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ISO 17025 Accredited Testing Laboratory

Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Miami Beach
Date Received/Processed: June 15, 2021
Report Generated: June 24, 2021

SM #	Sample ID	Date Collected	Time Collected	Analysis Requested	Marker Quantified	Result Unit
SM21F15167	7A	6/15/2021	3:10 PM	Dog_BacCan-UCD	9.52E+04	copies per 100ml
SM21F15168	4D	6/15/2021	3:04 PM	Dog_BacCan-UCD	6.72E+03	copies per 100ml
SM21F15169	4A	6/15/2021	2:54 PM	Dog_BacCan-UCD	1.02E+03	copies per 100ml
SM21F15170	4	6/15/2021	2:56 PM	Dog_BacCan-UCD	1.19E+03	copies per 100ml
SM21F15171	1 Ambient	6/15/2021	3:00 PM	Dog_BacCan-UCD	3.34E+03	copies per 100ml

Reported Results Authorized By: Anda Quintero, Quality Manager

Results reported herein apply only to the sample matrices as received.
Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 1.4
Effective Date 12/12/19

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SM #	Sample ID	Sample Type	Extraction Date	Analysis Date	Amount Processed	Amount Processed Unit	Analytical Volume (ul)
SM21F15167	7A	Water	6/18/2021	6/23/2021	100	ml	2
SM21F15168	4D	Water	6/18/2021	6/23/2021	100	ml	2
SM21F15169	4A	Water	6/18/2021	6/23/2021	100	ml	2
SM21F15170	4	Water	6/18/2021	6/23/2021	100	ml	2
SM21F15171	1 Ambient	Water	6/18/2021	6/23/2021	100	ml	2

Reported Results Authorized By: Anda Quintero, Quality Manager

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Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysi s.

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

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. The most reliable way to accurately test for contamination is to combine genetic testing with scientifically sound and adequate study design appropriate for the water quality questions to be answered or issues to be resolved.

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.

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of Source Molecular Corporation, as well as its agents or representatives, the liability of the company shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to Source Molecular Corp. The company shall not be liable for any damages, either direct or consequential. Source Molecular Corp. provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request. The sample(s) cited in this report may be used for research purposes after an archiving period of 3 months from the date of this report. Research includes, but is not limited to internal validation studies and peer-reviewed research publications. Anonymity of the sample(s), including the exact geographic location will be maintained by assigning an arbitrary internal reference. These anonymous samples will only be grouped by state / province of origin for research purposes. The client must contact Source Molecular in writing within 10 days from the date of this report if he/she does not wish for their submitted sample(s) to be used for any type of future research.

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.