4. Article Two, B.5- Water Quality Monitoring ("WQM") Plan – Report on whether any of the semi-annual (or annual) WQM required under Article Two, B.5, was conducted during the quarter, and if so, in which sewer basins, and report the results of that monitoring (both BST and fecal coliform) for each such sewer basin.

The 2014 annual round of water quality monitoring was conducted on April 2, 2014. All 26 of the sewer basins were sampled (semi-annual and annual). Human source fecal bacteria were identified by library-based Polymerase Chain Reaction (PCR) testing in one or more samples from sixteen sewer basins. Using an independent verification test, discussed below, human sources were confirmed in nine of these basins.

As part of continuing re-evaluation of the Water Quality Monitoring Plan and of the methodologies used, the Virginia Tech laboratory that provides the bacterial source tracking (BST) testing services now conducts a verification test for human bacterial sources using a library-independent analytical method (Human Bacteroides HF183) on all samples. These results are presented below.

The 2014 semi-annual round of water quality sampling is planned for September, and results will be reported in the Fourth Quarter of 2014.

Broad Creek-	-	cal Bacteria Results:
	BST Results: (E)	<u>nterococcus CFU/100</u> ml)
	Human Bacteroides HF183: Negat	tive
	BOX-PCR Source Determination:	
	Human – 0%	
BRC001 – downstream	Avian – 33%	
	Canine – 10%	50
(Henson Creek)	Deer – 9%	
	Horse – 0%	
	Misc. Wildlife – 23%	
	Non-human Unknown – 25%	
	Human Bacteroides HF183: Negat	tive
	(Too few bacteria for HF183)	
	BOX-PCR Source Determination:	
	Human – 0 (NA)%	
BRC002 – upstream	Avian – 34 (NA)%	22 (<10)
(Henson Creek)	Canine – 12 (NA)%	32 (<10)
	Deer – 11 (NA)%	
	Horse -0 (NA)%	
	Misc. Wildlife – 19 (NA)%	
	Non-human Unknown – 24 (NA)%

Basins Subject to Semi-Annual Monitoring and Reporting Requirements:

<u>Note</u>: Values in parentheses for station BRC002 are for field duplicate sample. NA: Too few bacteria for BOX-PCR source determination in duplicate sample.

b.	Cabin John-	Fe	ecal Bacteria Results:
		BST Results: (<i>E</i>	<i>Enterococcus</i> CFU/100ml)
		Human Bacteroides HF183: Posit	ive
		BOX-PCR Source Determination:	
		Human – 7%	
		Avian – 29%	
	CBJ001 – downstream	Canine – 5%	118
		Deer – 10%	
		Horse - 0%	
		Misc. Wildlife – 24%	
		Non-human Unknown – 25%	
		Human Bacteroides HF183: Posit	ive
		BOX-PCR Source Determination:	
		Human – 15%	
		Avian – 27%	
	CBJ002 – upstream	Canine – 6%	164
		Deer – 17%	
		Horse – 0%	
		Misc. Wildlife – 21%	
		Non-human Unknown – 14%	

Horsepen-	F	ecal Bacteria Results:
_	BST Results: (.	Enterococcus CFU/100ml)
	Human Bacteroides HF183: Neg	ative
	(Negative)	
	BOX-PCR Source Determination	1:
	Human – 6 (6)%	
HSP001 – downstream	Avian – 29 (27)%	73 (87)
	Canine – 10 (10)%	73 (87)
	Deer – 8 (9)%	
	Horse – 15 (15)%	
	Misc. Wildlife – 20 (19)%	
	Non-human Unknown – 12 (14)%
	Human Bacteroides HF183: Pos	tive
	BOX-PCR Source Determination	1:
	Human – 11%	
	Avian – 31%	
HSP002 – upstream	Canine – 6%	141
	Deer – 7%	
	Horse – 17%	
	Misc. Wildlife – 17%	
	Non-human Unknown – 11%	

Note: Values in parentheses for station HSP001 are for field duplicate sample.

e.

d.	Indian Creek-	Fecal	Bacteria Results:
		BST Results: (Enter	rococcus CFU/100ml)
		Human Bacteroides HF183: Negative	2
		BOX-PCR Source Determination:	
		Human – 9%	
		Avian – 33%	
	INC001 – downstream	Canine – 7%	111
		Deer – 6%	
		Horse – 11%	
		Misc. Wildlife – 19%	
		Non-human Unknown – 15%	
		Human Bacteroides HF183: Negative	•
		BOX-PCR Source Determination:	
		Human – 5%	
		Avian – 29%	
	INC002 – upstream	Canine – 8%	83
		Deer – 4%	
		Horse – 13%	
		Misc. Wildlife – 23%	
		Non-human Unknown – 18%	

Little Falls-	Fecal	Bacteria Results:
	BST Results: (Enter	rococcus CFU/100m
	Human Bacteroides HF183: Negative	e
	BOX-PCR Source Determination:	
	Human – 3%	
	Avian – 35%	
LFS001 - downstream	Canine – 4%	18
	Deer – 11%	
	Horse – 0%	
	Misc. Wildlife – 27%	
	Non-human Unknown – 20%	
	Human Bacteroides HF183: Positive	
	BOX-PCR Source Determination:	
	Human – 37%	
	Avian – 21%	
LFS002 – upstream	Canine – 9%	1,028
	Deer – 6%	
	Horse – 0%	
	Misc. Wildlife – 15%	
	Non-human Unknown – 12%	

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f.	Lower Anacostia-	I	Fecal Bacteria Results:
		BST Results: (Enterococcus CFU/100ml)
		Human Bacteroides HF183: Pos	itive
		BOX-PCR Source Determination	n:
		Human – 12%	
		Avian – 35%	
	ANA001 – downstream	Canine – 15%	336
		Deer – 4%	
		Horse – 0%	
		Misc. Wildlife – 21%	
		Non-human Unknown – 13%	
		Human Bacteroides HF183: Neg	ative
		BOX-PCR Source Determination	n:
		Human – 4%	
		Avian – 39%	
	ANA002 – upstream	Canine – 17%	94
	_	Deer – 4%	
		Horse – 0%	
		Misc. Wildlife – 22%	
		Non-human Unknown – 14%	

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Lower Beaverdam Creek-

	BST Results: (Entero	<i>pcoccus</i> CFU/100ml)
	Human Bacteroides HF183: Positive	
	BOX-PCR Source Determination:	
	Human – 18%	
	Avian – 27%	
LBD001 – downstream	Canine – 7%	102
	Deer – 12%	
	Horse – 0%	
	Misc. Wildlife – 24%	
	Non-human Unknown – 12%	
	Human Bacteroides HF183: Positive	
	BOX-PCR Source Determination:	
	Human – 24%	
	Avian – 21%	
LBD002 – upstream	Canine – 4%	295
	Deer – 14%	
	Horse – 0%	
	Misc. Wildlife – 21%	
	Non-human Unknown – 16%	

h.	Muddy Branch-	Fecal B	acteria Results:
	-	BST Results: (Enterow	coccus CFU/100ml)
		Human Bacteroides HF183: Negative	
		BOX-PCR Source Determination:	
		Human – 0%	
		Avian – 36%	
	MDB001 – downstream	Canine – 5%	16
		Deer – 14%	
		Horse – 0%	
		Misc. Wildlife – 28%	
		Non-human Unknown – 17%	
		Human Bacteroides HF183: Negative	
		BOX-PCR Source Determination:	
		Human – 0%	
		Avian – 33%	
	MDB002 – upstream	Canine – 1%	34
		Deer – 13%	
		Horse – 0%	
		Misc. Wildlife – 35%	
		Non-human Unknown – 18%	

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Northeast Branch-

Fecal Bacteria Results:

	BST Results: (Entero	<i>ococcus</i> CFU/100ml)
	Human Bacteroides HF183: Positive	
	BOX-PCR Source Determination:	
	Human – 12%	
	Avian – 37%	
NEB001 – upstream	Canine – 8%	67
	Deer – 14%	
	Horse – 0%	
	Misc. Wildlife – 19%	
	Non-human Unknown – 10%	
	Human Bacteroides HF183: Positive	
	(Positive)	
	BOX-PCR Source Determination:	
	Human – 33 (33)%	
NEB002 – downstream	Avian – 24 (22)%	566 (565)
NEB002 – downstream	Canine – 4 (5)%	566 (565)
	Deer $- 6 (6)\%$	
	Horse $-0 (0)\%$	
	Misc. Wildlife – 21 (21)%	
	Non-human Unknown – 12 (13)%	

Note: Values in parentheses for station NEB002 are for field duplicate sample.

Northwest Branch	- Feca	al Bacteria Results:
	BST Results: (Ent	erococcus CFU/100m
	Human Bacteroides HF183: Negativ	ve
	BOX-PCR Source Determination:	
	Human – 4%	
	Avian – 32%	
NWA001 – downstream	Canine – 15%	48
	Deer – 8%	
	Horse -0%	
	Misc. Wildlife – 24%	
	Non-human Unknown – 17%	
	Human Bacteroides HF183: Negativ	ve
	BOX-PCR Source Determination:	
	Human – 0%	
	Avian – 38%	
NWA002 – upstream	Canine – 6%	19
1 I	Deer – 7%	
	Horse – 0%	
	Misc. Wildlife – 29%	
	Non-human Unknown – 20%	

k.	Oxon Run-	Feca	l Bacteria Results:
		BST Results: (Ent	erococcus CFU/100ml)
		Human Bacteroides HF183: Negativ	/e
		BOX-PCR Source Determination:	
		Human – 3%	
		Avian – 30%	
	OXN001 – downstream	Canine – 9%	24
		Deer – 12%	
		Horse – 0%	
		Misc. Wildlife – 28%	
		Non-human Unknown – 18%	
		Human Bacteroides HF183: Negativ	/e
		BOX-PCR Source Determination:	
		Human – 5%	
	OXN002 upstream	Avian – 28%	
	OXN002 – upstream (Watts Branch)	Canine – 7%	27
		Deer – 10%	
		Horse – 0%	
		Misc. Wildlife – 26%	
		Non-human Unknown – 24%	

l.	Paint Branch-]	Fecal Bacteria Results:
		BST Results: ((Enterococcus CFU/100ml)
		Human Bacteroides HF183: Pos	sitive
		BOX-PCR Source Determinatio	n:
		Human – 11%	
		Avian – 31%	
	PNT001 – downstream	Canine – 10%	233
		Deer – 11%	
		Horse – 0%	
		Misc. Wildlife – 21%	
		Non-human Unknown – 16%	
		Human Bacteroides HF183: Neg	gative
		BOX-PCR Source Determinatio	n:
		Human – 3%	
		Avian – 30%	
	PNT002 – upstream	Canine – 11%	25
		Deer – 9%	
		Horse – 0%	
		Misc. Wildlife – 34%	
		Non-human Unknown – 13%	

m.	Parkway-	F	ecal Bacteria Results:	
	-	BST Results: ()	Enterococcus CFU/100m	l)
		Human Bacteroides HF183: Neg	ative	
		BOX-PCR Source Determination	1:	
		Human – 6%		
	PKY001 – downstream	Avian – 30%		
	(Bear Branch)	Canine – 13%	93	
	(Bear Branch)	Deer – 15%		
		Horse – 0%		
		Misc. Wildlife – 23%		
		Non-human Unknown – 13%		
		Human Bacteroides HF183:		
	PKY002 – upstream (Walker Branch)	Too few bacteria for HF183	<10	
		BOX-PCR Source Determination	1:	
		Too few bacteria for BOX-PCF	2	

n. Piscataway-

	BST Results:	(Enterococcus CFU/100ml)
	Human Bacteroides HF183: N BOX-PCR Source Determinati	8
PSW001 – downstream	Human – 0% Avian – 29% Canine – 9% Deer – 14%	21

	Horse – 0% Misc. Wildlife – 23% Non-human Unknown – 25%	
PSW002 – upstream	Human Bacteroides HF183: Too few bacteria for HF183 BOX-PCR Source Determination: Too few bacteria for BOX-PCR	<10

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Fecal Bacteria Results:

Rock Creek-	Fec	al Bacteria Results:
	BST Results: (En	<i>terococcus</i> CFU/100ml)
	Human Bacteroides HF183: Positiv	ve
	BOX-PCR Source Determination:	
	Human – 22%	
	Avian – 25%	
RKC001 – downstream	Canine – 7%	188
	Deer – 8%	
	Horse – 0%	
	Misc. Wildlife – 19%	
	Non-human Unknown – 19%	
	Human Bacteroides HF183: Positiv	ve
	BOX-PCR Source Determination:	
	Human – 6%	
	Avian – 34%	
RKC002 – upstream	Canine – 3%	227
	Deer – 11%	
	Horse – 0%	
	Misc. Wildlife – 28%	
	Non-human Unknown – 18%	

p.

Seneca Creek-	Feca	al Bacteria Results:
	BST Results: (<i>Ent</i>	<i>erococcus</i> CFU/100ml)
	Human Bacteroides HF183: Negativ	ve
	BOX-PCR Source Determination:	
	Human – 0%	
	Avian – 29%	
SNC001 – downstream	Canine – 8%	15
	Deer – 11%	
	Horse – 0%	
	Misc. Wildlife – 24%	
	Non-human Unknown – 28%	
	Human Bacteroides HF183: Negativ	ve
	BOX-PCR Source Determination:	
SNC002 – upstream	Human – 0%	27
	Avian – 31%	
	Canine – 12%	

Deer – 10% Horse – 0%	
Misc. Wildlife – 23%	
Non-human Unknown – 24%	

q.	Sligo Creek-	Feca	l Bacteria Results:
	-	BST Results: (Ente	erococcus CFU/100ml)
		Human Bacteroides HF183: Positive	e l
		BOX-PCR Source Determination:	
		Human – 21%	
		Avian – 25%	
	SLC001 – downstream	Canine – 10%	71
		Deer – 11%	
		Horse – 0%	
		Misc. Wildlife – 17%	
		Non-human Unknown – 16%	
		Human Bacteroides HF183: Positive	
		BOX-PCR Source Determination:	
		Human – 9%	
		Avian – 23%	
	SLC002 – upstream	Canine – 11%	69
		Deer – 12%	
		Horse – 0%	
		Misc. Wildlife – 24%	
		Non-human Unknown – 21%	

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Upper Beaverdam Creek-

Opper Deaverdam Creek-		recai Dacteria Results.
	BST Results:	(Enterococcus CFU/100ml)
	Human Bacteroides HF183: Ne	egative
	BOX-PCR Source Determination	on:
	Human – 0%	
	Avian – 36%	
UBD001 – downstream	Canine – 9%	28
	Deer – 13%	
	Horse – 0%	
	Misc. Wildlife – 25%	
	Non-human Unknown – 17%	,
	Human Bacteroides HF183:	
	Too few bacteria for HF183	
	(Negative)	
LIPD002 unstroom	BOX-PCR Source Determination	on:
UBD002 – upstream	Human – NA (0)%	<10 (21)
	Avian – NA (34)%	
	Canine – NA (10)%	
	Deer – NA (12)%	

	Horse – NA (0)%	
	Misc. Wildlife – NA (25)%	
	Non-human Unknown – NA (19)%	
AT / AT 1 / .1		1

<u>Note</u>: Values in parentheses for station UBD002 are for field duplicate sample. NA: Too few bacteria for BOX-PCR source determination in sample.

s. Watts Branch-

t.

Fecal Bacteria Results:

	BST Results: (4)	Enterococcus CFU/100ml)
	Human Bacteroides HF183: Neg	ative
	BOX-PCR Source Determination	n:
	Human – 0%	
	Avian – 36%	
WTB001 – downstream	Canine – 7%	22
	Deer – 8%	
	Horse – 0%	
	Misc. Wildlife – 24%	
	Non-human Unknown – 25%	
	Human Bacteroides HF183:	
WTB002 – upstream	Too few bacteria for HF183	<10
	BOX-PCR Source Determination	n: _10
	Too few bacteria for BOX-PCI	R

Western Branch-**Fecal Bacteria Results: BST Results:** (Enterococcus CFU/100ml) Human Bacteroides HF183: Negative **BOX-PCR** Source Determination: Human – 8% Avian -27%WNB001 - downstream Canine – 9% 25 Deer - 10% Horse -0%Misc. Wildlife – 28% Non-human Unknown - 18% Human Bacteroides HF183: Negative **BOX-PCR** Source Determination:

15

	Human – 4% Avian – 33%
WNB002 – upstream	Canine – 6%
	Deer – 9%
	Horse – 0%
	Misc. Wildlife – 25%
	Non-human Unknown – 23%

a.	Dulles Interceptor	- Fecal 1	Bacteria Results:
		BST Results: (Enter	<i>pcoccus</i> CFU/100ml)
		Human Bacteroides HF183: Negative	
		BOX-PCR Source Determination:	
		Human – 3%	
		Avian – 36%	
	DSI001	Canine – 8%	18
		Deer – 14%	
		Horse – 0%	
		Misc. Wildlife – 23%	
		Non-human Unknown – 16%	

Basins Subject to Annual Monitoring and Reporting Requirements-

b. Mattawoman Fecal Bacteria Results: BST Results: (Enterococcus CFU/100ml) MTW001 Human Bacteroides HF183: Too few bacteria for HF183 BOX-PCR Source Determination: Too few bacteria for BOX-PCR <10</td>

c.	Monocacy-	Fe	ecal Bacteria Results:
		BST Results: (<i>E</i>	<i>Enterococcus</i> CFU/100ml)
		Human Bacteroides HF183: Nega	tive
		BOX-PCR Source Determination	:
		Human – 0%	
		Avian – 28%	
	MCY001	Canine – 7%	20
		Deer – 15%	
		Horse – 0%	
		Misc. Wildlife – 27%	
		Non-human Unknown – 23%	

d. Patuxent Center-

	BST Results:	(Enterococcus CFU/100ml)
	Human Bacteroides HF183:	
PTC001	Too few bacteria for HF183	. <10
(Mill Branch)	BOX-PCR Source Determinat	ion:
	Too few bacteria for BOX-P	CR

e.	e. Patuxent North- Fee			ecal Bacteria Results:	
		BST Results: (Ent	terocc	occus CFU/100ml)	
PIN001		Human Bacteroides HF183: Negativ BOX-PCR Source Determination:	ve	18	
(Hawlings River)		Human – 4%			

f.

Avian – 33% Canine – 4%	
Deer – 10%	
Horse -0%	
Misc. Wildlife – 28%	
Non-human Unknown – 21%	

Rock Run-	I	Fecal Ba	cteria Results:
	BST Results: (.	Enteroco	occus CFU/100ml)
	Human Bacteroides HF183: Neg	gative	
	(Negative)		
	BOX-PCR Source Determination	n:	
	Human – 0 (0)%		
RCM001	Avian – 29 (30)%		21 (20)
KCM001	Canine – 12 (12)%		31 (29)
	Deer – 16 (17)%		
	Horse $-0(0)\%$		
	Misc. Wildlife – 21 (20)%		
	Non-human Unknown – 22 (21	1)%	

Note: Values in parentheses for station RCM001 are for field duplicate sample.