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Testing Laboratory

Bird Fecal Quantification ID

Detection of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Company A
Date Received: January 2, 2017
Report Generated: January 8, 2017

SM #	Sample ID	Analysis Requested	DNA Analytical Results
SM-6A00001	Sample 1	Bird Fecal ID	**Detected**
SM-6A00002	Sample 2	Bird Fecal ID	Not Detected
SM-6A00003	Sample 3	Bird Fecal ID	Not Detected
SM-6A00004	Sample 4	Bird Fecal ID	**Detected**
SM-6A00005	Sample 5	Bird Fecal ID	**Detected**
SM-6A00006	Sample 6	Bird Fecal ID	Not Detected

Revision 1.2
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Laboratory Comments

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Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is 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 and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Bird Fecal ID™ Quantification

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 Quantification 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 Quantification 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 Quantification 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 qPCR DNA technology. qPCR simulataneously confirms and quantifies the bird-associated gene biomarker. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genome to be detected. This qPCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus.
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 bird-associated *Helicobacter* gene biomarker is not present.

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.