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

Chicken Bacteroidetes ID™

Detection of the fecal Bacteroidetes Chicken Gene Biomarker for Chicken Fecal Contamination by QuantitativePolymerase Chain Reaction (qPCR) DNA Analytical Technology

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

SM #	Client #	Analysis Requested	DNA Analytical Results
SM 16302	01012011F	Chicken Bacteroidetes	**Positive**
SM 16302	01012011C	Chicken Bacteroidetes	**Positive**

Laboratory Comments

The submitted water samples were filtered for fecal *Bacteroidetes*. DNA from the filters 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 contaminates, including PCR inhibitors.

Samples 276 (Our Ref: SM 0226) and 278 (Our Ref: SM 0228) tested negative for the fecal *Bacteroidetes* chicken gene biomarker. It is important to note that a negative result does not mean that the sample definitely has no chicken contamination. The biomarkers serve as an indicator of the targeted fecal pollution, but the absence of the biomarker does not mean that you conclusively have no form of fecal pollution. Only repeated sampling events (both during wet and dry events) will you be able you to draw more definitive conclusions.

Samples 275 (Our Ref: SM 0225) and 277 (Our Ref: SM 0227) tested positive for the fecal *Bacteroidetes* chicken gene biomarker suggesting that chicken fecal contamination is present in these water samples. Nonetheless only repeated sampling events (both during wet and dry events) will you be able you to draw more definitive conclusions.

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 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.

For quality control purposes, a positive control consisting of chicken fecal 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. 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.

Chicken 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 1990s it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that are inhabitants 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 purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Chicken 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 detected in chickens. Within these *Bacteroidetes*, certain strains of the *Bacteroides* genus have been found in chickens. As such, these bacterial strains can be used as indicators of chicken fecal contamination.

One of the advantages of the Chicken Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomn effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of 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. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the chicken *Bacteroidetes* gene biomarker is not present.

References

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