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 2016 semi-annual sampling round was collected December 15, 2016, when twenty of the twenty-six sewer basins were sampled. Human source fecal bacteria were identified by Quantitative Polymerase Chain Reaction (qPCR) testing in one or more samples from eighteen sewer basins.

On October 3, 2016, justification for a change to the WQM Plan was submitted to the Regulators. On October 6, 2016, WSSC made a verbal request to MDE and EPA for an expedited review and approval of the requested changes. Per the verbal request, library-dependent BOX-PCR analysis for Microbial Source Tracking (MST), used previously, can no longer be supported by WSSC's contract laboratory due to extenuating circumstances. WSSC requested use of library-independent MST source tracking methods based on qPCR, effective immediately. MDE approved the justification on November 21, 2016, and EPA approved the justification on December 13, 2016. Human sources were analyzed using two specific qPCR markers, an EPA Developed Assay and *B. dorei*. Avian sources were analyzed for one specific qPCR marker, Bird Fecal ID^{TM}. Canine sources were analyzed for one specific qPCR marker, Dog Bacteroides ID^{TM}. Other animal sources (e.g., horse, bovine, general ruminant etc.) are still undergoing qPCR methods development.

The 2017 annual round of water quality sampling is planned between April and June, and results will be reported in the Third Quarter of 2017.

Basins Subject to Semi-Annual Monitoring and Reporting Requirements:

a. **Broad Creek-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL)   (*Enterococcus* CCE/100ml)

	BST Results: (gene copies/100mL)	Fecal Bacteria Results: (*Enterococcus* CCE/100ml)

BRC001 – downstream (Henson Creek)	qPCR Source Determination:
	  Human Bacteroides (EPA) = <LOQ
	  Human Bacteroides (Dorei) = **840**
	  Bird Fecal ID™ = ND
	  Dog Bacteroides ID™ = ND
		132

BRC002 – upstream (Henson Creek)	qPCR Source Determination:
	  Human Bacteroides (EPA) = ND
	  Human Bacteroides (Dorei) = <LOQ
	  Bird Fecal ID™ = <LOQ
	  Dog Bacteroides ID™ = <LOQ
		413

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

b. **Cabin John-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL)   (*Enterococcus* CCE/100ml)

	BST Results: (gene copies/100mL)	Fecal Bacteria Results: (*Enterococcus* CCE/100ml)

CBJ001 – downstream	qPCR Source Determination:
	  Human Bacteroides (EPA) = ND
	  Human Bacteroides (Dorei) = ND
	  Bird Fecal ID™ = ND
	  Dog Bacteroides ID™ = ND
		82

CBJ002 – upstream	qPCR Source Determination:
	  Human Bacteroides (EPA) = ND
	  Human Bacteroides (Dorei) = <LOQ
	  Bird Fecal ID™ = ND
	  Dog Bacteroides ID™ = ND
		99

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

c. **Horsepen-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL)   (*Enterococcus* CCE/100ml)

	BST Results: (gene copies/100mL)	Fecal Bacteria Results: (*Enterococcus* CCE/100ml)

HSP001 – downstream	qPCR Source Determination:
	  Human Bacteroides (EPA) = ND
	  Human Bacteroides (Dorei) = ND
	  Bird Fecal ID™ = ND
	  Dog Bacteroides ID™ = ND
		144

HSP002 – upstream	qPCR Source Determination:
	  Human Bacteroides (EPA) = ND
	  Human Bacteroides (Dorei) = ND
	  Bird Fecal ID™ = ND
	  Dog Bacteroides ID™ = ND
		186

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

1/30/2017

d. **Indian Creek-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
INC001 – downstream	Human Bacteroides (EPA) = <LOQ Human Bacteroides (Dorei) = **1,090** Bird Fecal ID™ = 631 Dog Bacteroides ID™ = ND	740
INC002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = ND Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = ND	257

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

e. **Little Falls-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
LFS001 – downstream	Human Bacteroides (EPA) = <LOQ Human Bacteroides (Dorei) = **1,180** Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = <LOQ	1,354
LFS002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = **380** Human Bacteroides (Dorei) = **1,900** Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = <LOQ	6,236

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

f. **Lower Anacostia-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
ANA001 – downstream	Human Bacteroides (EPA) = **1,550** Human Bacteroides (Dorei) = **23,500** Bird Fecal ID™ = 753 Dog Bacteroides ID™ = ND	703
ANA002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = **2,580** Human Bacteroides (Dorei) = **27,500** Bird Fecal ID™ = 256 Dog Bacteroides ID™ = <LOQ	800

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

g. **Lower Beaverdam Creek-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
LBD001 – downstream	Human Bacteroides (EPA) = **740**	8,345

	Human Bacteroides (Dorei) = **4,180** Bird Fecal ID™ = 778 Dog Bacteroides ID™ = ND	
LBD002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = <LOQ Bird Fecal ID™ = 354 Dog Bacteroides ID™ = ND	1,889

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

h.   Muddy Branch- | **BST Results:** (gene copies/100mL) | **Fecal Bacteria Results:** (*Enterococcus* CCE/100ml)

	BST Results	Fecal Bacteria Results
MDB001 – downstream	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = ND Bird Fecal ID™ = ND Dog Bacteroides ID™ = ND	40
MDB002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = <LOQ Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = <LOQ	197

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

i.   Northeast Branch- | **BST Results:** (gene copies/100mL) | **Fecal Bacteria Results:** (*Enterococcus* CCE/100ml)

	BST Results	Fecal Bacteria Results
NEB001 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = **429** Human Bacteroides (Dorei) = **5,950** Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = <LOQ	259
NEB002 – downstream	qPCR Source Determination: Human Bacteroides (EPA) = **287** Human Bacteroides (Dorei) = **3,650** Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = <LOQ	188

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

j.   Northwest Branch- | **BST Results:** (gene copies/100mL) | **Fecal Bacteria Results:** (*Enterococcus* CCE/100ml)

	BST Results	Fecal Bacteria Results
NWA001 – downstream	qPCR Source Determination: Human Bacteroides (EPA) = **4,510** Human Bacteroides (Dorei) = **40,800** Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = <LOQ	1,196
NWA002 – upstream	qPCR Source Determination:	149

	Human Bacteroides (EPA) = ND	
	Human Bacteroides (Dorei) = ND	
	Bird Fecal ID™ = ND	
	Dog Bacteroides ID™ = <LOQ	

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

k. **Oxon Run-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
OXN001 – downstream (Oxon Run)	Human Bacteroides (EPA) = **2,570** Human Bacteroides (Dorei) = **44,900** Bird Fecal ID™ = ND Dog Bacteroides ID™ = <LOQ	389
OXN002 – upstream (Watts Branch, tributary to Anacostia River)	qPCR Source Determination: Human Bacteroides (EPA) = **367** Human Bacteroides (Dorei) = **1,590** Bird Fecal ID™ = ND Dog Bacteroides ID™ = 874	213

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

l. **Paint Branch-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
PNT001 – downstream	Human Bacteroides (EPA) = ND (ND) Human Bacteroides (Dorei) = ND (ND) Bird Fecal ID™ = ND (ND) Dog Bacteroides ID™ = ND (ND)	147 (113)
PNT002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = **260** Human Bacteroides (Dorei) = **5,600** Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = ND	140

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents
<u>Note</u>: Values in parentheses for station PNT001 are for field duplicate sample.

m. **Parkway-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
PKY001 – downstream (Bear Branch)	Human Bacteroides (EPA) = <LOQ Human Bacteroides (Dorei) = **683** Bird Fecal ID™ = 307 Dog Bacteroides ID™ = ND	125
PKY002 – upstream (Walker Branch)	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = ND Bird Fecal ID™ = ND	140

1/30/2017

	Dog Bacteroides ID™ = ND	

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

n. **Piscataway-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
PSW001 – downstream	Human Bacteroides (EPA) = ND (ND) Human Bacteroides (Dorei) = ND (ND) Bird Fecal ID™ = <LOQ (<LOQ) Dog Bacteroides ID™ = ND (ND)	111 (178)
PSW002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = ND Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = ND	93

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents
<u>Note</u>: Values in parentheses for station PSW001 are for field duplicate sample.

o. **Rock Creek-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
RKC001 – downstream	Human Bacteroides (EPA) = **423 (410)** Human Bacteroides (Dorei) = **8,840 (9,070)** Bird Fecal ID™ = <LOQ (<LOQ) Dog Bacteroides ID™ = ND (<LOQ)	317 (248)
RKC002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = ND Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = ND	256

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents
<u>Note</u>: Values in parentheses for station RKC001 are for field duplicate sample.

p. **Seneca Creek-** **BST Results:** **Fecal Bacteria Results:**
(gene copies/100mL) (*Enterococcus* CCE/100ml)

	qPCR Source Determination:	
SNC001 – downstream (Seneca Creek)	Human Bacteroides (EPA) = <LOQ Human Bacteroides (Dorei) = **694** Bird Fecal ID™ = ND Dog Bacteroides ID™ = ND	48
SNC002 – upstream (Dry Seneca Creek)	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = ND Bird Fecal ID™ = ND Dog Bacteroides ID™ = <LOQ	191

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

1/30/2017

q.	Sligo Creek-	BST Results: (gene copies/100mL)	Fecal Bacteria Results: (*Enterococcus* CCE/100ml)

	SLC001 – downstream	qPCR Source Determination:
		  Human Bacteroides (EPA) = <LOQ
		  Human Bacteroides (Dorei) = **2,480**
		  Bird Fecal ID™ = <LOQ
		  Dog Bacteroides ID™ = <LOQ
			419

	SLC002 – upstream	qPCR Source Determination:
		  Human Bacteroides (EPA) = ND
		  Human Bacteroides (Dorei) = <LOQ
		  Bird Fecal ID™ = ND
		  Dog Bacteroides ID™ = ND
			282

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

r.	Upper Beaverdam Creek-	BST Results: (gene copies/100mL)	Fecal Bacteria Results: (*Enterococcus* CCE/100ml)

	UBD001 – downstream	qPCR Source Determination:
		  Human Bacteroides (EPA) = <LOQ
		  Human Bacteroides (Dorei) = **4,010**
		  Bird Fecal ID™ = <LOQ
		  Dog Bacteroides ID™ = ND
			3,625

	UBD002 – upstream	qPCR Source Determination:
		  Human Bacteroides (EPA) = **336**
		  Human Bacteroides (Dorei) = **12,500**
		  Bird Fecal ID™ = <LOQ
		  Dog Bacteroides ID™ = ND
			1,316

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents

s.	Watts Branch-	BST Results: (gene copies/100mL)	Fecal Bacteria Results: (*Enterococcus* CCE/100ml)

	WTB001 – downstream
	(Watts Branch)
		qPCR Source Determination:
		  Human Bacteroides (EPA) = ND
		  Human Bacteroides (Dorei) = <LOQ
		  Bird Fecal ID™ = <LOQ
		  Dog Bacteroides ID™ = ND
			139

	WTB002 – upstream
	(Sandy Branch)
		qPCR Source Determination:
		  Human Bacteroides (EPA) = ND (ND)
		  Human Bacteroides (Dorei) = ND (ND)
		  Bird Fecal ID™ = ND (ND)
		  Dog Bacteroides ID™ = ND (ND)
			184 (97)

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents
<u>Note</u>: Values in parentheses for station WTB002 are for field duplicate sample.

t.	Western Branch-	BST Results: (gene copies/100mL)	Fecal Bacteria Results: (*Enterococcus* CCE/100ml)
	WNB001 – downstream	qPCR Source Determination: Human Bacteroides (EPA) = **15,700 (13,000)** Human Bacteroides (Dorei) = **171,000 (161,000)** Bird Fecal ID™ = <LOQ (<LOQ) Dog Bacteroides ID™ = <LOQ (<LOQ)	10,090 (6,893)
	WNB002 – upstream	qPCR Source Determination: Human Bacteroides (EPA) = ND Human Bacteroides (Dorei) = <LOQ Bird Fecal ID™ = <LOQ Dog Bacteroides ID™ = ND	238

ND = Not Detected, <LOQ = Below limit of quantitation, CCE = Calibrator Cell Equivalents
<u>Note</u>: Values in parentheses for station WNB001 are for field duplicate sample.

Basins Subject to Annual Monitoring and Reporting Requirements-

      a.   Dulles Interceptor-

      b.   Mattawoman-

      c.   Monocacy-

      d.   Patuxent Center-

      e.   Patuxent North-

      f.   Rock Run-