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Bird Fecal ID™

Detection of Bird -Associated Fecal Indicator Bacteria by Real-Time Quantitative Polymerase Chain Reaction (qPCR)

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

SM #	Client #	Analysis Requested	DNA Analytical Results
SM 16298	01012011E	Bird Fecal ID	**Positive**
SM 16300	01012011A	Bird Fecal ID	Negative

Laboratory Comments

Each submitted water sample was filtered for *Helicobacter* spp. and DNA was 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.

Sample 1 (Our Ref: SM 12345) tested positive for the Bird Gene Biomarker suggesting that bird fecal contamination is present in this water sample. The biomarkers serve as an indicator of the targeted fecal pollution, but the absence of the biomarker does not signify conclusively the absence of that form of fecal pollution. Only repeated sampling events (both during wet and dry events) will enable you to draw more definitive conclusions.

Sample 2 (Our Ref: SM 12346) tested negative for the Bird Gene Biomarker. It is important to note that a negative result does not mean that the sample does not definitely have bird contamination. Only repeated sampling events (both during wet and dry events) will enable 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 in duplicate 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.

For quality control purposes, a positive control consisting of bird fecal 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 was detected and graphed in an amplification plot. If the Helicobacter strain was absent in the sample, this accumulation was not detected and the sample was considered negative. If accumulation of PCR product was detected, the sample was considered positive.

Bird Fecal ID™ Theory Explanation

The genus *Helicobacter* is a group of gram-negative, microaerophilic bacteria that were initially classified under the *Campylobacter* genus prior to 1989. Since then, they have been reclassified into the genus *Helicobacter* after 16S rRNA sequencing differentiated them from other *Campylobacter* species. This group of bacteria typically have a spiral, curved or fusiform morphology with multiple flagella allowing them to rapidly maneuver in the intestinal mucous lining of their hosts. *Helicobacter* species colonize the gastrointestinal tract of mammals and birds and are shed in feces. There are approximately 20 strains of *Helicobacter*[1]. Certain strains, such as *Helicobacter pylori,* are pathogenic to humans causing chronic gastritis, peptic ulcers and stomach cancer.

The Bird Fecal ID™ service is designed around the principle that certain DNA sequences contained within strains of the *Helicobacter* genus are specific to wild birds. These *Helicobacter* sequences can be used as indicators of bird fecal contamination. Several species have been isolated from specific animal hosts such as *H. fennelliae* from humans, *H. hepaticus* from mice and *H. felis* from cats and dogs.[1] The Bird Fecal ID™ service targets a bird-associated gene biomarker in *Helicobacter pametensis.*[2] The biomarker is present at different degrees in the feces of various birds including but not limited to gull, goose, chicken, pigeon and duck.

One of the advantages of the Bird Fecal ID™ service is that the entire population of *Helicobacter* 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 real-time (quantitative) PCR DNA technology. Real-time (quantitative) PCR allows small DNA sequences to be amplified exponentially and detected in real-time. DNA amplification is accomplished with small pieces of DNA called primers that are specific to the genomes of interest. Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers. The DNA is replicated to create exact copies of the desired DNA fragment (i.e. the gene biomarker). This process is repeated rapidly many times ensuring an exponential growth 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. With real-time (quantitative) PCR, the desired DNA fragments also bind to a fluorescent dye. Consequently, the more copies of the desired DNA fragments that are made, the stronger the fluorescent signal, thus allowing for a straightforward detection of the targeted gene in real-time.

References

[1] Goldman, E. and Green, L. H. (2009). *Practical Handbook of Microbiology* (2nd ed) . Boca Raton, FL: CRC Press.

[2] Seymour, C., Lewis, R.G., Kim, M., Gagnon, D.F., Fox, J.G., Dewhirst, F.E., and Paster, B.J. Isolation of *Helicobacter* Strains from Wild Bird and Swine Feces. Appl. Environ. Microbiol. (1994) 60:3, 1025-1028.