NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION

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February 5, 2019

Mayor Timothy Tenke
City of Glen Cove
9 Glen Street
Glen Cove, NY 11542

RE: Determination of Sources of Fecal Contamination using Real Time Polymerase Chain Reaction (qPCR) Assays from Samples Collected Near Crescent Beach, Nassau County Report

Dear Mayor Tenke:

This letter is to relay the USEPA report entitled, "Determination of Sources of Fecal Contamination using Real Time Polymerase Chain Reaction (qPCR) Assays from Samples Collected Near Crescent Beach, Nassau County Report" dated January 29, 2020. The report is a result of the 2018 and 2019 seasonal sampling conducted by EPA, DEC, and NCDOH to determine if the coliform levels detected in the area surrounding Crescent Beach, leading to beach closures, were of human source. Ultimately it was determined that the elevated levels of fecal indicator bacteria (FIB) are likely due to domestic, wildlife, or other environmental factors, with birds (including gull species) and canines as the most likely contributors.

As a result, the DEC is hereby closing our Crescent Beach investigation. Please contact Alyssa Carroll at (631) 444-0426 with any questions you may have.

Sincerely,

Tony Leung, P.E., M.B.A.
Regional Water Engineer

Encl: January 29, 2020 USEPA Report
Cc: J. Ferretti, USEPA R2
D. Irwin, NCDOH

NEW YORK STATE OF OPPORTUNITY | Department of Environmental Conservation

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Determination of Sources of Fecal Contamination using Real Time Polymerase Chain Reaction (qPCR) Assays from Samples Collected Near Crescent Beach, Nassau County

Prepared for:

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January 29, 2020

1.0 PROJECT SUMMARY

The objective of this project was to evaluate and identify potential sources of bacterial contamination near Crescent Beach, a bathing beach in Glen Cove, NY. Elevated fecal indicator bacteria (FIB) concentrations at Crescent Beach and adjacent surface flow has led to its closure by the Nassau County Department of Health (NCDOH). Prior investigations conducted by the City of Glen Cove as well as Nassau County Department of Public Works in 2013 and 2017 have detailed bacterial contamination throughout the length of a stream/wetlands system which ultimately discharges into Crescent Beach. A dye testing study of several septic systems was performed in 2009, but a definitive source of the bacterial pollution has yet to be identified. Data collected by USEPA and NYSDEC in 2018 exhibited elevated levels of fecal indicator bacteria, but source-specific DNA testing did not detect bacteria from human sources. The purpose of this study was to gain additional information regarding the bacterial concentrations of the area during periods of both wet and dry weather and to confirm conclusions from our previous study that human sources of fecal pollution were not contributing to the observed levels of FIB.

Concentrations of FIB in surface waters are used to assess water quality and the potential impacts to human health if contact with these waters occur. Common FIB used in water quality testing are Enterococcus sp., *Escherichia coli*, and fecal coliform. FIB can originate from a few sources due to a combination of wildlife, agricultural, and human inputs. However, traditional culture-based FIB testing does not discriminate among pollution sources, posing a challenge for regulators and water quality managers tasked to mitigate unacceptable bacteria levels.

Information regarding potential human and non-human fecal pollution sources were initially obtained by sampling 19 locations in the vicinity of Cresent Beach during one wet and one dry weather event in 2018 by USEPA and NYSDEC. Results from these two sampling events indicated the absence of human sewage in any sample (non-detect of human MST markers) and observed increased FIB concentrations throughout the study area after a rain event. Eight samples were selected for additional analysis of target non-human MST markers; 6 of these samples had low level concentrations of a general avian and/or dog DNA marker. For microbial source tracking studies, it is important to evaluate spatial and temporal trends of both the conventional bacterial and MST DNA markers as well as the specificity and sensitivity of the MST assays. MST markers may not be specific to the target, or there may be regional differences in the target source that may not be reflected in the MST marker used. Several MST studies have shown much variation in the presence/ absence of a particular marker over relatively short periods of time. Also, this study provided additional data to account for variability resulting from weather conditions, local land use practices, and wildlife inputs (e.g. bird migrations). Additional data to address these inherit factors in test results are important so that the most effective remediation options can be considered.

During this year's study, conventional bacteria and human DNA marker data was collected during six sampling events over the summer of 2019 at nine sampling stations in the

Crescent Beach area. Results from the six sampling events collected during this study were similar to the initial EPA/NYSDEC project conducted in 2018. Human related fecal contamination was not detected or detected at trace levels and FIB was generally elevated at most sampling areas, with FIB increasing at every station after a rain event. Overall, station SW-09, the surface water station closest to Crescent Beach and the site receiving contributions from all the other upstream locations, had the highest FIB concentrations relative to other sites during both the wet and dry weather sampling events.

The concentration of FIB during the final dry weather sampling event on 8/27/19 were highest at ST-01, a stream sample adjacent to 18 Valley Road and SG-C, an uncapped pipe from the Schein residence near Valley Road. Enterococcus levels in Glen Cove Bay at Station NY529956 met the WQS of 104 MPN/100 mL for bathing beaches in four of the six sampling events, including the three dry weather events.

Data from this study should help guide proposed remediations by local and state agencies with the goal to reopen Crescent Beach. The Town of Glen Cove is currently evaluating several processes to treat the surface water prior to entering Crescent Beach at the area near station SW-09. NYSDOH regulations require that any stream which discharges within 75 feet of a bathing beach must meet water quality standards (WQS) for bacteria or the adjacent beach must be closed. Since the stream discharging to Crescent Beach has regular exceedances, the receiving beach has been closed to swimming for nearly a decade. Data from MST DNA analysis suggest that bacterial inputs throughout the study area are from non-human sources. Extra filters were prepared and frozen from each sampling site and available if any additional DNA tests are requested.

Data from five non-human markers were evaluated on archive filters from Station SW-09 and results are included as Addendum A in this report.

2.0 METHODS

2.1 Sampling Stations

There was a total of 9 sampling locations that were sampled up to six times between June 27, 2019 and August 27, 2019 by NYSDEC Region 1 and Nassau County Department of Health. Three events were classified as dry weather events (no or trace precipitation for at least 48 hours prior to sampling) and three wet weather events (precipitation of approximately 0.25 inches of rain within 48 hours of sampling). The 9 sites are a subset of the 19 stations sampled in 2018 by USEPA and NYSDEC. The stations in 2019 were selected to represent flow from the study area (Figure 1) and included the established NYSDOH Bathing Beach Station NY529956 and a new sampling area, a seep area directly on Crescent Beach (GW-S2).

- SW-09 – End of stream sample, by the gate on the beach.
- GW-S – Groundwater seepage across from 16 Valley Road.

- GW-S2- Seep area directly on Glen Cove Beach, just north of the SW-09 discharge area to Crescent Beach and just north of NYSDOH Bathing Beach Monitoring Location NY529956.
- SW-03 – Outfall picking up water from drains, and sump pumps, on the Katz Cottage property (across from 16-22 Valley Road).
- SW-07 – Stormwater pipe from storm drain on corner of driveway to Schein property rear gate which receives overland flow.
- WO-1 – Stream sample and possible location of where the wetland is discharging into the stream.
- SG-C – Wetland sample from location of previously capped outfall pipe from Schein's residence septic system.
- ST-01 – Stream sample collected during the wet weather event upstream of K-02A.
- Crescent Beach (NY529956) – In the bay, due West of Station SW-09.

2.2 Analytical Methods

The following analyses were performed on each sample collected in this study:

Conventional Microbiological Analyses

Enterococcus via IDEXX Enterolert Quanti-Tray/2000

Escherichia coli/Total Coliform via IDEXX Colilert Quanti-Tray/2000

Fecal Coliform via IDEXX Colilert-18 Quanti-Tray/2000

DNA Based Markers via Real Time PCR (qPCR)

HF183/BacR287 Human Marker TaqMan Assay (SOP based on EPA Method 1696)

HumM2 Human Marker TaqMan Assay (SOP based on EPA Method 1697)

Non-Human Markers

Extra filters were prepared and archived from the 6 sampling events for supplemental DNA analyses on targeted non-human markers (general avian, geese, gull, dog, ruminant) if funds are available and data provides value to the study.

2.3 Conventional Microbiological Analyses

For the conventional bacteriological analysis, approved methods (CFR Part 136) were used in accordance with EPA Region 2 Standard Operating Procedures. Fecal Indicator Bacteria (FIB) concentrations were measured using defined substrate technology Most Probable Number tests via IDEXX Enterolert (for Enterococci), IDEXX Colilert (for total coliform and *E. coli*), and IDEXX Colilert-18 (for fecal coliform).

Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliform group can occur in human feces, but some can also be present in animal manure, soil, submerged wood and even outside the human body. The usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found are fecal and human in origin. Public health offices have used total coliforms and fecal coliforms as indicator organisms since the 1920's. For drinking water, total coliforms are still used because their presence indicates contamination of a water

supply by an outside source. For recreational waters, including bathing beaches, total coliforms are no longer recommended as an indicator.

Fecal coliforms are a subset of total coliform bacteria (Figure 2) and are more fecal specific in origin. However, this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper wastes. Fecal coliform measurements are still used in many state standards for pathogens based on the designated use of the water body (primary contact, secondary contact, etc.)

Escherichia coli or *E. Coli* is a specific fecal coliform commonly found in the intestines of warm-blooded animals, including humans. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination.

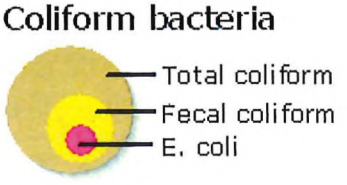


Figure 2. Relationship among Total Coliform, Fecal Coliform and *E. coli*

Enterococci are a subgroup within the fecal streptococcus group (not a coliform). Enterococci are distinguished by their ability to survive in salt water and they have been found to more closely mimic many pathogens than do the other indicator organisms. Enterococci are typically more human-specific than the larger fecal streptococcus group. Enterococci are an indicator organism in national and many state water quality standards in marine and freshwater recreational waters.

2.4 DNA Based Markers via Real Time PCR (qPCR)

The DNA (qPCR) method was performed in accordance with the EPA Region 2 Standard Operating Procedure adapted from a draft method developed by the USEPA Office of Research and Development (USEPA 2016). HumM2 and HF183/BacR287 are human-associated gene sequences from the bacteria genus *Bacteroides* commonly used as a marker in source tracking of human associated fecal inputs. Bacteroides human-associated gene sequences are commonly found in the feces of humans and other sources of human fecal pollution (sewage and septage). Although DNA from these organisms is sometimes present in other animal sources in low concentrations, their presence in environmental water is typically an indication of human fecal pollution.

Figure 1. Map of Crescent Beach MST Sampling Locations, June-August 2019, Glen Cove, NY

3.0 RESULTS

3.1 Conventional Bacteria Results Summary

Total Coliform results at all 9 stations exceeded the NYSDEC Surface Water and Groundwater Quality Standards for Class SA Waters among all 6 sampling events (Table 1). Total coliform levels were generally higher during the wet weather sampling events as compared to the three events sampled during dry weather. Total coliforms were the lowest in the station in Glen Cove Bay at Crescent Beach (Table 2). Since Total coliforms are still included with numerical standard in NYS WQS, they were included in this study. However, most of this report will focus on the three direct indicators of direct fecal inputs – Fecal Coliforms/*E. coli* and Enterococci.

Table 1. NYSDEC Surface Water and Groundwater Quality Standards and Groundwater Effluent Limitations for Class SA waters (6 NYCRR Part 703).

Total Coliform	70 MPN/100 mL
Fecal Coliform	200 MPN/100 mL
Enterococcus	35 MPN/100 mL
E. coli	No Standard Established
Human DNA Markers	No Standard Established

Table 1a. NYSDOH Bathing Beach Bacteria Water Quality Standard.

Enterococcus	104 MPN/100 mL

Station SW-09 had the highest concentrations of Fecal Coliform and *E. coli* during the first four sampling events (Table 2). The highest concentration of Fecal coliform and *E. coli* were measured during the last dry weather sampling event at Station ST-01 (adjacent to 18 Valley Road). Station SG-C, a wetland sample of an uncapped outfall pipe near the Schein residence on Valley Road, was sampled only during periods of flow. High concentrations of Enterococci were measured during the last 2 sampling events at this station (8620 MPN 100/mL on 8/20/19 and >241,960 MPN/100 mL during a dry weather sampling event on 8/27/19) (Table 2). Midway through the study period, a seep area directly on Crescent Beach was observed. This station (GW-S2) showed elevated results of FIB during the wet weather sampling event on 8/20/19. There was a trend deviation as *E. coli* results at this station as *E. coli* concentrations were almost 4x greater than Fecal Coliform results. *E. coli* bacteria are a subset of Fecal coliforms; however, fecal coliforms and *E. coli* were measured using independent tests. Natural test and bacterial variability can contribute to this type of result.

Overall, the amount of FIB was higher during the three wet weather sampling events as compared to the three dry weather events except for Station ST-01 and SG-C on 8/27/19 as depicted by Fecal Coliform concentration shown in Figure 3.

Table 2. Summary of Total, Fecal, *E. coli* and Enterococci Bacteria, Crescent Beach June-August 2019

NY529956/Glen Cove Bay				N 40°52.581'; W 73°38.532'		
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	52	10	110	31	148	< 10
Fecal Coliform (MPN/100 mL)	170	10	100	30	260	41
E. coli (MPN/100 mL)	201	41	411	984	850	1354
Total Coliform (MPN 100/mL)	6131	2909	4190	1882	4106	4710
HumM2 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
HF183 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
Rainfall within 24 hours (Inches)	0	0.23	0.53+	0	0.29+	0

SW-09/End of Stream Sample by Metal Gate				N 40°52.961'; W 73°38.821'		
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	809	2613	16310	934	3770	1043
Fecal Coliform (MPN/100 mL)	3300	8200	12000	1900	7100	2300
E. coli (MPN/100 mL)	1989	3290	7940	1005	2753	1259
Total Coliform (MPN 100/mL)	57940	> 241960	> 241960	> 241960	173290	> 241960
HumM2 Human DNA Marker (Log10 mean copies)	ND	ND	0.81	ND	ND	0.97
HF183 Human DNA Marker (Log10 mean copies)	ND	ND	0.93	ND	ND	ND
Rainfall within 24 hours (Inches)	0	0.23	0.53+	0	0.29+	0

WO-1/Stream Sample & Potential Wetland Discharge Point to Stream				N 40°52.927'; W 73°38.807'		
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	602	4106	11060	663	4106	565
Fecal Coliform (MPN/100 mL)	1500	4900	9300	880	4900	790
E. coli (MPN/100 mL)	1455	2381	7170	738	1602	689
Total Coliform (MPN 100/mL)	36540	> 241960	173290	241960	120330	129970
HumM2 Human DNA Marker (Log10 mean copies)	ND	ND	0.86	ND	ND	ND
HF183 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
Rainfall within 24 hours of sampling (Inches)	0	0.23	0.53+	0	0.29+	0

SW-07/Stormwater Pipe Schein Property Rear Gate				N 40°52.916'; W 73°38.788'		
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	30	591	689	10	74	41
Fecal Coliform (MPN/100 mL)	120	74	1200	41	1200	52
E. coli (MPN/100 mL)	110	134	1236	52	373	< 10
Total Coliform (MPN 100/mL)	8164	5210	64880	5794	41060	3873
HumM2 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
HF183 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
Rainfall within 24 hours of sampling (Inches)	0	0.23	0.53+	0	0.29+	0

ST-01/Stream Sample adjacent to 18 Valley Road				N 40°52.777'; W 73°38.806'		
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	1607	3654	1396	6867	3282	4140
Fecal Coliform (MPN/100 mL)	1200	2000	4100	880	11000	120000
E. coli (MPN/100 mL)	2613	3255	3654	637	3255	241960
Total Coliform (MPN 100/mL)	241960	120330	104620	57940	198630	> 241960
HumM2 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
HF183 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
Rainfall within 24 hours of sampling (Inches)	0	0.23	0.53+	0	0.29+	0

Table 2 (con't). Summary of Total, Fecal, *E. coli* and Enterococci Bacteria, Crescent Beach June-August 2019

SW-03/Stormwater Outfall influence by drains/sump pumps from 18 Valley Road N 40°52.737'; W 73°38.806'						
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	223	556	504	246	609	275
Fecal Coliform (MPN/100 mL)	470	480	660	400	860	85
E. coli (MPN/100 mL)	537	464	683	256	717	134
Total Coliform (MPN 100/mL)	13340	32820	19890	43520	30760	19350
HumM2 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
HF183 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
Rainfall within 24 hours of sampling (inches)	0	0.23	0.53+	0	0.29+	0

GW-S/Groundwater Seep across from 16 Valley Road				N 40°52.701'; W 73°38.784'		
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	460	243	> 241960	52	5710	173
Fecal Coliform (MPN/100 mL)	200	20	33000	63	16000	63
E. coli (MPN/100 mL)	323	31	3654	20	6488	75
Total Coliform (MPN 100/mL)	> 241960	38730	241960	5475	155310	6131
HumM2 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
HF183 Human DNA Marker (Log10 mean copies)	ND	ND	ND	ND	ND	ND
Rainfall within 24 hours of sampling (inches)	0	0.23	0.53+	0	0.29+	0

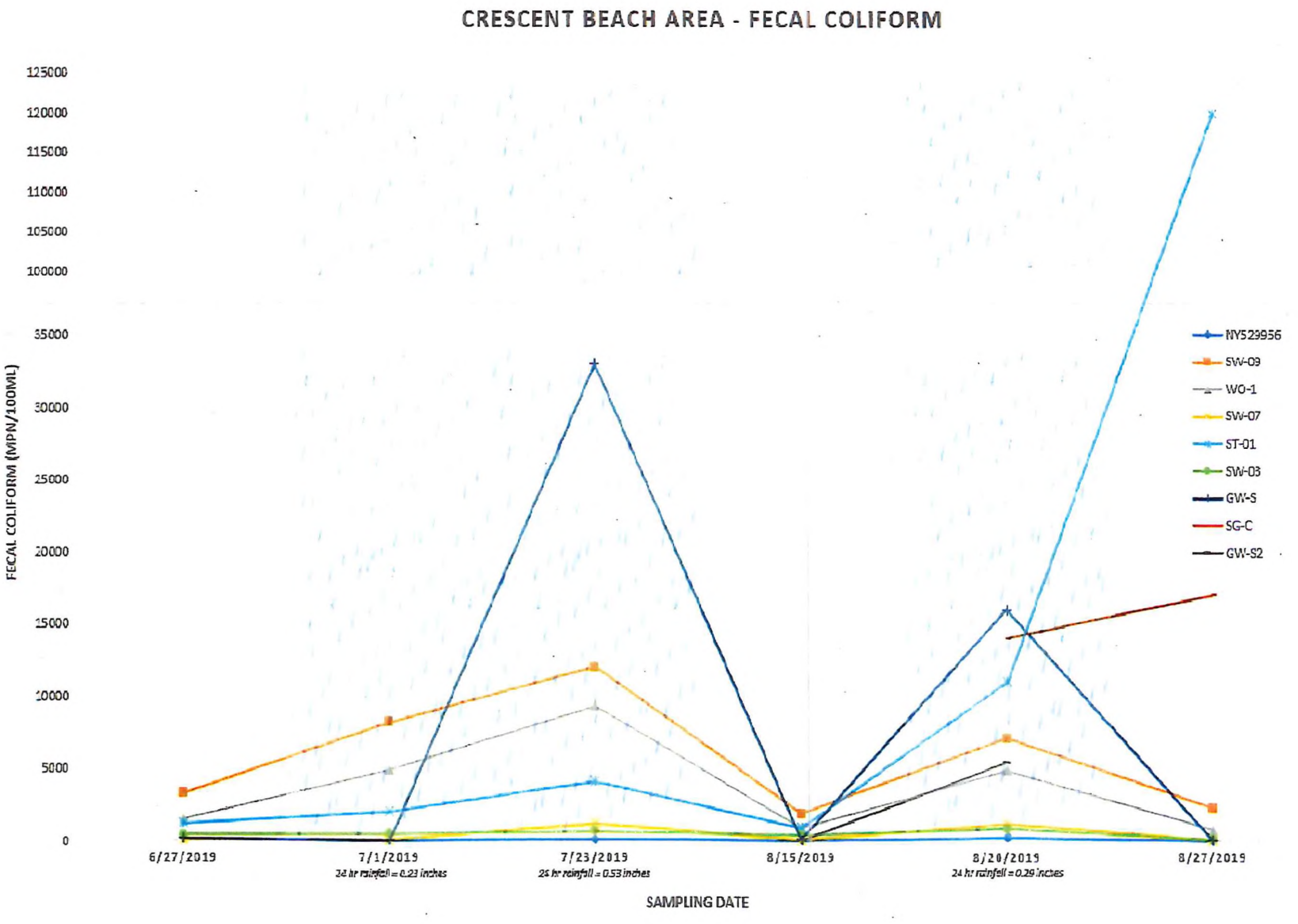
SG-C/Wetland sample of uncaped outfall pipe from Schein residence septic system N 40°52.883'; W 73°38.770'						
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	-	-	318	-	8620	> 241960
Fecal Coliform (MPN/100 mL)	-	-	580	-	14000	17000
E. coli (MPN/100 mL)	-	-	959	-	14390	3873
Total Coliform (MPN 100/mL)	-	-	173290	-	> 241960	> 241960
HumM2 Human DNA Marker (Log10 mean copies)	-	-	ND	-	ND	ND
HF183 Human DNA Marker (Log10 mean copies)	-	-	ND	-	ND	ND
Rainfall within 24 hours of sampling (inches)	-	0.23	0.53+	-	0.29+	0

GW-S2/Crescent Beach Intertidal Zone				N 40.883677 ; W 73.647305		
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Enterococcus (MPN/100 mL)	-	-	-	41	2850	-
Fecal Coliform (MPN/100 mL)	-	-	-	10	5400	-
E. coli (MPN/100 mL)	-	-	-	805	19610	-
Total Coliform (MPN 100/mL)	-	-	-	30760	>241960	-
HumM2 Human DNA Marker (Log10 mean copies)	-	-	-	ND	ND	-
HF183 Human DNA Marker (Log10 mean copies)	-	-	-	ND	ND	-
Rainfall within 24 hours of sampling (inches)	-	0.23	0.53+	0	0.29+	-

Lower Limit of Quantification (LLOQ)/Detection Limit						
Human Markers	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
HumM2 Human DNA Marker (Log10 mean copies)	0.81	0.86	0.63 except SW-07 = 0.73	0.91	0.92 except GW-S2 = 0.73; SW-03, GW-S = 0.81	0.78
HF183 Human DNA Marker (Log10 mean copies)	0.86	0.90	0.84 except SW-07 = 0.82	0.90	0.87 except GW-S2 = 0.82; SW-03, GW-S = 0.89	0.84

DNA results which were detected by the instrument but below the Lower Limit of Quantification

Figure 3. Fecal Coliform Results among Nine Stations Sampled in the Vicinity of Crescent Beach, Glen Cove NY June – August 2019



3.2 DNA Human Markers HF183/BacR287 and HumM2 Results Summary

Results of the DNA based qPCR analysis for human associated fecal bacteria suggest the source is not of human origin for samples collected during both the dry and wet weather sampling events. All qPCR results for the two human markers tested (HF183/BacR287 & HumM2) were not detected above the Lower Limit of Quantitation except for SW-09 and WO-1 on 7/23/19 and SW-09 on 8/27/19 (Table 2). The 7/23/19 sampling date represented the day with the highest rainfall amount prior to sampling with over one half of an inch of rain (Table 2). At station SW-09 on 7/23/19, human markers were detected at levels slightly above the Lowest Level of Quantification (LLOQ): 0.81 log10 copies per reaction for HumM2 (LLOQ of 0.63 log10 copies per reaction) and 0.93 log10 copies per reaction for the HF183/BacR287 (LLOQ of 0.84 log 10 copies per reaction). During the dry weather sampling event on 8/27/19 the HumM2 marker was also detected (0.97 log10 copies per reaction, LLOQ of 0.73 log 10 copies per reaction) but the HF183/BacR287 marker was not. At Station WO-1 on 7/23/19, the HumM2 human marker was detected (0.86 log10 copies per reaction, LLOQ of 0.63 log10 copies per reaction) but the HF183/BacR287 was not. Station WO-1 is south of SW-09 and is influenced by the wetland area southwest of SW-09.

There were also 13 samples in which one or both human DNA markers were detected but were below the LLOQ and were too low for quantification (Stations SW-09, WO-1, SW-7, and Glen Cove Bay/Crescent Beach- NY529956). These results are indicated by a yellow-shaded box in the Human Marker summary table (Table 2).

None of these results indicate a significant or discrete source of human fecal contamination. Also, given the potential for cross reactivity, host source specificity, and marker sensitivity, the human marker results at these identified areas are not considered significant as measured in this study.

3.3 Quality Control Summary

3.3.1 Fecal Indicator Bacteria QC Summary

There were no QC anomalies in any of the samples tested for Total Coliform, *E. coli*, Enterococci and Fecal Coliforms. In some of the samples, *E. coli* were higher than fecal coliforms. However, the two analyses were run using independent methods and the differences were not outside the typical variability of these types of tests.

3.3.2 Human Markers QC Summary

Calibration curve performance metrics are used to evaluate the suitability of the model for making target DNA sequence concentration estimates in unknown test samples. Performance is measured based on two metrics including R^2 and amplification efficiency (E). R^2 values should range between 0.98 and 1.0. E is derived from the slope parameter in the fitted curve and is defined as follows $E = (10^{-1/slope}) - 1$. E values should range from 0.90 to 1.10. LLOQ is the 95% prediction upper limit at the 1 $\log_{10}$ copy per

reaction standard dilution. The calibration curve metrics for both markers were within the required ranges.

No Template Controls (NTC) were included with each instrument run performed. These controls ensure that the reagents and/or laboratory environment did not introduce contaminants that could result in false positives. The NTC data were acceptable and show that no true positive logarithmic amplification traces.

Method Extraction Blanks (MEB) were included with each analytical batch of samples. (MEB). The MEBs were prepared by filtering 100mL of laboratory grade RNA-free water. The absence of a fluorescence amplification growth curve from the human-associated assays during qPCR analysis of these controls indicates the absence of contaminant target DNA. For HF183/BacR287 and HumM2, the MEB tests showed no evidence of contamination.

Internal Amplification Control (IAC): Amplification interference can result from either inhibition (interference from substances that persist in the filter DNA extract after DNA purification) or competition between the native human-associated target sequence and the IAC spike. The criterion for establishing the presence or absence of inhibition in an environmental sample DNA extract relies on data from the standard curve, NTC reactions, and environmental test samples from an instrument run. Inhibition of amplification was observed in only 1 of 144 test sample replicates in wet weather samples, and 0 of 138 test sample replicates in wet weather samples (each sample is analyzed in triplicate). However, replicates which display inhibition or competition are rejected from the study and not used to calculate final Cq values.

Sample Processing Control (SPC): While extracting the DNA, there are a few factors that can impact the efficiency of DNA recovery. This may include loss of DNA, interference from the test sample matrix, and/or poor laboratory technique which could result in a bias in target sequence concentration estimates. Each environmental test filter DNA preparation must therefore be screened for potential inconsistencies in DNA recovery to make reliable target sequence concentration estimates. Variability in sample processing efficiency is measured in each environmental sample filter and method extraction blank controls (MEB) with a spike preparation consisting of a fixed concentration of salmon sperm DNA called a Sample Processing Control (SPC). The resulting DNA elute is tested with the Sketa22 qPCR assay. The demonstration of consistent spiked DNA recovery from one filter DNA extract to the next is achieved by establishing a SPC acceptance threshold based on acceptable MEB results. Sample Processing Control Proficiency is obtained when MEB Cq standard deviation is ≤ 0.62. There was only one sample which failed to meet the SPC Proficiency (GW-S2 sampled during the wet weather event on 8/20/19). This sample had a high amount of suspended solids (and possibly dissolved solids) likely leading to the failure of the SPC due to interference caused by the matrix. The result for both human DNA markers was non-detect but was qualified to account for the QC failure.

The final raw data report with results of the fecal indicator bacteria and the DNA human marker analyses is included as Attachment 1.

4.0 CONCLUSIONS

- Results from the six sampling events during this study were similar to the initial EPA/NYSDEC project conducted in 2018. Human related fecal contamination was not detected or detected at trace levels and FIB was generally elevated at most sampling areas with FIB increasing at every station after a rain event.
- Overall, Station SW-09, the surface water station closest to Crescent Beach and the site receiving contributions from all the other upstream locations, had the highest FIB concentrations relative to other sites during both the wet and dry weather sampling events.
- The concentration of FIB during the final dry weather sampling event on 8/27/19 were highest at ST-01, a stream sample adjacent to 18 Valley Road and SC-C an uncapped pipe from the Schein residence.
- Enterococcus levels in Glen Cove Bay at Station NY529956 met the WQS of 104 MPN/100 mL for bathing beaches in 4 of the six sampling events, including the three dry weather events.
- Results of the DNA based qPCR analysis for human associated fecal bacteria suggest the source is not of human origin for all the samples collected during both the dry and wet weather sampling events. There were only 3 instances where the marker was detected slightly above the Lower Limit of Quantitation (SW-09 and WO-1 on 7/23/19 and SW-09 on 8/27/19) however this does not indicate a significant or discrete source of human fecal contamination given the potential for cross reactivity, host source specificity, and marker sensitivity.
- Further examination of archived samples for non-human markers including geese, gulls, dogs, and deer were requested by NYSDEC after receipt of the original report. Archive filters from Station SW-09 from all six sampling events were shipped by the EPA Region 2 Lab on dry ice to Source Molecular for analysis of 5 non- human markers. Results from this set of samples were amended to this report as Addendum 1.
- Low level concentrations of a general avian and dog marker were detected in all samples evaluated for target non-human MST markers. The detection of general bird, gull, and canine confirm results from the EPA study from 2018 but were detected in higher amounts during the 2019 study.

The 2018 and 2019 studies were designed to address the following two factors:

- <u>Spatial/Temporal Trends of both conventional and MST DNA markers</u>:
  Weather conditions, agricultural practices and wildlife including bird migrations often contribute to variability of FIB concentrations. MST studies have shown much variation in the presence/absence of a particular marker over relatively short periods of time.

- Variability/Specificity of the MST assays:
  MST markers use the DNA of different bacteria than are measured typically by FIB. Although there are many QC samples analyzed with each batch of MST samples, MST markers may either not be specific to the target, or there may be regional differences in the target source that may not be reflected in the MST marker used. Local reference sources were not tested against target MST markers in this study, but the markers used generally have good reproducibility throughout the US.

- Based on the data and observations from this study, the source of the observed FIB exceedances is likely due to domestic, wildlife, or other environmental factors, with birds (including gull species) and canines as most likely contributors based on the markers evaluated in this study.

5.0 REFERENCES

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Addendum A

Determination of Human and Non-Human-Associated Fecal Contamination using Real Time Polymerase Chain Reaction (qPCR) Assays from Samples Collected Near Crescent Beach, Nassau County

Non-Human MST Marker Data – January 2020

Introduction

In the MST study conducted at Crescent Beach in 2019, Station SW-09 was the surface water station closest to Crescent Beach and the site receiving contributions from most of the other upstream locations. Bacteria levels from this Station have generally supported higher bacteria levels than the adjacent beach and had the overall highest fecal indicator bacteria (FIB) concentrations relative to other sites in this study. Results of the DNA-based qPCR analysis for human associated fecal bacteria suggest the source is not of human origin for all samples collected during both the dry and wet weather sampling events. Further examination of archived samples for non-human markers was conducted to provide insight into potential contributors of fecal pollution into Crescent Beach.

DNA duplicate filters collected at SW-09 from each of the six 2019 sampling events were shipped to a commercial laboratory for quantification of non-human markers for general avian spp., Canada Goose, gulls, ruminants (including deer), and dogs. These markers were selected based on most likely non-human sources from the study area.

The specific marker and target organism evaluated for each potential source is identified below:

- Bird/GFD (Heliobacter)
- Canada Goose/CGOF1-Bac (Bacteroides)
- Gull/Gull4 (*C. marimammalium*)
- Ruminant/Rum2Bac (Bacteroides)
- Dog/BacCan-UCD (Bacteriodes)

Results

A summary of the fecal indicator bacteria, human and non-human marker results for Stations SW-09 is listed in Table A-1. The raw data report submitted by Source Molecular is included in this report as Attachment 2. The human markers evaluated by the USEPA Region 2 Laboratory is reported in Log_{10} gene copies per 25 µL reaction. The contract laboratory also reported their results in Log_{10} gene copies per 20µL reaction to assist with data comparison with the EPA Laboratory Human Marker data (the endpoints in the raw data report for the non-human markers are reported as copies per 100 mL but were converted to log10 copies to allow for more direct comparisons with the EPA human marker results in this addendum). For all markers, the amount of PCR DNA volume tested is the same (2µL) except for the bird marker (GFD) which the contract laboratory was required to dilute (to 0.2µL) to obtain quantifiable results. Due to differences in the reagents used in the reaction volumes, the results of the human and non-human marker assays cannot be directly compared. However, relative abundance based on densities and respective reporting limits within each marker provides needed information to determine potential sources in a set of samples.

Both the EPA Region 2 Laboratory and the commercial laboratory provide information if a marker was detected but below established reporting limits. Reporting detection of a signal below the analytical reporting limit is acceptable for these types of data. Any results detected but not quantifiable would be categorized as a trace concentration and not a significant or discrete source given the potential for cross reactivity, host source specificity, and marker sensitivity. The reporting limits for both laboratories can be found at the bottom of Table A-1.

Table A-1
Summary of Results for Crescent Beach Sampling Site SW-09
Dry and Rain Events, 6/27/19 to 8/27/19
Conventional Bacteria, Human, and Non-Human MST Markers

SW-09 (Located at end of stream by metal gate)	N 40°52.961'; W 73°38.821'					
Date of Sampling Event	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Rainfall within 24 hours (inches)	0	0.23	0.53+	0	0.29+	0
Type of Sampling Event	DRY	WET	WET	DRY	WET	DRY
Enterococcus (MPN/100 mL)	809	2613	16310	934	3770	1043
Fecal Coliform (MPN/100 mL)	3300	8200	12000	1900	7100	2300
E. coli (MPN/100 mL)	1989	3290	7940	1005	2753	1259
Total Coliform (MPN/100 mL)	57940	> 241960	> 241960	> 241960	173290	> 241960
Human DNA Marker-*HumM2* (Log_{10} copies per reaction)	ND	ND	0.81	ND	ND	0.97
Human DNA Marker-*HF183/BacR287* (Log_{10} copies per reaction)	ND	ND	0.93	ND	ND	ND
Dog DNA Marker-*BacCan-UCD* (copies/100 mL)	1.11	1.38	1.90	1.18	1.33	ND
Ruminant DNA Marker-*Rum2Bac* (copies/100 mL)	ND	ND	ND	ND	ND	ND
General Avian DNA Marker-*GFD* (copies/100 mL)	1.95	2.27	2.09	1.95	1.94	1.81
Gull DNA Marker-*Gull4* (copies/100 mL)	ND	ND	ND	1.08	1.01	1.49
Canada Goose DNA Marker-*CGOF1-Bac* (copies/100 mL)	ND	ND	ND	ND	ND	ND

Lower Limit of Quantification (LLOQ)/Detection Limit						
	6/27/2019	7/1/2019	7/23/2019	8/15/2019	8/20/2019	8/27/2019
Human DNA Marker-*HumM2* (Log_{10} copies per reaction)	0.81	0.86	0.63	0.91	0.92	0.78
Human DNA Marker-*HF183/BacR287* (Log_{10} copies per reaction)	0.86	0.90	0.84	0.90	0.87	0.84
Non-Human DNA Markers (Log_{10} copies per reaction)	1.00	1.00	1.00	1.00	1.00	1.00

DNA results which were detected by the instrument but below the Lower Limit of Quantification

The highest concentrations of non-human markers were that of the general avian/bird marker (GFD). This marker was detected at SW-09 throughout the study period and results were similar regardless among all sampling events whether wet or dry. The gull marker (Gull4) was not detected for the first three events but was detected during the final three sampling events. Results were non-detect for the Canada Goose (CGOF1-Bac) marker for all events except for two dry weather dates which were detected but below the reporting limit.

The dog marker (BacCan-UCD) was detected at SW-09 throughout the study period with results detect above the reporting limit during the first five sampling events and detected but below the detection limit on 8/27/19.

Results of the ruminant marker (Rum2Bac) were non detect for all sampling events at SW-09 suggesting that wildlife such as deer are not contributing to the observed fecal pollution at Crescent Beach.

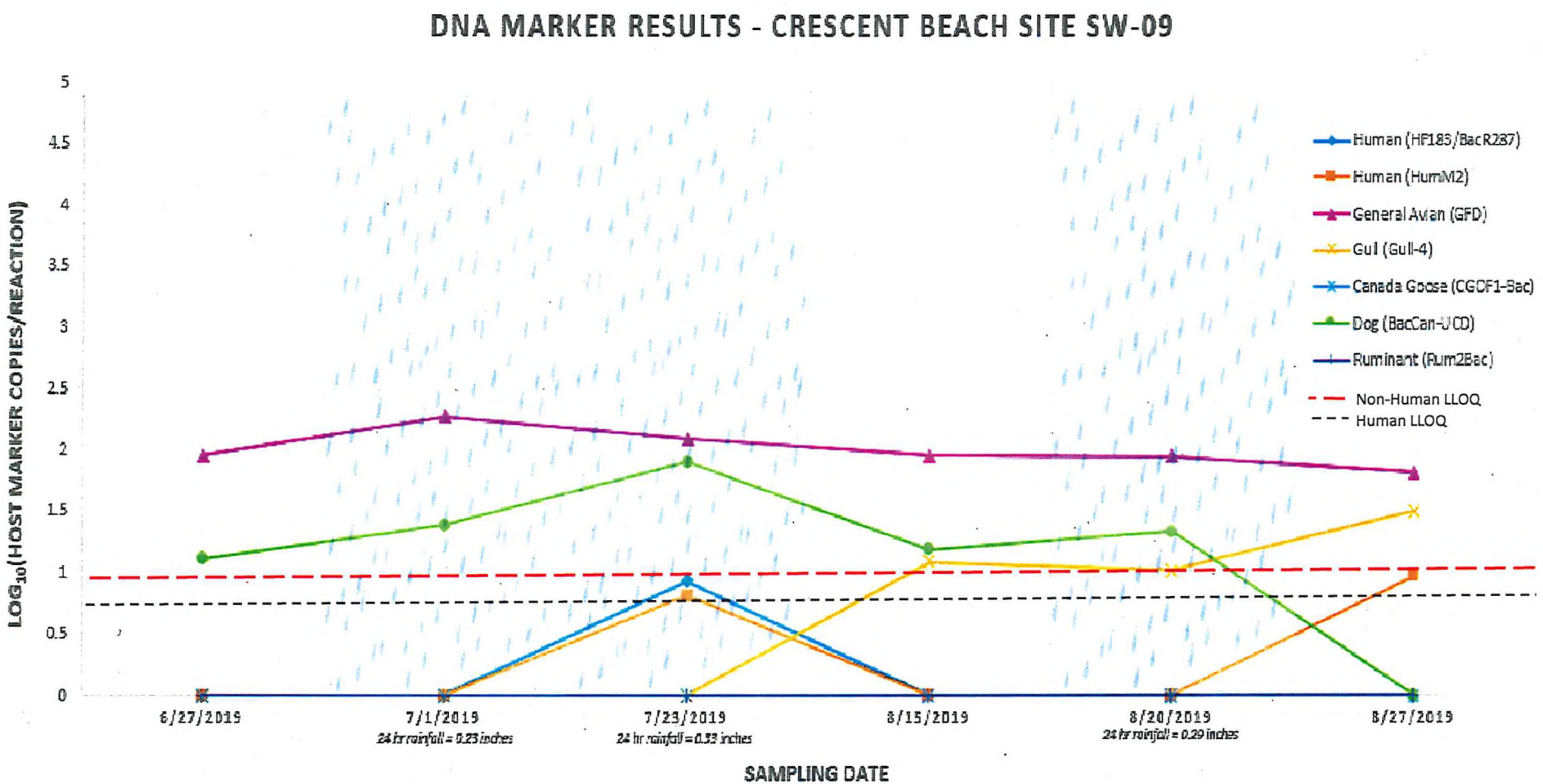

Figure A-1: Results of human and non-human DNA markers at Crescent Beach site SW-09 from wet and dry sampling events. The dashed lines on the chart represent the LLOQ for the host markers. The LLOQ for the human markers are run dependent and varied throughout the study period. For illustration purposes, the LLOQ depicted here represents the average LLOQ for HF183/BacR287 and HumM2 of runs which had quantifiable results (approximately 0.8 Log_{10} copies/reaction).

Non-Human Markers Quality Control Summary

The five non-human DNA markers were analyzed by Source Molecular in Miami Lakes, Florida (ISO/IEC 17025:2005). The laboratory utilizes quality control procedures to monitor the validity of test results and ensures that only valid results are reported by continuously monitoring and reviewing the performance of tests. All QC criteria must be met for the results to be considered valid and reportable. Analysis are repeated on test samples that do not meet QC requirements and if they do not pass a second time, are flagged.

Listed below is a table of the Quality Control activities established for the six samples collected at SW-09 during the 2019 wet and dry weather sampling events (Table A-2). All evaluated QC criteria were met for this batch of samples. Samples analyzed using the general avian marker (GFD) were diluted to account for sample matrix affects.

Table A-2. QC Requirements for Non-Human MST qPCR Markers, Source Molecular

QC Item/Activity	Data Quality Indicator and Purpose	Frequency	Measurement Performance Criteria
Standard Curve	*Accuracy/Bias, Comparability, Sensitivity* -Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range for accurate quantification	One curve in duplicate per PCR plate, per test if result is quantifiable and requires quantification	R^2: ≥0.98 Efficiency: 80-110% Slope: -3.0 - -4.0 Sample unknown must be within the linear dynamic range limits
Extraction blank	*Accuracy/Bias* - Evaluates contamination during DNA extraction/purification	At least one for every batch of samples extracted	No detection or detection at least 3 CT units above sample CT values (presence/absence tests)
Sample duplicates	*Precision, Comparability* -Ensures precision and confidence in data	Every sample analyzed	± 1.0 standard deviation unless CT value ≥33
Diluted sample	*Accuracy/Bias* -Monitors for sample matrix inhibition affects	Every sample analyzed	CT value must be greater than that of un-diluted sample
Positive control	*Accuracy/Bias* -Monitors for false negatives	At least one reaction per PCR plate, per test	Detection
Negative control	*Accuracy/Bias* -Monitors for false positives	Three reactions per PCR plate, per test	No detection or detection at least 3 CT units above sample CT

ATTACHMENT 1- DATA REPORT

HUMAN DNA AND CONVENTIONAL MICROBIOLOGICAL PARAMETERS

Six Sampling Events Between June 27, 2019 and August 27, 2019

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 2 Laboratory
2890 Woodbridge Avenue
Edison , New Jersey 08837
732-906-6886 Phone
732-906-6165 Fax

November 14, 2019

Alyssa Carroll
New York State Dept. of Environmental Conservation
SUNY @ Stony Brook, 50 Circle Road
Stony Brook, NY 11790-3409

RE: Crescent Beach MST- 2019 - 1906060

Enclosed are the results of analyses for samples received by the laboratory between 6/27/2019 and 8/27/2019. The signature below reflects the laboratory's approval of the reported results. If you have any questions concerning this report, please refer to Project Number 1906060 and contact the laboratory.

Sincerely,

John R. Bourbon
Chief, DESA/LB

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 2 Laboratory

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Project Narrative:

The National Environmental Laboratory Accreditation Conference Institute (TNI) is a voluntary environmental laboratory accreditation association of State and Federal agencies. TNI established and promoted a National Environmental Laboratory Accreditation Program (NELAP) that provides a uniform set of standards for the generation of environmental data that are of known and defensible quality. The EPA Region 2 Laboratory is NELAP accredited. The Laboratory tests that are accredited have met all the requirements established under the TNI Standards.

Condition Comments

None

Comment(s):

The "Sample Analysis Date and Time" is included in the results section for any analyte with a prescribed holding time of 72 hours or less.

MST Human Marker HumM2
HumM2 and HF183 are human-associated gene sequences from the bacteria genus Bacteriodes commonly used as a marker in source tracking of human associated fecal inputs. *Bacteroides* human-associated gene sequences are commonly found in the feces of humans and other sources of human fecal pollution (sewage and septage). Although DNA from these organisms is sometimes present in other animal sources in low concentrations, their presence in environmental water is typically an indication of human fecal pollution.

HF183 Samples 1906060-01 to -02; 1908050-01; 1907066-03; 1907066-04; 1908079-04; and 1908059-01 and -05 were detected but below the Lower Limit of Quantitation (LLOQ).
HumM2 Samples 1907036-02 to -03; 1907066-04; 1908079-03; and 1908059-02 and -04 were detected but below the LLOQ.

Human DNA Markers HumM2 and HF183: Due to a high amount of solids, sample 1908059-03 (GW-S2) was shaken then allowed to settle prior to filtration. Sample 1907066-04 (SW-07) also had a high amount of solids and was slow to filter, but did not require settling prior to filtration. To reduce potential matrix interferences, both GW-S2 and SW-07 were subjected to additional centrifugation during extraction. However, GW-S2 failed the Sample Processing Control acceptance threshold for the spiked salmon DNA recovery (Sketa22 assay). Due to the failing QC metric likely caused by matrix inference therefore the sample (1908059-03) result was qualified with "J."

Data Qualifier(s):

U- The analyte was not detected at or above the Reporting Limit.
J- The identification of the analyte is acceptable; the reported value is an estimate.
K- The identification of the analyte is acceptable; the reported value may be biased high.
L- The identification of the analyte is acceptable; the reported value may be biased low.
NJ- There is presumptive evidence that the analyte is present; the analyte is reported as a tentative identification. The reported value is an estimate.

Reporting Limit(s):

The Laboratory was able to achieve the appropriate limits for each analyte requested.

Reporting Limit(s):

The Laboratory was able to achieve the appropriate limit for each analyte requested.

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

SUMMARY REPORT FOR SAMPLES

Field ID	Laboratory ID	Matrix	Date Sampled	Date Received
NY529956	1906060-01	Aqueous	06/27/2019 08:33	06/27/2019 11:35
SW-09	1906060-02	Aqueous	06/27/2019 08:41	06/27/2019 11:35
WO-01	1906060-03	Aqueous	06/27/2019 08:51	06/27/2019 11:35
SW-07	1906060-04	Aqueous	06/27/2019 08:58	06/27/2019 11:35
ST-01	1906060-05	Aqueous	06/27/2019 09:19	06/27/2019 11:35
SW-03	1906060-06	Aqueous	06/27/2019 09:28	06/27/2019 11:35
GW-S	1906060-07	Aqueous	06/27/2019 09:40	06/27/2019 11:35
NY529956	1907036-01	Aqueous	07/01/2019 08:11	07/01/2019 10:30
SW-09	1907036-02	Aqueous	07/01/2019 08:22	07/01/2019 10:30
WO-1	1907036-03	Aqueous	07/01/2019 08:28	07/01/2019 10:30
SW-07	1907036-04	Aqueous	07/01/2019 08:34	07/01/2019 10:30
ST-01	1907036-05	Aqueous	07/01/2019 08:49	07/01/2019 10:30
SW-03	1907036-06	Aqueous	07/01/2019 08:58	07/01/2019 10:30
GW-S	1907036-07	Aqueous	07/01/2019 09:06	07/01/2019 10:30
NY529956	1907066-01	Aqueous	07/23/2019 10:04	07/23/2019 13:45
SW-09	1907066-02	Aqueous	07/23/2019 10:08	07/23/2019 13:45
WO-1	1907066-03	Aqueous	07/23/2019 10:24	07/23/2019 13:45
SW-07	1907066-04	Aqueous	07/23/2019 10:30	07/23/2019 13:45
SG-C	1907066-05	Aqueous	07/23/2019 10:40	07/23/2019 13:45
ST-01	1907066-06	Aqueous	07/23/2019 10:55	07/23/2019 13:45
SW-03	1907066-07	Aqueous	07/23/2019 11:05	07/23/2019 13:45
GW-S	1907066-08	Aqueous	07/23/2019 11:15	07/23/2019 13:45
NY529956	1908050-01	Aqueous	08/15/2019 07:45	08/15/2019 10:18
GW-S2	1908050-02	Aqueous	08/15/2019 08:02	08/15/2019 10:18
SW-09	1908050-03	Aqueous	08/15/2019 08:07	08/15/2019 10:18
WO-1	1908050-04	Aqueous	08/15/2019 08:17	08/15/2019 10:18
SW-07	1908050-05	Aqueous	08/15/2019 08:24	08/15/2019 10:18
ST-01	1908050-06	Aqueous	08/15/2019 08:41	08/15/2019 10:18

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 2 Laboratory

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

SUMMARY REPORT FOR SAMPLES

Field ID	Laboratory ID	Matrix	Date Sampled	Date Received
SW-03	1908050-07	Aqueous	08/15/2019 08 49	08/15/2019 10 18
GW-S	1908050-08	Aqueous	08/15/2019 08 59	08/15/2019 10 18
NY529956	1908059-01	Aqueous	08/20/2019 10 15	08/20/2019 13 07
SW-09	1908059-02	Aqueous	08/20/2019 10 20	08/20/2019 13 07
GW-S2	1908059-03	Aqueous	08/20/2019 10 25	08/20/2019 13 07
WO-1	1908059-04	Aqueous	08/20/2019 10 30	08/20/2019 13 07
SW-07	1908059-05	Aqueous	08/20/2019 10 45	08/20/2019 13 07
SG-C	1908059-06	Aqueous	08/20/2019 11 10	08/20/2019 13 07
ST-01	1908059-07	Aqueous	08/20/2019 11 25	08/20/2019 13 07
SW-03	1908059-08	Aqueous	08/20/2019 11 35	08/20/2019 13 07
GW-S	1908059-09	Aqueous	08/20/2019 11 45	08/20/2019 13 07
NY529956	1908079-01	Aqueous	08/27/2019 07 47	08/27/2019 09 52
SW-09	1908079-02	Aqueous	08/27/2019 07 53	08/27/2019 09 52
WO-1	1908079-03	Aqueous	08/27/2019 08 02	08/27/2019 09 52
SW-07	1908079-04	Aqueous	08/27/2019 08 08	08/27/2019 09 52
SG-C	1908079-05	Aqueous	08/27/2019 08 13	08/27/2019 09 52
ST-01	1908079-06	Aqueous	08/27/2019 08 27	08/27/2019 09 52
SW-03	1908079-07	Aqueous	08/27/2019 08 36	08/27/2019 09 52
GW-S	1908079-08	Aqueous	08/27/2019 08 40	08/27/2019 09 52

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 2 Laboratory

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

SUMMARY REPORT FOR METHODS

Analysis	Method	Certification	Matrix
Colilert, Quanti-Tray	SM 9223 B SOP B-46 Rev 3.1	NELAP	Aqueous
Colilert-18, Quantitray, Fecal Coliform	SM 9223 B SOP B-46 Rev 3.1		Aqueous
Enterococcus	Enterolert-IDEXX SOP B-44 Rev1.3	NELAP	Aqueous
MST-Human-HF183 Marker	Human Bacteroides by QPCR / SOP B-49 Rev		Aqueous
MST-Human-HumM2 Marker	Human Bacteroides by QPCR / SOP B-49 Rev		Aqueous

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 2 Laboratory

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: NY529956				**Sample ID: 1906060-01**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.86	mean Log10 copies	6/27/2019 1:25:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	6/27/2019 1:25:00PM
Microbiology, Colilert					
E. coli	201		10	MPN/100 mL	6/27/2019 2:00:00PM
Coliform, Total	6131		10	MPN/100 mL	6/27/2019 2:00:00PM
Microbiology, Enterolert					
Enterococcus	52		10	MPN/100 mL	6/27/2019 2:15:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	171		10	MPN/100 mL	6/27/2019 2:30:00PM
Field ID: SW-09				**Sample ID: 1906060-02**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.86	mean Log10 copies	6/27/2019 1:35:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	6/27/2019 1:35:00PM
Microbiology, Colilert					
E. coli	1989		10	MPN/100 mL	6/27/2019 2:00:00PM
Coliform, Total	57940		100	MPN/100 mL	6/27/2019 2:00:00PM
Microbiology, Enterolert					
Enterococcus	809		10	MPN/100 mL	6/27/2019 2:15:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	3255		10	MPN/100 mL	6/27/2019 2:30:00PM
Field ID: WO-01				**Sample ID: 1906060-03**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.86	mean Log10 copies	6/27/2019 1:40:00PM

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: WO-01				**Sample ID: 1906060-03**	
Microbiology					
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	6/27/2019 1:40:00PM
Microbiology, Colilert					
E. coli	1455		10	MPN/100 mL	6/27/2019 2:00:00PM
Coliform, Total	36540		100	MPN/100 mL	6/27/2019 2:00:00PM
Microbiology, Enterolert					
Enterococcus	602		10	MPN/100 mL	6/27/2019 2:15:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	1515		10	MPN/100 mL	6/27/2019 2:30:00PM
Field ID: SW-07				**Sample ID: 1906060-04**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.86	mean Log10 copies	6/27/2019 1:50:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	6/27/2019 1:50:00PM
Microbiology, Colilert					
E. coli	110		10	MPN/100 mL	6/27/2019 2:00:00PM
Coliform, Total	8164		10	MPN/100 mL	6/27/2019 2:00:00PM
Microbiology, Enterolert					
Enterococcus	30		10	MPN/100 mL	6/27/2019 2:15:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	122		10	MPN/100 mL	6/27/2019 2:30:00PM
Field ID: ST-01				**Sample ID: 1906060-05**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.86	mean Log10 copies	6/27/2019 2:05:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	6/27/2019 2:05:00PM

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: ST-01				**Sample ID: 1906060-05**	
Microbiology, Colilert					
E. coli	2613		10	MPN/100 mL	6/27/2019 2:00:00PM
Coliform, Total	241960		100	MPN/100 mL	6/27/2019 2:00:00PM
Microbiology, Enterolert					
Enterococcus	1607		10	MPN/100 mL	6/27/2019 2:15:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	1246		10	MPN/100 mL	6/27/2019 2:30:00PM
Field ID: SW-03				**Sample ID: 1906060-06**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.86	mean Log10 copies	6/27/2019 2:15:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	6/27/2019 2:15:00PM
Microbiology, Colilert					
E. coli	537		10	MPN/100 mL	6/27/2019 2:00:00PM
Coliform, Total	13340		100	MPN/100 mL	6/27/2019 2:00:00PM
Microbiology, Enterolert					
Enterococcus	223		10	MPN/100 mL	6/27/2019 2:15:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	473		10	MPN/100 mL	6/27/2019 2:30:00PM
Field ID: GW-S				**Sample ID: 1906060-07**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.86	mean Log10 copies	6/27/2019 2:20:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	6/27/2019 2:20:00PM
Microbiology, Colilert					
E. coli	323		10	MPN/100 mL	6/27/2019 2:00:00PM
Coliform, Total	>241960		100	MPN/100 mL	6/27/2019 2:00:00PM

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Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: GW-S				**Sample ID: 1906060-07**	
Microbiology, Enterolert					
Enterococcus	460		10	MPN/100 mL	6/27/2019 2:15:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	195		10	MPN/100 mL	6/27/2019 2:30:00PM
Field ID: NY529956				**Sample ID: 1907036-01**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	7/1/2019 12:35:00PM
MST-Human-HumM2 Marker	---	U	0.86	mean Log10 copies	7/1/2019 12:35:00PM
Microbiology, Colilert					
E. coli	41		10	MPN/100 mL	7/1/2019 12:55:00PM
Coliform, Total	2909		10	MPN/100 mL	7/1/2019 12:55:00PM
Microbiology, Enterolert					
Enterococcus	10		10	MPN/100 mL	7/1/2019 12:45:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	10		10	MPN/100 mL	7/1/2019 1:35:00PM
Field ID: SW-09				**Sample ID: 1907036-02**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	7/1/2019 12:40:00PM
MST-Human-HumM2 Marker	---	U	0.86	mean Log10 copies	7/1/2019 12:40:00PM
Microbiology, Colilert					
E. coli	3290		100	MPN/100 mL	7/1/2019 12:55:00PM
Coliform, Total	>241960		100	MPN/100 mL	7/1/2019 12:55:00PM
Microbiology, Enterolert					
Enterococcus	2613		10	MPN/100 mL	7/1/2019 12:45:00PM
Microbiology, Colilert 18, Fecal Coliform					

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Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SW-09				**Sample ID: 1907036-02**	
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	8164		10	MPN/100 mL	7/1/2019 1:35:00PM
Field ID: WO-1				**Sample ID: 1907036-03**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	7/1/2019 12:45:00PM
MST-Human-HumM2 Marker	---	U	0.86	mean Log10 copies	7/1/2019 12:45:00PM
Microbiology, Colilert					
E. coli	2381		10	MPN/100 mL	7/1/2019 12:55:00PM
Coliform, Total	>241960		100	MPN/100 mL	7/1/2019 12:55:00PM
Microbiology, Enterolert					
Enterococcus	4106		10	MPN/100 mL	7/1/2019 12:45:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	4884		10	MPN/100 mL	7/1/2019 1:35:00PM
Field ID: SW-07				**Sample ID: 1907036-04**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	7/1/2019 1:00:00PM
MST-Human-HumM2 Marker	---	U	0.86	mean Log10 copies	7/1/2019 1:00:00PM
Microbiology, Colilert					
E. coli	134		10	MPN/100 mL	7/1/2019 12:55:00PM
Coliform, Total	5210		100	MPN/100 mL	7/1/2019 12:55:00PM
Microbiology, Enterolert					
Enterococcus	591		10	MPN/100 mL	7/1/2019 12:45:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	74		10	MPN/100 mL	7/1/2019 1:35:00PM

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Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: ST-01				**Sample ID: 1907036-05**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	7/1/2019 1:10:00PM
MST-Human-HumM2 Marker	---	U	0.86	mean Log10 copies	7/1/2019 1:10:00PM
Microbiology, Colilert					
E. coli	3255		10	MPN/100 mL	7/1/2019 12:55:00PM
Coliform, Total	120330		100	MPN/100 mL	7/1/2019 12:55:00PM
Microbiology, Enterolert					
Enterococcus	3654		10	MPN/100 mL	7/1/2019 12:45:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	2014		10	MPN/100 mL	7/1/2019 1:35:00PM
Field ID: SW-03				**Sample ID: 1907036-06**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	7/1/2019 1:15:00PM
MST-Human-HumM2 Marker	---	U	0.86	mean Log10 copies	7/1/2019 1:15:00PM
Microbiology, Colilert					
E. coli	464		10	MPN/100 mL	7/1/2019 12:55:00PM
Coliform, Total	32820		100	MPN/100 mL	7/1/2019 12:55:00PM
Microbiology, Enterolert					
Enterococcus	556		10	MPN/100 mL	7/1/2019 12:45:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	480		10	MPN/100 mL	7/1/2019 1:35:00PM
Field ID: GW-S				**Sample ID: 1907036-07**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	7/1/2019 1:20:00PM

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Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: GW-S				**Sample ID: 1907036-07**	
Microbiology					
MST-Human-HumM2 Marker	---	U	0.86	mean Log10 copies	7/1/2019 1:20:00PM
Microbiology, Colilert					
E. coli	31		10	MPN/100 mL	7/1/2019 12:55:00PM
Coliform, Total	38730		100	MPN/100 mL	7/1/2019 12:55:00PM
Microbiology, Enterolert					
Enterococcus	243		10	MPN/100 mL	7/1/2019 12:45:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	20		10	MPN/100 mL	7/1/2019 1:35:00PM
Field ID: NY529956				**Sample ID: 1907066-01**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	7/23/2019 2:05:00PM
MST-Human-HumM2 Marker	---	U	0.63	mean Log10 copies	7/23/2019 2:05:00PM
Microbiology, Colilert					
E. coli	411		10	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	4190		100	MPN/100 mL	7/23/2019 2:22:00PM
Microbiology, Enterolert					
Enterococcus	110		10	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	105		10	MPN/100 mL	7/23/2019 2:35:00PM
Field ID: SW-09				**Sample ID: 1907066-02**	
Microbiology					
MST-Human-HF183 Marker	0.93		0.84	mean Log10 copies	7/23/2019 2:10:00PM
MST-Human-HumM2 Marker	0.81		0.63	mean Log10 copies	7/23/2019 2:10:00PM

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Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SW-09				**Sample ID: 1907066-02**	
Microbiology, Colilert					
E. coli	7940		100	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	>241960		100	MPN/100 mL	7/23/2019 2:22:00PM
Microbiology, Enterolert					
Enterococcus	16310		100	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	11530		100	MPN/100 mL	7/23/2019 2:35:00PM
Field ID: WO-1				**Sample ID: 1907066-03**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	7/23/2019 2:15:00PM
MST-Human-HumM2 Marker	0.86		0.63	mean Log10 copies	7/23/2019 2:15:00PM
Microbiology, Colilert					
E. coli	7170		100	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	173290		100	MPN/100 mL	7/23/2019 2:22:00PM
Microbiology, Enterolert					
Enterococcus	11060		100	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	9330		100	MPN/100 mL	7/23/2019 2:35:00PM
Field ID: SW-07				**Sample ID: 1907066-04**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.82	mean Log10 copies	7/23/2019 2:55:00PM
MST-Human-HumM2 Marker	---	U	0.73	mean Log10 copies	7/23/2019 2:55:00PM
Microbiology, Colilert					
E. coli	1236		10	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	64880		100	MPN/100 mL	7/23/2019 2:22:00PM

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Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SW-07				**Sample ID: 1907066-04**	
Microbiology, Enterolert					
Enterococcus	689		10	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	1223		10	MPN/100 mL	7/23/2019 2:35:00PM
Field ID: SG-C				**Sample ID: 1907066-05**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	7/23/2019 3:00:00PM
MST-Human-HumM2 Marker	---	U	0.63	mean Log10 copies	7/23/2019 3:00:00PM
Microbiology, Colilert					
E. coli	959		10	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	173290		100	MPN/100 mL	7/23/2019 2:22:00PM
Microbiology, Enterolert					
Enterococcus	318		10	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	583		10	MPN/100 mL	7/23/2019 2:35:00PM
Field ID: ST-01				**Sample ID: 1907066-06**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	7/23/2019 3:15:00PM
MST-Human-HumM2 Marker	---	U	0.63	mean Log10 copies	7/23/2019 3:15:00PM
Microbiology, Colilert					
E. coli	3654		10	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	104620		100	MPN/100 mL	7/23/2019 2:22:00PM
Microbiology, Enterolert					
Enterococcus	1396		10	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					

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Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: ST-01				**Sample ID: 1907066-06**	
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	4106		10	MPN/100 mL	7/23/2019 2:35:00PM
Field ID: SW-03				**Sample ID: 1907066-07**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	7/23/2019 3:20:00PM
MST-Human-HumM2 Marker	---	U	0.63	mean Log10 copies	7/23/2019 3:20:00PM
Microbiology, Colilert					
E. coli	683		10	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	19890		100	MPN/100 mL	7/23/2019 2:22:00PM
Microbiology, Enterolert					
Enterococcus	504		10	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	657		10	MPN/100 mL	7/23/2019 2:35:00PM
Field ID: GW-S				**Sample ID: 1907066-08**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	7/23/2019 3:25:00PM
MST-Human-HumM2 Marker	---	U	0.63	mean Log10 copies	7/23/2019 3:25:00PM
Microbiology, Colilert					
E. coli	3654		10	MPN/100 mL	7/23/2019 2:22:00PM
Coliform, Total	241960		100	MPN/100 mL	7/23/2019 2:22:00PM
Microbiology, Enterolert					
Enterococcus	>241960		100	MPN/100 mL	7/23/2019 2:32:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	32550		100	MPN/100 mL	7/23/2019 2:35:00PM

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Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: NY529956				**Sample ID: 1908050-01**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 1:15:00PM
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 1:15:00PM
Microbiology, Colilert					
E. coli	984		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	1882		10	MPN/100 mL	8/15/2019 12:40:00PM
Microbiology, Enterolert					
Enterococcus	31		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	30		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: GW-S2				**Sample ID: 1908050-02**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 1:40:00PM
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 1:40:00PM
Microbiology, Colilert					
E. coli	805		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	30760		100	MPN/100 mL	8/15/2019 12:40:00PM
Microbiology, Enterolert					
Enterococcus	41		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	10		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: SW-09				**Sample ID: 1908050-03**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 1:42:00PM

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Project: Crescent Beach MST- 2019 - 1906060

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Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SW-09				**Sample ID: 1908050-03**	
Microbiology					
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 1:42:00PM
Microbiology, Colilert					
E. coli	1005		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	>241960		100	MPN/100 mL	8/15/2019 12:40:00PM
Microbiology, Enterolert					
Enterococcus	934		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	1918		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: WO-1				**Sample ID: 1908050-04**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 1:55:00PM
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 1:55:00PM
Microbiology, Colilert					
E. coli	738		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	241960		100	MPN/100 mL	8/15/2019 12:40:00PM
Microbiology, Enterolert					
Enterococcus	663		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	884		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: SW-07				**Sample ID: 1908050-05**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 2:00:00PM
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 2:00:00PM

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Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SW-07				**Sample ID: 1908050-05**	
Microbiology, Colilert					
E. coli	52		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	5794		10	MPN/100 mL	8/15/2019 12:40:00PM
Microbiology, Enterolert					
Enterococcus	10		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	41		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: ST-01				**Sample ID: 1908050-06**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 2:15:00PM
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 2:15:00PM
Microbiology, Colilert					
E. coli	637		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	57940		100	MPN/100 mL	8/15/2019 12:40:00PM
Microbiology, Enterolert					
Enterococcus	6867		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	882		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: SW-03				**Sample ID: 1908050-07**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 2:20:00PM
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 2:20:00PM
Microbiology, Colilert					
E. coli	256		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	43520		100	MPN/100 mL	8/15/2019 12:40:00PM

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Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SW-03				**Sample ID: 1908050-07**	
Microbiology, Enterolert					
Enterococcus	246		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	399		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: GW-S				**Sample ID: 1908050-08**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.90	mean Log10 copies	8/15/2019 2:25:00PM
MST-Human-HumM2 Marker	---	U	0.91	mean Log10 copies	8/15/2019 2:25:00PM
Microbiology, Colilert					
E. coli	20		10	MPN/100 mL	8/15/2019 12:40:00PM
Coliform, Total	5475		10	MPN/100 mL	8/15/2019 12:40:00PM
Microbiology, Enterolert					
Enterococcus	52		10	MPN/100 mL	8/15/2019 12:55:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	63		10	MPN/100 mL	8/15/2019 1:35:00PM
Field ID: NY529956				**Sample ID: 1908059-01**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.87	mean Log10 copies	8/20/2019 2:45:00PM
MST-Human-HumM2 Marker	---	U	0.92	mean Log10 copies	8/20/2019 2:45:00PM
Microbiology, Colilert					
E. coli	850		10	MPN/100 mL	8/20/2019 3:18:00PM
Coliform, Total	4106		10	MPN/100 mL	8/20/2019 3:18:00PM
Microbiology, Enterolert					
Enterococcus	148		10	MPN/100 mL	8/20/2019 3:13:00PM
Microbiology, Colilert 18, Fecal Coliform					

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Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: NY529956				**Sample ID: 1908059-01**	
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	256		10	MPN/100 mL	8/20/2019 3:25:00PM
Field ID: SW-09				**Sample ID: 1908059-02**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.87	mean Log10 copies	8/20/2019 2:50:00PM
MST-Human-HumM2 Marker	---	U	0.92	mean Log10 copies	8/20/2019 2:50:00PM
Microbiology, Colilert					
E. coli	2753		10	MPN/100 mL	8/20/2019 3:18:00PM
Coliform, Total	173290		100	MPN/100 mL	8/20/2019 3:18:00PM
Microbiology, Enterolert					
Enterococcus	3770		100	MPN/100 mL	8/20/2019 3:13:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	7120		100	MPN/100 mL	8/20/2019 3:25:00PM
Field ID: GW-S2				**Sample ID: 1908059-03**	
Microbiology					
MST-Human-HF183 Marker	---	U J	0.82	mean Log10 copies	8/20/2019 3:20:00PM
MST-Human-HumM2 Marker	---	U J	0.73	mean Log10 copies	8/20/2019 3:20:00PM
Microbiology, Colilert					
E. coli	19610		100	MPN/100 mL	8/20/2019 3:18:00PM
Coliform, Total	>241960		100	MPN/100 mL	8/20/2019 3:18:00PM
Microbiology, Enterolert					
Enterococcus	2850		100	MPN/100 mL	8/20/2019 3:13:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	5450		100	MPN/100 mL	8/20/2019 3:25:00PM

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Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*	
Field ID: WO-1				**Sample ID: 1908059-04**		
Microbiology						
MST-Human-HF183 Marker	---	U	0.87	mean Log10 copies	8/20/2019	2:55:00PM
MST-Human-HumM2 Marker	---	U	0.92	mean Log10 copies	8/20/2019	2:55:00PM
Microbiology, Colilert						
E. coli	1602		10	MPN/100 mL	8/20/2019	3:18:00PM
Coliform, Total	120330		100	MPN/100 mL	8/20/2019	3:18:00PM
Microbiology, Enterolert						
Enterococcus	4106		10	MPN/100 mL	8/20/2019	3:13:00PM
Microbiology, Colilert 18, Fecal Coliform						
Coliform, Fecal	4870		100	MPN/100 mL	8/20/2019	3:25:00PM
Field ID: SW-07				**Sample ID: 1908059-05**		
Microbiology						
MST-Human-HF183 Marker	---	U	0.87	mean Log10 copies	8/20/2019	3:00:00PM
MST-Human-HumM2 Marker	---	U	0.92	mean Log10 copies	8/20/2019	3:00:00PM
Microbiology, Colilert						
E. coli	373		10	MPN/100 mL	8/20/2019	3:18:00PM
Coliform, Total	41060		100	MPN/100 mL	8/20/2019	3:18:00PM
Microbiology, Enterolert						
Enterococcus	74		10	MPN/100 mL	8/20/2019	3:13:00PM
Microbiology, Colilert 18, Fecal Coliform						
Coliform, Fecal	1178		10	MPN/100 mL	8/20/2019	3:25:00PM
Field ID: SG-C				**Sample ID: 1908059-06**		
Microbiology						
MST-Human-HF183 Marker	---	U	0.87	mean Log10 copies	8/20/2019	3:25:00PM

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SG-C				**Sample ID: 1908059-06**	
Microbiology					
MST-Human-HumM2 Marker	---	U	0.92	mean Log10 copies	8/20/2019 3:25:00PM
Microbiology, Colilert					
E. coli	14390		100	MPN/100 mL	8/20/2019 3:18:00PM
Coliform, Total	>241960		100	MPN/100 mL	8/20/2019 3:18:00PM
Microbiology, Enterolert					
Enterococcus	8620		100	MPN/100 mL	8/20/2019 3:13:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	13740		100	MPN/100 mL	8/20/2019 3:25:00PM
Field ID: ST-01				**Sample ID: 1908059-07**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.87	mean Log10 copies	8/20/2019 3:05:00PM
MST-Human-HumM2 Marker	---	U	0.92	mean Log10 copies	8/20/2019 3:05:00PM
Microbiology, Colilert					
E. coli	3255		10	MPN/100 mL	8/20/2019 3:18:00PM
Coliform, Total	198630		100	MPN/100 mL	8/20/2019 3:18:00PM
Microbiology, Enterolert					
Enterococcus	3282		10	MPN/100 mL	8/20/2019 3:13:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	10670		100	MPN/100 mL	8/20/2019 3:25:00PM
Field ID: SW-03				**Sample ID: 1908059-08**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.89	mean Log10 copies	8/20/2019 3:10:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	8/20/2019 3:10:00PM

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: SW-03				**Sample ID: 1908059-08**	
Microbiology, Colilert					
E. coli	717		10	MPN/100 mL	8/20/2019 3:18:00PM
Coliform, Total	30760		100	MPN/100 mL	8/20/2019 3:18:00PM
Microbiology, Enterolert					
Enterococcus	609		10	MPN/100 mL	8/20/2019 3:13:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	860		10	MPN/100 mL	8/20/2019 3:25:00PM
Field ID: GW-S				**Sample ID: 1908059-09**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.89	mean Log10 copies	8/20/2019 3:20:00PM
MST-Human-HumM2 Marker	---	U	0.81	mean Log10 copies	8/20/2019 3:20:00PM
Microbiology, Colilert					
E. coli	6488		10	MPN/100 mL	8/20/2019 3:18:00PM
Coliform, Total	155310		100	MPN/100 mL	8/20/2019 3:18:00PM
Microbiology, Enterolert					
Enterococcus	5710		100	MPN/100 mL	8/20/2019 3:13:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	16070		100	MPN/100 mL	8/20/2019 3:25:00PM
Field ID: NY529956				**Sample ID: 1908079-01**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.82	mean Log10 copies	8/27/2019 12:40:00PM
MST-Human-HumM2 Marker	---	U	0.73	mean Log10 copies	8/27/2019 12:40:00PM
Microbiology, Colilert					
E. coli	1354		10	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	4710		100	MPN/100 mL	8/27/2019 12:15:00PM

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: NY529956				**Sample ID: 1908079-01**	
Microbiology, Enterolert					
Enterococcus	<10		10	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	41		10	MPN/100 mL	8/27/2019 2:05:00PM
Field ID: SW-09				**Sample ID: 1908079-02**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.82	mean Log10 copies	8/27/2019 12:45:00PM
MST-Human-HumM2 Marker	0.97		0.73	mean Log10 copies	8/27/2019 12:45:00PM
Microbiology, Colilert					
E. coli	1259		10	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	>241960		100	MPN/100 mL	8/27/2019 12:15:00PM
Microbiology, Enterolert					
Enterococcus	1043		10	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	2282		10	MPN/100 mL	8/27/2019 2:05:00PM
Field ID: WO-1				**Sample ID: 1908079-03**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	8/27/2019 12:50:00PM
MST-Human-HumM2 Marker	---	U	0.78	mean Log10 copies	8/27/2019 12:50:00PM
Microbiology, Colilert					
E. coli	689		10	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	129970		100	MPN/100 mL	8/27/2019 12:15:00PM
Microbiology, Enterolert					
Enterococcus	565		10	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					

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Region 2 Laboratory

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: WO-1				**Sample ID: 1908079-03**	
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	794		10	MPN/100 mL	8/27/2019 2:05:00PM
Field ID: SW-07				**Sample ID: 1908079-04**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	8/27/2019 1:00:00PM
MST-Human-HumM2 Marker	---	U	0.78	mean Log10 copies	8/27/2019 1:00:00PM
Microbiology, Colilert					
E. coli	<10		10	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	3873		10	MPN/100 mL	8/27/2019 12:15:00PM
Microbiology, Enterolert					
Enterococcus	41		10	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	52		10	MPN/100 mL	8/27/2019 2:05:00PM
Field ID: SG-C				**Sample ID: 1908079-05**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	8/27/2019 1:05:00PM
MST-Human-HumM2 Marker	---	U	0.78	mean Log10 copies	8/27/2019 1:05:00PM
Microbiology, Colilert					
E. coli	3873		10	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	>241960		100	MPN/100 mL	8/27/2019 12:15:00PM
Microbiology, Enterolert					
Enterococcus	>241960		100	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	16700		100	MPN/100 mL	8/27/2019 2:05:00PM

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: ST-01				**Sample ID: 1908079-06**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	8/27/2019 1:20:00PM
MST-Human-HumM2 Marker	---	U	0.78	mean Log10 copies	8/27/2019 1:20:00PM
Microbiology, Colilert					
E. coli	241960		100	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	>241960		100	MPN/100 mL	8/27/2019 12:15:00PM
Microbiology, Enterolert					
Enterococcus	4140		100	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	120330		100	MPN/100 mL	8/27/2019 2:05:00PM
Field ID: SW-03				**Sample ID: 1908079-07**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	8/27/2019 1:25:00PM
MST-Human-HumM2 Marker	---	U	0.78	mean Log10 copies	8/27/2019 1:25:00PM
Microbiology, Colilert					
E. coli	134		10	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	19350		100	MPN/100 mL	8/27/2019 12:15:00PM
Microbiology, Enterolert					
Enterococcus	275		10	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	85		10	MPN/100 mL	8/27/2019 2:05:00PM
Field ID: GW-S				**Sample ID: 1908079-08**	
Microbiology					
MST-Human-HF183 Marker	---	U	0.84	mean Log10 copies	8/27/2019 1:30:00PM

Final Report

Project: Crescent Beach MST- 2019 - 1906060

Project Number: 1906060

Analyte	Result	Qualifier	Reporting Limit	Units	Date and Time of Analysis*
Field ID: GW-S				**Sample ID: 1908079-08**	
Microbiology					
MST-Human-HumM2 Marker	---	U	0.78	mean Log10 copies	8/27/2019 1:30:00PM
Microbiology, Colilert					
E. coli	75		10	MPN/100 mL	8/27/2019 12:15:00PM
Coliform, Total	6131		10	MPN/100 mL	8/27/2019 12:15:00PM
Microbiology, Enterolert					
Enterococcus	173		10	MPN/100 mL	8/27/2019 12:33:00PM
Microbiology, Colilert 18, Fecal Coliform					
Coliform, Fecal	63		10	MPN/100 mL	8/27/2019 2:05:00PM

ATTACHMENT 2- RAW DATA REPORT

Non-Human Markers Raw Data from Station SW-09

Six Sampling Events Between June 27, 2019 and August 27, 2019

as provided by Source Molecular

Source Molecular

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Ruminant Fecal Quantification ID Test Results

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

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

ND: Not Detected

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM-9L04030	SW-09-Event 1-DRY	Ruminant Fecal ID: Target 1	ND	Filter
SM-9L04032	SW-09-Event 2-WET	Ruminant Fecal ID: Target 1	ND	Filter
SM-9L04033	SW-09-Event 3-WET	Ruminant Fecal ID: Target 1	ND	Filter
SM-9L04034	SW-09-Event 4-DRY	Ruminant Fecal ID: Target 1	ND	Filter
SM-9L04035	SW-09-Event 5-WET	Ruminant Fecal ID: Target 1	ND	Filter
SM-9L04036	SW-09-Event 6-DRY	Ruminant Fecal ID: Target 1	ND	Filter

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 analysi s.

Revision 1.4
Effective Date 12/12/19

Source Molecular

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Ruminant Fecal Quantification ID Details of Sample Tested

Details of the sample material tested for the of the fecal associated Ruminant gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

SM #	Sample ID	Extraction Date	Analysis Date	Filtration Volume (ml)	Analytical Volume (ul)
SM-9L04030	SW-09-Event 1-DRY	12/9/2019	12/9/2019	100	2
SM-9L04032	SW-09-Event 2-WET	12/9/2019	12/9/2019	100	2
SM-9L04033	SW-09-Event 3-WET	12/9/2019	12/9/2019	100	2
SM-9L04034	SW-09-Event 4-DRY	12/9/2019	12/9/2019	100	2
SM-9L04035	SW-09-Event 5-WET	12/9/2019	12/9/2019	100	2
SM-9L04036	SW-09-Event 6-DRY	12/9/2019	12/9/2019	100	2

Reported Results Authorized By: Anda Quintero, Quality Manager

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Gull Fecal Quantification ID Test Results Report

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

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

ND Not Detected

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM-9L04007	SW-09-Event 1-DRY	Gull Fecal ID	ND	Filter
SM-9L04008	SW-09-Event 2-WET	Gull Fecal ID	ND	Filter
SM-9L04009	SW-09-Event 3-WET	Gull Fecal ID	ND	Filter
SM-9L04010	SW-09-Event 4-DRY	Gull Fecal ID	5.94E+02	Filter
SM-9L04012	SW-09-Event 5-WET	Gull Fecal ID	5.13E+02	Filter
SM-9L04013	SW-09-Event 6-DRY	Gull Fecal ID	1.56E+03	Filter

Reported Results Authorized By: Anda Quintero, Quality Manager

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Gull Fecal Quantification ID Details of Sample Tested

Details of the sample material tested for the of the fecal associated Gull gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

SM #	Sample ID	Extraction Date	Analysis Date	Filtration Volume (ml)	Analytical Volume (ul)
SM-9L04007	SW-09-Event 1-DRY	12/9/2019	12/9/2019	100	2
SM-9L04008	SW-09-Event 2-WET	12/9/2019	12/9/2019	100	2
SM-9L04009	SW-09-Event 3-WET	12/9/2019	12/9/2019	100	2
SM-9L04010	SW-09-Event 4-DRY	12/9/2019	12/9/2019	100	2
SM-9L04012	SW-09-Event 5-WET	12/9/2019	12/9/2019	100	2
SM-9L04013	SW-09-Event 6-DRY	12/9/2019	12/9/2019	100	2

Reported Results Authorized By: Anda Quintero, Quality Manager

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Goose Fecal Quantification ID Test Results Report

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

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

ND: Not Detected
DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM-9L04014	SW-09-Event 1-DRY	Goose Bacteroidetes ID: Target 1	ND	Filter
SM-9L04016	SW-09-Event 2-WET	Goose Bacteroidetes ID: Target 1	ND	Filter
SM-9L04018	SW-09-Event 3-WET	Goose Bacteroidetes ID: Target 1	ND	Filter
SM-9L04019	SW-09-Event 4-DRY	Goose Bacteroidetes ID: Target 1	DNQ	Filter
SM-9L04020	SW-09-Event 5-WET	Goose Bacteroidetes ID: Target 1	ND	Filter
SM-9L04022	SW-09-Event 6-DRY	Goose Bacteroidetes ID: Target 1	DNQ	Filter

Reported Results Authorized By: Anda Quintero, Quality Manager

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Revision 1.4
Effective Date 12/12/19

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Goose Fecal Quantification ID Details of Sample Tested

Details of the sample material tested for the of the fecal associated Goose gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

SM #	Sample ID	Extraction Date	Analysis Date	Filtration Volume (ml)	Analytical Volume (ul)
SM-9L04014	SW-09-Event 1-DRY	12/9/2019	12/10/2019	100	2
SM-9L04016	SW-09-Event 2-WET	12/9/2019	12/10/2019	100	2
SM-9L04018	SW-09-Event 3-WET	12/9/2019	12/10/2019	100	2
SM-9L04019	SW-09-Event 4-DRY	12/9/2019	12/10/2019	100	2
SM-9L04020	SW-09-Event 5-WET	12/9/2019	12/10/2019	100	2
SM-9L04022	SW-09-Event 6-DRY	12/9/2019	12/10/2019	100	2

Reported Results Authorized By: Anda Quintero, Quality Manager

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Dog Fecal Quantification ID Test Results Report

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

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM-9L04023	SW-09-Event 1-DRY	Dog Bacteroidetes ID: Target 1	6.52E+02	Filter
SM-9L04025	SW-09-Event 2-WET	Dog Bacteroidetes ID: Target 1	1.20E+03	Filter
SM-9L04026	SW-09-Event 3-WET	Dog Bacteroidetes ID: Target 1	3.98E+03	Filter
SM-9L04027	SW-09-Event 4-DRY	Dog Bacteroidetes ID: Target 1	7.56E+02	Filter
SM-9L04028	SW-09-Event 5-WET	Dog Bacteroidetes ID: Target 1	1.06E+03	Filter
SM-9L04029	SW-09-Event 6-DRY	Dog Bacteroidetes ID: Target 1	DNQ	Filter

Reported Results Authorized By: Anda Quintero, Quality Manager

Results reported herein apply only to the sample matrices as received.
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Revision 1.4
Effective Date 12/12/19

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Dog Fecal Quantification ID Details of Sample Tested

Details of the sample material tested for the of the fecal associated Dog gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

SM #	Sample ID	Extraction Date	Analysis Date	Filtration Volume (ml)	Analytical Volume (ul)
SM-9L04023	SW-09-Event 1-DRY	12/9/2019	12/9/2019	100	2
SM-9L04025	SW-09-Event 2-WET	12/9/2019	12/9/2019	100	2
SM-9L04026	SW-09-Event 3-WET	12/9/2019	12/9/2019	100	2
SM-9L04027	SW-09-Event 4-DRY	12/9/2019	12/9/2019	100	2
SM-9L04028	SW-09-Event 5-WET	12/9/2019	12/9/2019	100	2
SM-9L04029	SW-09-Event 6-DRY	12/9/2019	12/9/2019	100	2

Reported Results Authorized By: Anda Quintero, Quality Manager

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Source Molecular

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Bird Fecal Quantification ID Test Results Report

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

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM-9L04001	SW-09-Event 1-DRY	Bird Fecal ID	4.48E+04	Filter
SM-9L04002	SW-09-Event 2-WET	Bird Fecal ID	9.35E+04	Filter
SM-9L04003	SW-09-Event 3-WET	Bird Fecal ID	6.15E+04	Filter
SM-9L04004	SW-09-Event 4-DRY	Bird Fecal ID	4.43E+04	Filter
SM-9L04005	SW-09-Event 5-WET	Bird Fecal ID	4.36E+04	Filter
SM-9L04006	SW-09-Event 6-DRY	Bird Fecal ID	3.24E+04	Filter

Reported Results Authorized By: Anda Quintero, Quality Manager

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Revision 1.4
Effective Date 12/12/19

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Bird Fecal Quantification ID Details of Sample Tested

Details of the sample material tested for the of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: USEPA Region 2
Date Received/Processed: December 4, 2019
Report Generated: December 13, 2019

SM #	Sample ID	Extraction Date	Analysis Date	Filtration Volume (ml)	Analytical Volume (ul)
SM-9L04001	SW-09-Event 1-DRY	12/9/2019	12/10/2019	100	0.2
SM-9L04002	SW-09-Event 2-WET	12/9/2019	12/10/2019	100	0.2
SM-9L04003	SW-09-Event 3-WET	12/9/2019	12/10/2019	100	0.2
SM-9L04004	SW-09-Event 4-DRY	12/9/2019	12/10/2019	100	0.2
SM-9L04005	SW-09-Event 5-WET	12/9/2019	12/10/2019	100	0.2
SM-9L04006	SW-09-Event 6-DRY	12/9/2019	12/10/2019	100	0.2

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.

Laboratory Comments

Submitter: USEPA Region 2

Report Generated: December 13, 2019

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.