

**2017 FINAL PROJECT REPORT**

**Microbial Source Tracking and Virginia's Beach Monitoring Program**

**MEMORANDUM OF AGREEMENT: HRSDMST617FY17**

between

**Virginia Department of Health**  
Office of Epidemiology  
Waterborne Hazards Control Program  
109 Governor Street, Madison Building  
Post Office Box 2448, Suite 516 East  
Richmond, Virginia, 23219

and

**Hampton Roads Sanitation District**  
Technical Services Division  
1434 Air Rail Avenue  
Virginia Beach, VA 23507

**Raul Gonzalez**, Environmental Scientist  
**Danny Barker**, Environmental Scientist

## **Introduction:**

Hampton Roads Sanitation District (HRSD) took over microbial source tracking (MST) analyses for the Virginia Beach Monitoring Program from Virginia Tech after the 2016 beach monitoring season. Previously Virginia Tech quantified the culturable enterococci and the human-associated MST marker, HF183, on Virginia Department of Health (VDH) health districts water samples that exceeded the recreational water quality standard. HRSD processing MST markers for the 2017 beach monitoring season brought a shortened time between sample collection and processing due to closer proximity of the HRSD's Central Environmental Laboratory (CEL) to the health districts.

Water samples from 2017 collected by health districts were chosen for MST analyses at VDH's discretion when regulatory exceedances of enterococci concentrations occurred. Samples were picked up at contract laboratories used for culture enterococci analysis then brought to CEL. Upon receipt, each sample was immediately filtered to collect total DNA then stored in a -80°C freezer until downstream molecular processing within the week. Extraction of samples was completed with an automated total nucleic acid extraction system (bioMerieux NucliSENS easyMag) to minimize contamination and technician variability. HF183 and enterococci molecular markers were quantified using droplet digital PCR according to the protocol in Cao et al. (2015). Previous beach monitoring season MST used qPCR to provide relative quantification of molecular markers. Droplet digital PCR is the latest generation of PCR providing multiple advantages over qPCR—absolute quantification, improved handling of matrix inhibition, lower limits of detection. Molecular marker concentrations are reported as gene copies (copies) per 100 mL. For simplicity positive results were anything quantifiable above the assay limit of detection, while negative results were below limits of detection.

The HF183 molecular marker is associated with recent human fecal contamination. HF183 is the most frequently recommended and used human-associated fecal contamination marker to identify human sewage pollution in surface waters (Griffith et al., 2013; Ahmed et al., 2016). A recent study suggested a HF183 threshold of 4200 copies/100 mL to represent the benchmark illness rate of 30 gastrointestinal illnesses per 1000 swimmers (the current recreational acceptable risk level; Boehm et al., 2015). However for the purposes of detecting the potential presence of human fecal contamination, any significant positive detection defines the susceptibility of a water body to contamination. For reference HF183 is found in the range of  $10^7$  –  $10^8$  copies/100 mL in Hampton Roads raw sewage, but can be lower in sewer collection systems. The enterococci assay is based on the 2012 Recreational Water Quality Criteria (RWQC) and EPA method 1611. Virginia has yet to adopt the 2012 RWQC and the use of molecular enterococci assay. Molecular detection of enterococci has the advantage of a shorter time from sample collection to beach posting; as low as 4 hours (compared to the 18 to 24 hours for the culture test). For interpretation of molecular enterococci concentrations, EPA recommends the threshold of 2000 copies/100 mL for an acceptable illness rate of 36/1000 primary contact recreators.

## **Microbial Source Tracking Results:**

Fifty samples were submitted to HRSD for molecular analyses—48 samples from the Peninsula Health District and 2 samples from Virginia Beach. While the total number of samples submitted for MST analyses did not vary drastically from 2016 to 2017 (53 versus 50 samples). In 2016 samples were submitted predominantly from the Peninsula Health District with additional samples from the Eastern Shore and Hampton Health Districts. Samples from 2017 submitted to HRSD included 8 from Anderson Beach, 12 Hilton Beach, 5 from Hilton Beach outfall, 6 from Huntington Beach, 10 from King-Lincoln Park, 2 from King Lincoln outfall, 3 from Yorktown Beach-1, 2 from Yorktown Beach-2, and 2 from Virginia Beach 20 Lesner.

### *Human Fecal Contamination Marker*

The human-associated HF183 marker was detected in 6 of 50 samples (Table 1). The Hilton Beach outfall had 3 (out of 5 samples received) positive detections, King-Lincoln Park had 2 (out of 10 samples received), and Anderson Beach had 1 (out of 8 samples received). Even with the lower limits of detection and better handling of matrix inhibition with droplet digital PCR overall positive detection of the HF183 marker occurred at a lower detection frequency than in 2016—83% in 2016 versus 12% in 2017. This reduction is likely due to the numerous sewer infrastructure repair and replacement projects that have occurred over the last 2 years in Newport News (Garrett, 2017). Previous studies have documented the presence of human-associated fecal contamination markers present at the Hilton Beach outfall (Gonzalez 2014a; Gonzalez 2014b; Badgley 2016). Much of the collaborative work completed by the City of Newport News, Virginia Health Department, Hampton Roads Sanitation District, and Brown and Caldwell focused primarily in the Hilton Beach outfall watershed. Even though Hilton Beach outfall had the most HF183 detection during the swimming season (3), the magnitude was insignificant and well below the estimated HF183 threshold developed by Boehm et al. (2015) using the RWQC acceptable human health risk. The number of advisories remained the same from 2016 to 2017 (Table 2) while the frequency and magnitude of HF183 detections were drastically reduced. Environmental and animal sources transported to receiving waters via stormwater are a likely major source of elevated enterococci concentrations. It is well known that enterococci are not-host/human specific, can be persistent and thrive in different environmental matrices, and are ubiquitous in nature. Future work investigating chronic exceedances at beaches should consider non-human MST markers to help identify potential sources when human-associated markers are not present. Additionally since the HF183 marker is still being detected at certain beaches, more targeted MST work should be undertaken upstream and around those sites.

### *Alternative Recreational Water Quality Indicators*

The 2012 RWQC has not been adopted by Virginia but VDH Beach Monitoring Program did perform an enterococci qPCR performance and demonstration project in 2013. While results of

EPA method 1611 (use of the molecular enterococci marker) and logistics to implement the more rapid technology did not warrant adaptation at the time, the technology has improved drastically since 2012. As a result of the droplet digital PCR HF183 assay being a multiplexed assay with enterococci, an opportunity presented itself to re-examine the alternative indicator. This is especially timely since EPA is on the verge of releasing new and/or updated RWQC criteria in 2018 (EPA 2015, EPA 2016).

Table 3 highlights the agreement in 2017 beach management decisions between the current ‘gold standard’ (culture enterococci) quantification method with the molecular enterococci quantification based on EPA method 1611. There were a total of 43 submitted samples that had both culture and molecular enterococci data. A King-Lincoln outfall sample was not processed for culture enterococci and was not included in this section. Stormwater outfall data was also excluded. Beach advisories would occur if the culture result exceeded the 104 MPN/100 mL threshold or if the molecular result exceeded the 2000 copies/100 mL threshold. Twenty-two (51%) samples had similar beach management advisory decisions. All 22 of these samples were in agreement to not post an advisory. Twenty-one (49%) samples were not in agreement with regard to the beach management decision. Of these 21 samples 20 would have posted an advisory with culture results when molecular results would have caused no advisories. Based on this limited data less beach advisories would have occurred if the alternative molecular method would have been used. HF183 detection in the samples (as a proxy for human enteric pathogens) was used to gauge if the samples where beach management decisions disagreed posed a greater human health risk and should have been posted. Contact with human fecally contaminated waters pose a greater risk than water contact with waters without human fecal contamination. Of the 6 positive HF183 detections, only 3 occurred where there was disagreement. The 3 HF183 positive samples occurred in samples where culture results exceeded the RWQC threshold but molecular enterococci results did not. However these HF183 results were all very low in concentration (see Table 1)—under the estimated HF183 human health risk level from Boehm et al. (2015). Caution should be used when interpreting these results since these samples were generally sent to HRSD when there was an exceedance. These samples were a small proportion of those collected at monitored beaches and were collected under certain conditions, skewing potential comparisons. This comparison could benefit from a more structured side-by-side study where a wide range of enterococci and HF183 concentrations is collected and analyzed. Furthermore, future work should focus on evaluating a side-by-side study using coliphage as an indicator compared to enterococci. EPA is currently considering new coliphage (bacteriophage that infect *E. coli* bacteria) because recent literature has suggested that enteric viruses (i.e. norovirus) are the greater risk to recreational swimmers (Soller et al., 2010). Coliphage would likely be more representative of enteric viruses than enterococci because coliphage would have more similar fate and transport characteristics to enteric viruses than a bacterial indicator.

## Recommendations:

- Future MST studies should target the few beach sites with positive human-associated marker detections for intensive upstream investigations to identify any potential compromised infrastructure. Relevant stakeholders should be involved in study planning (e.g. municipalities, health districts, wastewater authorities).
- Currently beach monitoring samples are collected regardless of rainfall. Stormwater is known to transport larger microbial loads to downstream receiving waters than during dry weather. Conditional beach advisories based on specific rainfall amounts (derived from historical data) in tandem with a more dynamic sample collection scheme would minimize risk.
- Caution should be used when interpreting beach water quality data that does not accurately characterize the entire beach. Sampling scheme should be designed according to EPA (2010) when beaches are affected by infrastructure (e.g. stormwater outfalls).
- Future work should evaluate the effectiveness of alternative RWQC (e.g. coliphage) that EPA is considering.

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**Tables:**

Table 1. Results of the human-associated HF183 marker for samples sent to HRSD. Samples were reported as negative when results were below assay limit of detection. Positive samples were those where results were above the assay limit of detection.

<b>Date</b>	<b>Location</b>	<b>Sample Volume (mL)</b>	<b>HF183 copies/100 mL</b>	<b>Report As</b>
5/23	King-Lincoln Park	100	<168	Negative
5/24	King-Lincoln Park	200	78	Positive
6/6	Hilton Beach	108	<156	Negative
6/6	Huntington Beach	106	<162	Negative
6/7	Hilton Beach	400	<42	Negative
6/7	Huntington Beach	400	<42	Negative
6/14	Hilton Outfall	400	106	Positive
6/14	Hilton Beach	400	<42	Negative
6/21	Hilton Beach	400	<42	Negative
6/21	Hilton Outfall	400	167	Positive
6/21	Huntington Beach	400	<42	Negative
6/21	Anderson Beach	400	<42	Negative
6/22	Hilton Beach	400	<42	Negative
6/22	Hilton Outfall	400	<42	Negative
6/28	Anderson Beach	400	<42	Negative
6/28	King-Lincoln Park	400	<42	Negative
6/28	Huntington Beach	400	<42	Negative
6/28	Hilton Outfall	400	<42	Negative
7/6	King-Lincoln Park	400	<42	Negative
7/28	King-Lincoln Park	400	<42	Negative
8/8	VB20 Lesner	90	<187	Negative
8/9	VB20 Lesner	200	<84	Negative
8/9	Hilton Beach	97	<173	Negative
8/9	Huntington Beach	97	<173	Negative
8/9	King-Lincoln Park	100	<168	Negative
8/9	Anderson Beach	92	<183	Negative
8/9	Yorktown Beach-1	100	<168	Negative
8/9	Yorktown Beach-2	75	<224	Negative
8/10	King-Lincoln	400	<42	Negative
8/10	Hilton Beach	400	<42	Negative
8/29	Hilton Outfall	200	411	Positive
8/29	Hilton Beach	200	<84	Negative
8/29	Huntington Beach	200	<84	Negative
8/29	King-Lincoln Park	200	767	Positive
8/29	Anderson Beach	200	<84	Negative

8/29	Yorktown Beach-1	200	<84	Negative
8/29	King-Lincoln Outfall	200	<84	Negative
8/30	Hilton Beach	200	<84	Negative
8/30	King-Lincoln Park	200	<84	Negative
8/30	Anderson Beach	200	133	Positive
8/30	Yorktown Beach-1	200	<84	Negative
8/30	Yorktown Beach-2	200	<84	Negative
8/30	King-Lincoln Outfall	200	<84	Negative
8/31	Hilton Beach	200	<84	Negative
8/31	King-Lincoln Park	200	<84	Negative
8/31	Anderson Beach	200	<84	Negative
9/5	Hilton Beach	82	<205	Negative
9/5	Anderson Beach	106	<158	Negative
9/6	Hilton Beach	200	<84	Negative
9/6	Anderson Beach	200	<84	Negative



Table 2. Number of beach advisories by year.

<b>Year</b>	<b># of Advisories</b>	<b>Days under Advisory</b>	<b># of Beaches</b>
2004	27	147	11
2005	14	42	8
2006	8	43	4
2007	14	50	8
2008	10	29	6
2009	14	51	9
2010	38	81	16
2011	28	69	15
2012	23	29	17
2013	21	30	13
2014	52	113	32
2015	26	97	4
2016	42	96	16
2017	42	67	18

Table 3. Beach management decision agreement between culture and molecular enterococci quantification methods.

Date	Location	VDH Enterococci MPN/100 mL	Enterococci copies/100 mL	Was there a		Would there be an advisory using molecular method?
				VDH advisory using culture method?	Agreement b/w methods?	
5/23	King-Lincoln Park	169/134	133	Yes	No	No
5/24	King-Lincoln Park	211/132	200	Yes	No	No
6/6	Hilton Beach	2755/2909	556	Yes	No	No
6/6	Huntington Beach	2046/2613	<135	Yes	No	No
6/7	Hilton Beach	63/85	<36	No	Yes	No
6/7	Huntington Beach	97/31	<36	No	Yes	No
6/14	Hilton Beach	663/657	<36	Yes	No	No
6/21	Hilton Beach	1354/1376	228	Yes	No	No
6/21	Huntington Beach	30/63	<36	No	Yes	No
6/21	Anderson Beach	52/85	328	No	Yes	No
6/22	Hilton Beach	41/31	<36	No	Yes	No
6/28	Anderson Beach	41/10	100	No	Yes	No
6/28	King-Lincoln Park	10/41	<36	No	Yes	No
6/28	Huntington Beach	<10/20	<36	No	Yes	No
7/6	King-Lincoln Park	<10/10	<36	No	Yes	No
7/28	King-Lincoln Park	<10/10	72	No	Yes	No
8/8	VB20 Lesner	934	1040	Yes	No	No
8/9	VB20 Lesner	<10	<236.5	No	Yes	No
8/9	Hilton Beach	171/189	1510	Yes	No	No
8/9	Huntington Beach	10/20	2430	No	No	Yes
8/9	King-Lincoln Park	119/98	844	Yes	No	No
8/9	Anderson Beach	20/31	411	No	Yes	No
8/9	Yorktown Beach-1	31/63	<143	No	Yes	No
8/9	Yorktown Beach-2	<10/20	<191	No	Yes	No
8/10	King-Lincoln	41 /63	178	No	Yes	No
8/10	Hilton Beach	31/20	883	No	Yes	No
8/29	Hilton Beach	2014/2481	433	Yes	No	No
8/29	Huntington Beach	97/85	<72	No	Yes	No
8/29	King-Lincoln Park	>24196/>24196	933	Yes	No	No
8/29	Anderson Beach	15531 />24196	711	Yes	No	No
8/29	Yorktown Beach-1	1396/1421	411	Yes	No	No
8/30	Hilton Beach	288/295	<72	Yes	No	No
8/30	King-Lincoln Park	187/199	<72	Yes	No	No
8/30	Anderson Beach	399/223	<72	Yes	No	No
8/30	Yorktown Beach-1	20/20	<72	No	Yes	No
8/30	Yorktown Beach-2	52/52	<72	No	Yes	No
8/31	Hilton Beach	203/145	100	Yes	No	No
8/31	King-Lincoln Park	52/85	122	No	Yes	No
8/31	Anderson Beach	323/379	<72	Yes	No	No
9/5	Hilton Beach	134/109	379	Yes	No	No
9/5	Anderson Beach	288/364	377	Yes	No	No
9/6	Hilton Beach	41/52	189	No	Yes	No
9/6	Anderson Beach	20/10	289	No	Yes	No