM 16302	01012011F	**Major Contributor**	High levels of cow biomarker detected

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Cow "Quantification" ID™

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

Submitter: ABC Company
Date Received: October 3, 2011
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SM #	Client #	Analysis Requested	General Marker Quantified*	Cow Specific Marker Quantified*	DNA Analytical Results
SM 16294	01012011A	Cow Bacteroidetes ID			Negative
SM 16295	01012011B	Cow Bacteroidetes ID			Negative
SM 16296	01012011C	Cow Bacteroidetes ID	5.97E+02	9.66E+01	**Positive**
SM 16297	01012011D	Cow Enterococcus ID	4570	9.66E+01	**Positive**
SM 16298	01012011E	Cow Enterococcus ID	5.44E+03	3.44E+02	**Positive**
SM 16302	01012011F	Cow Enterococcus ID	5.65E+03	1.29E+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. All reagents, chemicals and apparatus 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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-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 tubes containing a unique mix of beads and lysis buffer and 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 to detect the target gene biomarker 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: 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 target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control consisting of cow fecal DNA or plasmid 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 Cow 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 Cow Bacteroidetes "Quantification" ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[1,2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately found in cows. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found to be specific to cows.[2,3] As such, these bacterial strains can be used as indicators of cow 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. Absolute quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of plasmid DNA containing a known amount of the cow-specific biomarker.

The Cow Bacteroidetes "Quantification" ID™ service uses qPCR to simultaneously confirm and quantify the cattle-specific fecal Bacteroidetes genetic biomarker. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. This data should serve only as a preliminary indicator of cow pollution in the water sample. To strengthen the validity of the results, the Cow Bacteroidetes "Quantification" ID™ service should also be combined with other DNA analytical services such as the Cow Enterococcus "Quantification" ID™.

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Theory Explanation of Human Enterococcus "Quantification" ID™

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 and recreational waters as indicators of potential health risks and swimming-related gastroenteritis.[1,2,3,4,5] Enterococci are benign bacteria when they reside in their normal habitat such as the gastrointestinal tracts of human or animals.

The Cow Enterococcus "Quantification" ID™ service is designed around the principle that certain strains of the Enterococcus genus are specific to cows.[5] These Enterococci can be used as indicators of cow fecal contamination. The Cow Enterococcus "Quantification" ID™ service targets the cattle gene biomarker in Enterococcus hirae.[6] One of the advantages of the Cow Enterococcus "Quantification" ID™ service is that the entire population of Enterococci of the selected portion of the water sample is screened.

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 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. Absolute quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of plasmid DNA containing a known amount of the cow-specific biomarker.

The Cow Enterococcus "Quantification" ID™ service uses qPCR to simultaneously confirm and quantify the cattle-specific fecal Enterococcus genetic biomarker. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus. This data should serve only as a preliminary indicator of cow pollution in the water sample. To strengthen the validity of the results, the Cow Enterococcus "Quantification" ID™ service should also be combined with other DNA analytical services such as the Cow Bacteroidetes "Quantification" ID™.

References

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