2013 FINAL PROJECT REPORT

Microbial Source Tracking And Virginia's Beach Monitoring Program

MEMORANDUM OF AGREEMENT: (VPI-BCH617V66479-1-2013)

Between

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Office of Epidemiology
Waterborne Hazards Control Program
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and

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Annual Project Report on Activities Relating to Sample Collection, Monitoring and Source Tracking Analyses at Virginia's Public Beaches, 2013.

1. Overview of 2013 Advisories

The VDH-VT Beaches Project began in 2004 when VDH initiated weekly monitoring and implemented EPA-approved sampling protocols. Beach statistics for each year are compiled by VDH and are available at: http://www.vdh.virginia.gov/Epidemiology/dee/beachmonitoring/.

For the 2013 swimming season there were 21 total advisories covering 13 beaches and 30 days under advisory (Tables 1 and 2). Fairview Beach (on the Potomac River), an annual problem every year, was surprisingly not problematic in 2013, with just 2 advisories and 5 days under advisory. In addition to Fairview (King George), five locations at Norfolk had 6 advisories and 6 days under advisory, four locations at Newport News had 9 advisories and 15 days under advisory, and three locations at Virginia Beach had 4 advisories and 4 days under advisory, (Table 1). One of the advisories at Fairview Beach (6/3-6/6) was precautionary and was issued during a severe rain event.

Table 1. Virginia's public beach advisories for the 2013 season

Sample Date (Lab. Report)	Sampling Site	Results (cfu/100ml)	Action Taken
Norfolk			
7/1/13	Captains Quarters	1521	Advisory
7/2/13	Captains Quarters	1	Lifted
7/1/13	Capeview Ave., North	1408	Advisory
7/2/13	Capeview Ave., North	15	Lifted
7/1/13	13th View, North	13084	Advisory
7/2/13	13th View, North	25	Lifted
6/10/13	21st Bay Street	116	Advisory
6/11/13	21st Bay Street	1	Lifted
7/1/13	21st Bay Street	148	Advisory
7/2/13	21st Bay Street	1	Lifted
6/24/13	E. Community Beach	181	Advisory
6/25/13	E. Community Beach	1	Lifted
Newport News			
6/11/13	Huntington	1463	Advisory
6/12/13	Huntington	1	Lifted
7/23/13	Huntington	1818	Advisory
7/24/13	Huntington	1	Lifted
5/21/13	Hilton	592	Advisory
5/22/13	Hilton	57	Lifted
7/9/13	Hilton	191	Advisory
7/10/13	Hilton	11	Lifted
7/23-7/26/13	Hilton	7766, 211, 528, 1397	Advisory
7/31/13	Hilton	15	Lifted

8/6-8/7/13	Hilton	227, 311	Advisory
8/8/13	Hilton	21	Lifted
8/13/13	Hilton	563	Advisory
8/14/13	Hilton	36	Lifted
7/23/13	Anderson's Beach	181	Advisory
7/24/13	Anderson's Beach	47	Lifted
7/23/13	King/Lincoln Park	128	Advisory
7/24/13	King/Lincoln Park	42	Lifted
King George			
6/3/13	Fairview Beach	Precautionary	Advisory
6/5/13	Fairview Beach	79	Lifted
6/10/13	Fairview Beach	144	Advisory
6/12/13	Fairview Beach	59	Lifted
Virginia Beach			
9/17/13	Chick's Beach	122	Advisory
9/18/13	Chick's Beach	20	Lifted
6/11/13	Lesner Bridge East	187	Advisory
6/12/13	Lesner Bridge East	10	Lifted
9/17/13	Lesner Bridge East	201	Advisory
9/18/13	Lesner Bridge East	1	Lifted
7/9/13	63rd Street	169	Advisory
7/10/13	63rd Street	1	Lifted

For the 21 advisories, 17 were one day events and 4 were multi-day events; two at Fairview Beach, (the precautionary advisory on 6/3-6/5, and 6/10-6/12), and two at Hilton Beach (7/23-7/31 and 8/6-8/8). The advisories often come in clusters, sometimes connected by proximity, sometimes spread over beaches where there is no proximity, and some one-day advisories just affect a single beach or two. Examples of these will be discussed in turn below.

To put the 2013 beach monitoring results in a historical perspective, Table 2 shows the advisory data for 2004 through 2013. The number of advisories and the number of beaches involved remains problematic over these years, but the number of days under advisory is showing improvement, especially when comparing 2010-2011 against 2012-2013.

Table 2. Advisory results for years 2004 through 2013, all beaches

Year	# of Advisories	Days under Advisory*	# of Beaches
2004	27	147 (122)	11
2005	14	42 (34)	8
2006	8	43 (10)	4

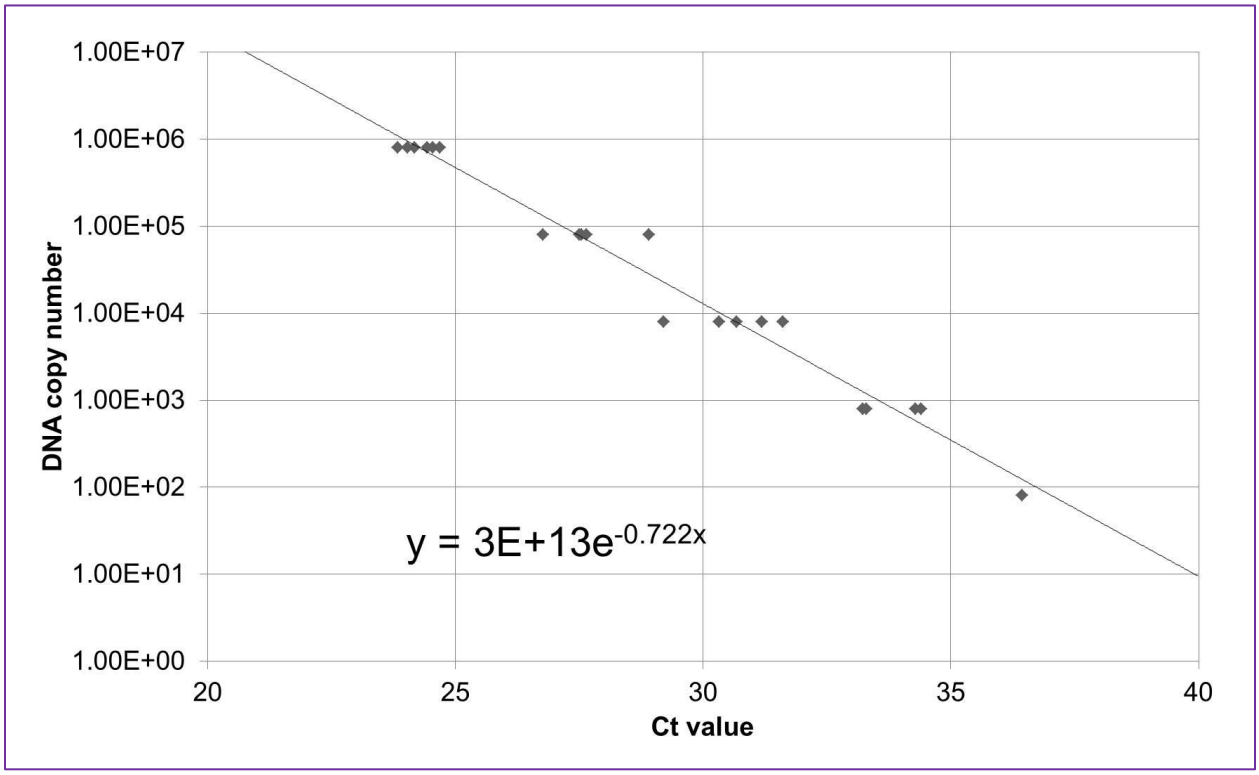
2007	14	50 (18)	8
2008	10	29 (5)	6
2009	14	51 (35)	9
2010	38	81 (63)	16
2011	28	69 (47)	15
2012	23	29 (19)	17
2013	21	30 (25)	13

*The number in parentheses indicates number of days under advisory for all beaches with Fairview Beach removed.

2. DNA-marker Methodology

To test for the presence of human-source pollution at beaches that experienced advisories, water samples collected during the advisories were shipped to the Hagedorn lab at VT over the course of the 2013 season. Samples were tested for two DNA-based markers, GenBac, the general *Bacteroides* indicator of fecal pollution, and HF183, a human-specific DNA marker. The detection and quantification of both markers is based on polymerase chain reaction (PCR) technology. In quantitative PCR (qPCR), the procedure involves 42 amplification cycles where the target DNA sequence, if present, is amplified (doubled) at each cycle. If a lot of the target DNA is present, then little amplification is needed and a positive result is visible at a low cycle number threshold (Ct), say 21 for example. If little target DNA is present, then it takes more cycles to amplify the DNA so the qPCR machine can read the amount (amplify to above the level of detection). What is actually detected is the number of copies of target DNA in the sample. The MST community now typically publishes qPCR results as copy number and those are included for HF183 in this year's report. The following figure is an example of an HF183 standard curve; this is used (formula in the figure) to convert Ct to copy number. Usually a standard curve is performed with every batch of samples so the copy number values will vary slightly from run to run. The standard curve is a log scale because each amplification cycle in qPCR doubles the amount of DNA present if a given marker is detected. A "hit" at cycle 21 for example, would indicate the sample contained about 10 million copies of HF183 DNA. This is a very large amount, a level normally found in raw sewage. A "hit" at cycle 35, for example (out of 42 cycles) indicates a much smaller amount, approx. 573 copies of HF183 DNA.

Figure 1. Example of a HF183 standard curve comparing Ct and DNA copy number



At present, the MST community is reporting amplification at cycles 40, 41, and 42 as <10 copies, or below limits of quantification (BLQ), indicating very weak and suspect (indeterminate) result. Cycles 36 through 39 are currently reported as <100; still positive but with a weak signal. Cycles 35 and lower are considered definite positive results and the number of copies of target DNA increases exponentially. There is no way at present to convert DNA copy number to some type of percentage, like what was done with the library-based approach. However, the MST community is actively involved in research to develop an algorithm that can convert copy number to a percentage. How well this will ultimately work is unknown at this time, but the MST community is attempting to develop such a capability. For water samples, qPCR results where the amplification cycle is 40, 41, or 42, the HF183 marker is essentially absent or below limits of detection (BLQ). Cycle numbers lower than 40 are considered positive for HF183.

3. Results for 2013

A. Norfolk Beaches: There was a one day advisory on 7/1-7/2 that involved four beaches, (Captain's Quarters, Capeview Ave., North, 13th View, North, and 21st Bay Street). No other public beaches experienced advisories or elevated counts over these dates. This was a substantial event along the Norfolk shoreline as indicated by the high numbers in the monitoring results for the four beaches, except for 21st Bay Street, where an *Enterococcus* count of only 148 was obtained (Table 3).

Table 3. Monitoring results for Norfolk beaches (Table 2), plus qPCR results for DNA markers

Date	Location	Counts	Status	Gen Bac	HF183	HF183 Ct	HF183 copy #
7/1/13	Captains Quarters	1521	Advisory	P	P	37	265
7/2/13	Captains Quarters	1	Lifted	N	N	--	--
7/1/13	Capeview Ave., North	1408	Advisory	P	P	37	265
7/2/13	Capeview Ave., North	15	Lifted	N	N	--	--
7/1/13	13th View, North	13084	Advisory	P	P	31	8674
7/2/13	13th View, North	25	Lifted	P	P	40	10
7/1/13	21st Bay Street	148	Advisory	P	P	38	51
7/2/13	21st Bay Street	1	Lifted	N	N	--	--

Monitoring results were typically <10 (reported as 1) for the ten Norfolk beaches sampled in 2013 (VDH monitoring data). On July 1, there were *Enterococccus* counts greater than 10 recorded for seven of the ten beaches, while the numbers were above state water quality standard of 104 for the four beaches in Table 3. However, on 7/2 the advisory was lifted on the four beaches and eight of the ten beaches had results of "1." Only Capeview and 13th View had results above 1 on 7/2. Such single-day events, especially when counts go from 13,084 to 25 as happened at 13th View, can be difficult to explain. The VDH sample collectors noted that there was dredging activity near 13th View in late June and early July, but it is hard to see how the dredging was related to the counts that were elevated for just a single day. That could only have been ascertained if the daily activities of the dredging operation was known, and if samples had been more intensively collected throughout the time the dredging was occurring. The GenBac results confirmed that fecal contamination occurred at the four beaches and the HF183 results confirmed that there was human-origin pollution present for the four beaches on 7/1 (Table 3). By 7/2 the markers were negative for all beaches except 13th View, where there was residual pollution as both GenBac and HF183 were positive, but HF183 had a Ct of 40, and a copy number that was BLQ. All four Norfolk beaches reported results of 1 for sampling the following week.

The only other advisories for Norfolk were a one-day exceedence on 6/10 that was lifted on 6/11. No samples were analyzed by VT for that advisory. The remaining was another single-day advisory that occurred on 6/24-6/25 (Table 4).

Table 4. Monitoring results for single-day advisory plus qPCR results for DNA markers

Date	Location	Counts	Status	Gen Bac	HF183	HF183 Ct	HF183 copy #
6/24/13	E. Community Beach	181	Advisory	P	N	--	--
6/25/13	E. Community Beach	1	Lifted	P	P	41	<10

The samples sent to VT were positive for fecal contamination (GenBac) on both days and the sample on 6/25 provided a result for HF183 but BLQ and indeterminate (less than 10 copies). The count on 6/24 was not large (181) and no suspected cause was identified for this advisory.

B. Newport News Beaches:
There were numerous advisories at the Newport News Beaches, especially Hilton and Huntington; and extra sampling was conducted in 2013. In addition, the City of Newport News and the Hampton Roads Sanitation District (HRSD) were involved in infrastructure upgrades and conducted sampling in August to determine if that work was impacting the beaches (see August results and discussion below).

The results for the Newport News beaches are as follows:
5/21-5/22 Single-day advisory at Hilton Beach, count of 592, no samples sent to VT.
6/11-6/12 Single-day advisory at Huntington Beach, counts of 1,463, no samples sent to VT.
7/9-7/10 Single-day advisory at Hilton Beach, counts of 191, no samples sent to VT.
7/23 Five beaches sampled, advisories at four beaches, Hilton, Huntington, Anderson, and King-Lincoln, samples sent to VT.

Table 5. Monitoring and DNA marker results for 7/23

	SAMPLE	ENTEROCOCCI
Sample Description	TIME	#/100mL
Yorktown Beach	10:20 AM	31/31
Huntington Beach	8:35 AM	1956/1679
Hilton Beach	8:20 AM	6867/8664
King-Lincoln Park	9:05 AM	185/71
Anderson Beach	9:25 AM	158/201

VT Samples					
Site	**Date**	**GenBac**	**HF183**	**HF183 Ct**	**HF183 Copy number**
Hilton	23-Jul	Pos.	Pos.	31	8732
Huntington	23-Jul	Pos.	Pos.	35	573
Anderson	23-Jul	Pos.	Pos.	39	28
King-Lincoln	23-Jul	Pos.	Neg.	--	--

The 7/23 counts were very high for Huntington and Hilton (both on the James River), but much lower for Anderson and King-Lincoln (Bay beaches). The HF183 results were strongly positive for human pollution at Hilton and Huntington, and less strong but still positive for Anderson Beach, and negative for King-Lincoln. The high counts at Hilton and Huntington indicate some major source of human fecal pollution that occurred on or before 7/23. There is no direct connection between the bay beaches (Anderson and King-Lincoln) and the river beaches (Hilton and Huntington), making it unlikely that the pollution source that was responsible for the high counts on the river beaches also impacted the bay beaches.

7/24 Four beaches resampled, advisory continued at Hilton, other beaches clear, Hilton sample sent to VT.

Table 6. Monitoring and DNA marker results for 7/24

	SAMPLE	ENTEROCOCCI
Sample Description	**TIME**	**#/100mL**
Huntington Beach	9:25 AM	31/20
Hilton Beach	9:10 AM	187/235
King-Lincoln Park	9:50 AM	52/31
Anderson Beach	10:15 AM	63/31

VT Sample					
Site	**Date**	**GenBac**	**HF183**	**HF183 Ct**	**HF183 Copy number**
Hilton	24-Jul	Pos.	Pos.	39	28

On 7/24 the counts at Hilton had fallen from an average of 7765 to 211, and the HF183 copy number results dropped from 8732 to 38, indicating that the pollution on 7/23 was not continuous.

7/25 Hilton resampled, advisory continued (counts of 528), no sample sent to VT.
7/26 Hilton resampled, advisory continued (counts of 1397, no sample sent to VT.

7/31 Five beaches sampled, none under advisory, 7/23-7/26 advisory at Hilton lifted (counts of 15). Samples also collected from two stormwater pipes at Hilton Beach.

Table 7. Monitoring and DNA marker results for 7/31

	SAMPLE	ENTEROCOCCI
Sample Description	**TIME**	**#/100mL**
Yorktown Beach	9:40 AM	<10/10
Huntington Beach	8:10 AM	<10/<10
Hilton Beach	7:30 AM	10/20
King-Lincoln Park	8:20 AM	20/10
Anderson Beach	8:50 AM	30/31
Hilton Stormwater Pipe #1	7:45 AM	285/320
Hilton Stormwater Pipe #2	7:35 AM	20/10

VT Samples					
Site	**Date**	**GenBac**	**HF183**	**HF183 Ct**	**HF183 Copy number**
Hilton	31-Jul	Neg.	Neg.	--	--
Hilton stormwater pipe 1	31-Jul	Pos.	Pos.	38	63
Hilton stormwater pipe 2	31-Jul	Neg.	Neg.	--	--

Stormwater pipe 1 had counts averaging 303 and was positive for HF183, but at a low level. Both Hilton and stormwater pipe 2 had counts averaging 15 and were negative for HF183. The advisory at Hilton that covered 7/23 to 7/31 appeared to stem from a large pollution event that was first detected on 7/23 with the high counts and positive results for HF183. Since the stormwater pipes were not sampled until 7/31, it's not possible to determine if the pollution detected previously originated from one or both of the stormwater pipes. The pipes are certainly implicated as possible sources and needing further investigation since pipe 1 had counts above the standard and was positive for HF183 on 7/31.

8/6 Five beaches sampled, Hilton under advisory (average count of 227), sample sent to VT.

Table 8. Monitoring and DNA marker results for 8/6

	SAMPLE	**ENTEROCOCCI**
Sample Description	**TIME**	**#/100mL**
Yorktown Beach	10:30 AM	10/10
Huntington Beach	9:00 AM	10/<10
Hilton Beach	8:45 AM	278/176
King-Lincoln Park	9:25 AM	<10/10
Anderson Beach	9:35 AM	<10/<10

VT Sample					
Site number	**Date**	**GenBac**	**HF183**	**HF183 Ct**	**HF183 Copy number**
Hilton	6-Aug	Pos.	Pos.	38	63

Counts were very low for four of the five beaches with the exception of Hilton, but the counts were not high and only averaged 27. The Hilton sample was positive for HF183, but at a low level, once again indicating that the persistent source of fecal pollution at Hilton Beach in the summer of 2013 had a human component.

8/7 Hilton resampled, stormpipe 1 sampled, both over standard, samples sent to VT.

Table 9. Monitoring and DNA marker results for 8/7

	SAMPLE	**ENTEROCOCCI**
Sample Description	**TIME**	**#/100mL**
Hilton Beach	9:50 AM	313/309
Hilton- Stormwater Pipe 1	9:50 AM	613/223

VT Samples					
Site number	**Date**	**GenBac**	**HF183**	**HF183 Ct**	**HF183 Copy number**
Hilton	7-Aug	Pos.	Pos.	37	84
Hilton stormwater pipe 1	7-Aug	Pos.	Pos.	36	275

8/8 Hilton resampled, result below standard (average count of 21), advisory lifted at Hilton.
8/13 Five beaches sampled, only Hilton under advisory (avg count 563). No sample sent to VT.

8/14 Hilton resampled, result below standard (average count 36), advisory lifted at Hilton, no sample sent to VT.

8/20 Five beaches sampled, all below standard and no advisories. Both stormpipes also sampled and sent to VT.

Table 10. Monitoring and DNA marker results for 8/20

	SAMPLE	**ENTEROCOCCI**
Sample Description	**TIME**	**#/100mL**
Hilton Beach	7:55 AM	<10
Stormwater Pipe #1	7:40 AM	301/213
Stormwater Pipe #2	7:50 AM	337/275

VT Samples				
Site	**GenBac**	**HF183**	**HF183 Ct**	**HF183 Copy number**
Stormwater pipe 1	Pos.	Pos.	38	63
Stormwater pipe 2	Pos.	Pos.	40	<10

Both stormpipes had counts above standard and both, plus the Hilton Beach sample, were positive for GenBac. The beach and stormwater pipe 2 samples were indeterminate for HF183 (very low copy number), while stormwater pipe 1 was positive for HF183.

8/29 Hilton Beach and four additional samples collected and sent to VT.

Table 11. Additional samples collected 8/29 and sent to VT

VT Samples							
Site	**Date**	**#/100mL**	**FSU**	**GenBac**	**HF183**	**HF183 Ct**	**HF183 Copy number**
Hilton #1	29-Aug	304	56.3	Pos.	Pos.	38	63
Hilton #3	29-Aug	<10	24	Neg.	Neg.	0	0
Hilton #4	29-Aug	<10	22.5	Neg.	Neg.	0	0
Hilton #5	29-Aug	205	40.6	Pos.	Neg.	0	0
Hilton Beach	29-Aug	<10	23.3	Neg.	Neg.	0	0

On 8/29, Hilton Beach was below the standard (count of <10) and two of the four additional samples also had counts of <10 (Hilton 3, downriver from the stormwater pipe; and Hilton 4, upriver next to the fishing pier). Two samples recorded counts above the standard, 304 for Hilton 1 (the stormwater pipe) and 205 for Hilton 5 (an open ditch west of the elementary school). These two samples were positive for GenBac but only Hilton 1 was positive for HF183, but at a low level – in keeping with the low regulatory counts. Fluorometry results (FSU) were typical for river water samples and only slightly elevated for Hilton 1 and 5. Clearly the stormwater pipe and the ditch near the school warrant further investigation in 2014.

There were no further advisories at any Newport New beaches after 8/13 and no additional samples sent to VT after 8/29.

D. VDH and VT Collaboration with City of Newport News / HRSD at Hilton Beach:

During 2013, five swimming advisories were issued at Hilton Beach for a total of 11 days, most of which occurred during July and August. These findings warranted further investigation to determine possible sources that may have contributed to exceedances during 2013. In order to systematically approach this problem, a collaborative effort was formed among experts from VDH, VT, Hampton Roads Sanitation District (HRSD), and the City of Newport News. HRSD maintains the sewer pump station and distribution system near the Hilton Beach area, the City of Newport News maintains the storm water system, and Virginia Tech has expertise in the molecular analysis of water samples using microbial source tracking methods. Water samples were collected on four days during August 2013 by the City of Newport News and HRSD from the storm water system and other locations surrounding Hilton Beach. These water samples were analyzed by HRSD for *E. coli* and enterococci bacteria, fluorescent material, salinity, and turbidity. These samples were also analyzed by Virginia Tech using BacHum and HF183 to assess whether the bacterial source was human or non-human (Table 12).

Table 12: Newport News storm water samples - Analyzed by HRSD & Virginia Tech

Site No.[1]	Date	Enterococci (MPN/100mL)	E. Coli (MPN/100mL)	BacHum	HF183	HF183 Cycle Number	HF183 Copy Number[2]	FM (fsu)	Latitude	Longitude
1	8/5/2013	1730	2720	Positive	Positive	35	573	79	37.027849	-76.464426
2	8/5/2013	3260	1990	Positive	Positive	33	1849	80	37.028054	-76.464383
3	8/5/2013	9210	4790	Positive	Positive	31	8732	75	37.028366	-76.464494
4	8/5/2013	1350	1310	Positive	Positive	36	275	85	37.028314	-76.464047
5	8/5/2013	106	10	Negative	Negative	0	0	32	37.025949	-76.462365
6	8/5/2013	820	990	Positive	Positive	36	275	74	37.021432	-76.455495
7	8/5/2013	400	275	Positive	Positive	38	63	170	37.035914	-76.477311
8	8/5/2013	1460	529	Positive	Positive	36	275	133	37.036419	-76.480683
9	8/9/2013	776	465	Positive	Positive	36	275	59.1	37.028921	-76.464743
10	8/9/2013	>24200	6870	Positive	Positive	27	101,210	621	37.028998	-76.465112
11	8/9/2013	1840	733	Positive	Positive	35	573	38.1	37.02929	-76.464235
12	8/9/2013	7270	2910	Positive	Positive	32	4973	149	37.029859	-76.464844
13	8/9/2013	266	315	Positive	Positive	38	63	71.2	37.03013	-76.465629
1	8/20/2013	426	3650	Negative	Negative	0	0	90.3	37.027849	-76.464426
2	8/20/2013	402	4610	Positive	Negative	0	0	82.3	37.028054	-76.464383
3	8/20/2013	712	3260	Negative	Negative	0	0	76.3	37.028366	-76.464494
4	8/20/2013	512	1300	Negative	Negative	0	0	133	37.028314	-76.464047
9	8/20/2013	1110	3650	Positive	Positive	37	93	56.5	37.028921	-76.464743
10	8/20/2013	1670	24200	Positive	Positive	35	611	488	37.028998	-76.465112
11	8/20/2013	441	1050	Negative	Negative	0	0	40.1	37.02929	-76.464235
12	8/20/2013	1480	24200	Positive	Positive	36	283	263	37.029859	-76.464844
13	8/20/2013	313	6130	Positive	Positive	38	54	80.5	37.03013	-76.465629
16	8/27/2013	5790	75	Positive	Positive	32	5,214	35	37.030572	-76.465924
17	8/27/2013	>24200	2910	Positive	Positive	27	102,300	721	37.030622	-76.466165
19	8/27/2013	24200	6130	Positive	Positive	27	102,300	1.7	37.031717	-76.467147
21	8/27/2013	13000	3870	Positive	Positive	30	14,126	422	37.032613	-76.464597

[1] The Site Number locations can be found by clicking here. It is necessary to use a Gmail account to view the map.

[2] HF183 Copy Number:

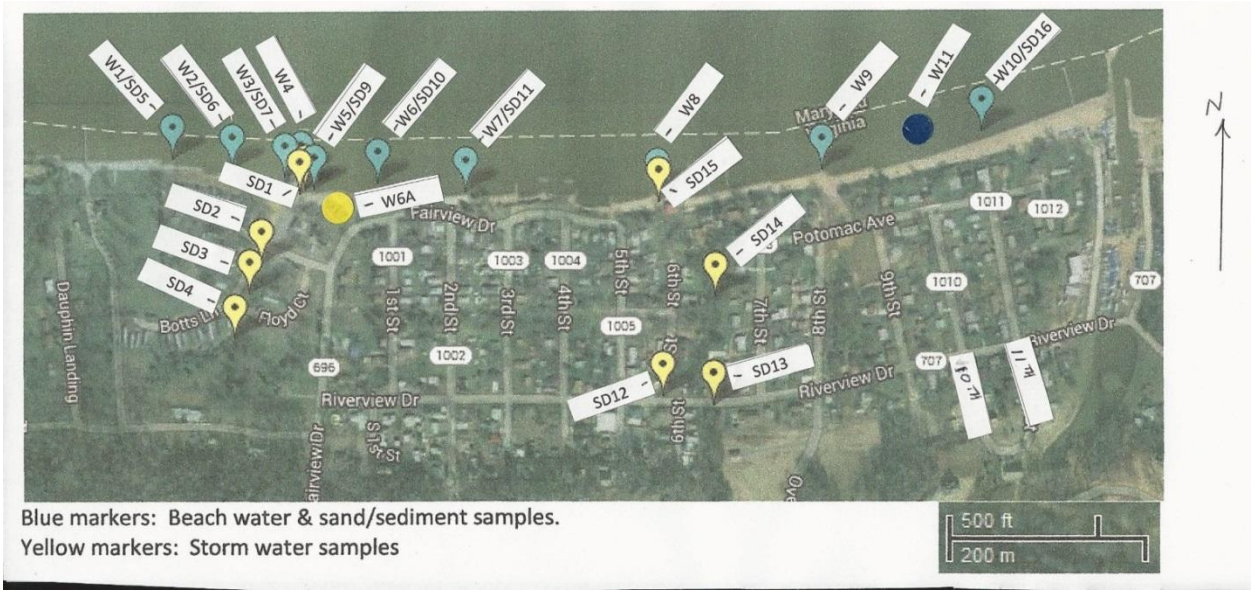
Cts < or = to 29 are strong positive reactions indicative of abundant target nucleic acid in the sample.
Cts of 30 to 37 are positive reactions indicative of moderate amounts of target nucleic acid.
Cts of 38-40 are weak reactions indicative of minimal amounts of target DNA and are indeterminate.

Approximately 63.3% and 71.9% of all samples were positive for HF183 and BacHum respectively; indicating that most samples contained bacteria that were of human origin. This information suggests that human pollution sources were contributing to the bacterial concentrations in the Newport News storm water system and the Hilton Beach water of the James River.

During previous discussions, it was hypothesized that rainfall and tidal cycles may impact the bacterial levels at Hilton Beach. VDH collected daily cumulative precipitation and tidal cycle data from the National Oceanic and Atmospheric Administration (NOAA) and analyzed the data to determine any relationships. The precipitation and tidal cycles were compared to VDH enterococci results from 2010-2013 and 2011-2013 respectively. There was no observed relationship between precipitation and VDH enterococci results. Also, when tidal cycles were compared with enterococci levels, the odds of observing high bacterial level during low tide were 1.99 times that of observing high bacterial level during high tide within the 95% confidence interval ranging from (0.51 to 7.79). The confidence interval is too wide; therefore it can be concluded that no relationship exists between tidal cycles and enterococci levels with the information available.

D. King George (Fairview Beach): There were two advisories at Fairview Beach, both in June as shown in Table 1, and no samples were sent to VT. The first advisory (6/3-6/6) was precautionary and not based on high counts while the second advisory only resulted in counts of 144 on 6/10 and the advisory was lifted with a count of 59 on 6/12. There were no advisories at Fairview Beach after these early June dates in 2013. To obtain a baseline at Fairview Beach, VDH and VT collected water and sediment samples on November 18, 2013 at various locations as shown in Figure 2. The sampling was designed to evaluate two intermittent waterways along SD1 to SD4 and SD12 to SD15 that had been implicated as pollution sources in previous years.

Figure 2. Sample locations at Fairview Beach for 11/18 collection.



For the water samples, only two sites had counts above the standard, W6A and W8 (Table 13). Fluorometry was positive for both samples (70 and above being considered positive), and both samples were also positive for HF183, indicating a human pollution component.

Table 13. Results for 11/18 sampling at Fairview Beach

Sample Type	**Sample ID**	**#/100mL**	**FSU**	**HF183**	**HF183Ct**	**HF183 Copy number**
Water	W1	<10	30.3	Neg.	--	--
	W2	<10	29.6	Neg.	--	--
	W3	<10	30.4	Neg.	--	--
	W4	<10	29.3	Neg.	--	--
	W5	12	29.2	Neg.	--	--
	W6	27	30.2	Neg.	--	--
	W6A	**2200**	**98.2**	**Pos.**	36	275
	W7	18	32.6	Neg.	--	--
	W8	**830**	**88.1**	**Pos.**	38	63
	W9	18	29.5	Neg.	--	--
	W10	11	33.3	Neg.	--	--
	W11	<10	29.1	Neg.	--	--
Sediment	SD1	**1460**		Mud only, no water	--	--
	SD2	**6300**		same	--	--
	SD3	**3000**		same	--	--
	SD4	**6000**		same	--	--
	SD5	60		Neg.	--	--
	SD6	60		Neg.	--	--
	SD7	<10		Neg.	--	--
	SD9	140		Neg.	--	--
	SD10	20		Neg.	--	--
	SD11	20		Neg.	--	--
	SD12	**800**		**Pos.**	38	63
	SD13	**3050**		**Pos.**	35	573
	SD14	**2400**		**Pos.**	36	275
	SD15	**2800**		**Pos.**	35	573
	SD16	100		Neg.	--	--

For the sediment samples, SD1 through SD4 had counts that ranged from 1,460 to 6,300. No DNA could be extracted as these were mud samples that were nearly dry, so HF183 could not be run on these samples. Fluorometry could not be measured in the sediment samples due to problems extracting optical brighteners from soil and mud. It was surprising to see such high number of enterococci in the mud samples in mid-November, indicating that either the strains of

enterococci present were capable of long-term persistence in soil and mud, or that rainfall that occurred the previous week had deposited the bacteria there from some pollution source when water was flowing. The counts alone further implicate this drainage waterway as a problem with pollution at Fairview Beach. It is not clear where the waterway enters the river when there is flowing water as the drainage goes under a parking lot and there is no visible structure evident. There has been assumption that the waterway drains out of a stormwater pipe at W5, but this has never been positively confirmed. The site at W6A was standing water at the base of the bluff above site W5 and had both a high count and positive results for fluorometry and HF183. Standing water had not been observed here before by those present when the samples were collected on 11/18 and a sediment sample was not collected here because it was anticipated (wrongly) that there would be nothing in the sample. It is possible that a connection exists between this site and the drainage waterway represented by SD1 through SD4.

Like the beach water samples (except for W6A and W8), the sediments from those samples were all negative for HF183. There were some minor reservoirs of enterococci in the river water sediments, as indicted by site 9, where the water sample (W9) had a count of 18 and the sediment sample (SD9) had a count of 140 (Table 13). Both were negative for HF183. For samples SD12 through SD15, there was a small amount of flowing water in this drainage ditch so it was possible to extract DNA from the samples. These samples had counts that ranged from 800 to 3,050 and all four were positive for HF183, implicating this waterway as a source of human pollution as well. As this waterway was flowing in enters the Potomac River at site W8 (Figure 2) and explains the high counts and positive HF183 results for W8.

This survey sampling on 11/18 of Fairview Beach reconfirmed results from previous years, that the two intermittent waterways were still problems and represent sources of fecal pollution that can impact the river water samples collected in the summer at Fairview.

E. Virginia Beach:
There were four advisories at Virginia Beach and all were one-day events (Table 1). One was in June at Lesner Bridge, one was in July at 63rd Street, and two were in mid-September at Lesner Bridge and Chick's Beach. The counts ranged from 122 to 201 and all advisories were lifted with the resample on the following day. No samples from these were sent to VT.

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