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Pathogenic Bacteria and Microbial-Source Tracking Markers in Brandywine Creek Basin, Pennsylvania and Delaware, 2009–10

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U.S. Department of the Interior
U.S. Geological Survey

Cover: View from the U.S. Geological Survey streamflow-gaging station at West Branch Brandywine Creek at Coatesville, Pennsylvania, during high flow. (Photograph by Andrew Reif, U.S. Geological Survey.)

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Conversion Factors

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
mile (mi)	1.609	kilometer (km)
yard (yd)	0.9144	meter (m)
	Volume	
gallon (gal)	3.785	liter (L)
milliliter (mL)	1,000	microliter (µL)
	Flow rate	
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
cubic foot per second (ft^3/s)	283.168	deciliters per second (dL/s)
	Mass	
pound, avoirdupois (lb)	0.4536	kilogram (kg)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
°F=(1.8×°C)+32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:
°C=(°F-32)/1.8

Concentrations of fecal-indicator bacteria are given in colony-forming units per 100 milliliters (CFU/100 mL).

Concentrations of quantitative microbial-source tracking gene markers are given in copies per 100 milliliters (copies/100 mL).

Abbreviations used in report

BMP	best-management practices
C	Celsius
CDC	Centers for Disease Control and Prevention
CSO	combined sewer overflow
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EPA	U.S. Environmental Protection Agency
FIB	fecal-indicator bacteria
LLQ	lower limit of quantification
LOD	limit of detection
MST	microbial-source tracking
NTNC	no-template negative control
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
RSD	relative standard deviation
rRNA	ribosomal ribonucleic acid
RWQC	recreational water-quality criteria
STEC	Shiga-toxin producing *Escherichia coli*
ULQ	upper limit of quantification
USGS	U.S. Geological Survey

Pathogenic Bacteria and Microbial-Source Tracking Markers in Brandywine Creek Basin, Pennsylvania and Delaware, 2009–10

By Joseph W. Duris, Andrew G. Reif, Leif E. Olson, Heather E. Johnson

Abstract

The City of Wilmington, Delaware, is in the downstream part of the Brandywine Creek Basin, on the main stem of Brandywine Creek. Wilmington uses this stream, which drains a mixed-land-use area upstream, for its main drinking-water supply. Because the stream is used for drinking water, Wilmington is in need of information about the occurrence and distribution of specific fecally derived pathogenic bacteria (disease-causing bacteria) and their relations to commonly measured fecal-indicator bacteria (FIB), as well as information regarding the potential sources of the fecal pollution and pathogens in the basin.

This study focused on five routinely sampled sites within the basin, one each on the West Branch and the East Branch of Brandywine Creek and at three on the main stem below the confluence of the West and East Branches. These sites were sampled monthly for 1 year. Targeted event samples were collected on two occasions during high flow and two occasions during normal flow.

On the basis of this study, high flows in the Brandywine Creek Basin were related to increases in FIB densities, and in the frequency of selected pathogen and source markers, in the West Branch and main stem of Brandywine Creek, but not in the East Branch. Water exceeding the moderate full-body-contact single-sample recreational water-quality criteria (RWQC) for *Escherichia coli* (*E. coli*) was more likely to contain selected markers for pathogenic *E. coli* (*eaeA*, stx_1, and rfb_{O157} gene markers) and bovine fecal sources (*E. hirae* and LTIIa gene markers), whereas samples exceeding the enterococci RWQC were more likely to contain the same pathogenic markers but also were more likely to carry a marker indicative of human source (*esp* gene marker).

On four sample dates, during high flow between October and March, the West Branch was the only observed potential contributor of selected pathogen and bovine source markers to the main stem of Brandywine Creek. Indeed, the stx_2 marker, which indicates a highly virulent type of pathogenic *E. coli,* was found only in the West Branch and main stem at high flow but was not found in the East Branch under similar conditions. However, it must be noted that throughout the entire year of sampling there were occasions, during both high and normal flows, when both the East and West Branches were potential contributors of pathogen and microbial-source tracking markers to the main stem. Therefore, this study indicates that under selected conditions (high flow, October through March), West Branch Brandywine Creek Basin was the most likely source of elevated FIB densities in the main stem. These elevated densities are associated with more frequent detection of selected pathogenic *E. coli* markers (rfb_{O157}, stx_1) and are associated with MST markers of bovine source. However, during other times of the year, both the West Branch and East Branch Basins are acting as potential sources of FIB and fecally derived pathogens.

Introduction

Brandywine Creek drains a mixed-land-use basin that contains agricultural, urban, and suburban areas and has within it several wastewater-treatment discharges and industrial and public-supply withdrawals. The City of Wilmington, Delaware, which is in the downstream part of the basin on the main stem of Brandywine Creek, uses the stream for its main drinking-water supply. Because of the stream's use as drinking water, Wilmington is in need of information about the occurrence and distribution of specific fecally derived pathogenic bacteria (disease-causing bacteria) and their relations to commonly measured fecal-indicator bacteria (FIB), as well as information regarding the potential sources of the fecal pollution and pathogens in the basin. Potential contaminant sources include waste from humans, domestic animals, farm animals, and wildlife. This information could be used by the City of Wilmington, Del., to develop a best-management-practice (BMP) strategy based on the fecal pathogens and potential fecal sources within the Brandywine Creek Basin. Since 2005, Wilmington has been investing in BMP projects in the Brandywine headwaters to control sediments and pollutants, including bacteria that could adversely affect the drinking-water supply.

Water-quality studies by the U.S. Geological Survey (USGS) Pennsylvania Water Science Center demonstrated that in certain reaches of West Branch Brandywine Creek near Coatesville, Pennsylvania, elevated concentrations of FIB were related to leaking sewer pipes, failed septic systems, combined sewer overflows, and illegal discharges. In addition, resuspension of fluvial sediments was cited as a potential source for elevated concentrations of FIB, but neither the origin of the FIB in sediments nor the potential for redeposition of this sediment in the stream system was assessed (Cinotto, 2005). A study of the historical trends in FIB concentrations in the Brandywine Creek Basin showed higher median concentrations of both fecal coliform bacteria and *Escherichia coli* (*E. coli)* in West Branch Brandywine Creek than in East Branch Brandywine Creek and the main stem (Town, 2001).

Although the USGS did preliminary surveys of FIB sources along select sections of the Brandywine Creek Basin and has done routine monitoring of the basin for FIB, allowing for historical perspectives on concentrations, no work has been done to assess the specific fecal sources of FIB or the occurrence of fecally derived pathogenic bacteria within the basin. Recently issued reports indicate that commonly measured FIB have a variable relation with pathogenic bacteria, suggesting sampling location and spatial-temporal differences in distributions of FIB and pathogens are primary influencing factors in the relation (Duris and others, 2009; Smith and others, 2009).

Current (2011) methods used to assess microbial water quality rely on commonly measured FIB (fecal coliform bacteria, *E. coli*, and enterococci). The presence of FIB indicates possible contamination by fecal waste, which may also contain pathogens from human or animal fecal sources. Most of these indicator organisms are not pathogens themselves; they simply indicate the possible presence of pathogens. There are, however, specific groups of bacteria within the broader FIB group that are known human pathogens. Among the fecal coliform bacteria is a class of *E. coli* known as the Shiga-toxin producing *E. coli* (STEC), including *E. coli* O157:H7. The genus *Shigella*, which includes the pathogen *Shigella sonnei*, is also in the broad fecal coliform bacteria group. The total coliform indicator bacteria group contains the well-known fecal pathogen *Salmonella enterica*. *Campylobacter jejuni* and other *Campylobacter* species are also fecally derived pathogens but are not part of any commonly measured FIB group.

The four fecally derived pathogens STEC, *Shigella*, *Salmonella,* and *Campylobacter* are noted by the Centers for Disease Control and Prevention (CDC) to be dominant bacterial causes of fecal-associated waterborne disease (Lee and others, 2002). All these organisms can be easily enriched from water samples via selective media, including common FIB culturing media, and this approach serves as a simple platform for detection of these organisms from water samples. Once a water sample is enriched for the specific bacterial group, the bacterial growth can be used as a basis for specific pathogen detection with the DNA-based tool polymerase chain reaction (PCR). Additionally, bacterial DNA can be extracted directly from water samples without an enrichment step and used in quantitative PCR (qPCR) reactions.

STEC can cause a range of intestinal illnesses, from common watery “traveler’s diarrhea” to severe bloody diarrhea (hemorrhagic colitis) that can lead to kidney failure (hemolytic uremic syndrome) and death. The *E. coli* O157 serotype can be identified by the presence of the rfb_{O157} gene (Maurer and others, 1999). Genes found in pathogenic *E. coli* include *eaeA*, stx_1, and stx_2 (Fagan and others, 1999). In addition, specific genes that encode the heat-labile toxin IIa (LTIIa gene) and the heat-stable toxin (STh gene) have been demonstrated to be specific for pathogenic *E. coli* from cattle and human sources, respectively (Duris and Beeler, 2008; Jiang and others, 2007).

Shigella sonnei is another common bacterial pathogen belonging to the fecal coliform bacteria group. Many *Shigella* species can cause shigellosis, an intestinal infection in humans that manifests as disease ranging from mild watery diarrhea to severe hemorrhagic colitis. Symptoms are similar to, but typically less severe than, diarrhea resulting from STEC infection (Schroeder and Hilbi, 2008).

Salmonella species are one of the most prevalent causes of recreational illness in the United States and are occasionally associated with drinking-water-related illness (Lee and others, 2002). *Salmonella enterica* serotypes typically cause self-limiting enteritis, but extreme cases can lead to severe complications including blood and heart infections (Chiu and others, 2004).

Campylobacter jejuni (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are emerging enteric pathogens that have been increasingly observed in drinking-water-related illness (Lee and others, 2002). *Campylobacter* causes diarrheal disease similar to that resulting from *Shigella* and STEC (Centers for Disease Control and Prevention, 2008).

In addition to determining what fecal pathogens are present, knowing the source of the fecal contamination to a water system is useful. Microbial source tracking (MST) is a widely used tool for identifying the probable sources of fecal pollution that are found in surface waters (Santo Domingo and others, 2007). This technology identifies specific differences among bacteria present in the feces of different animal species. Time, diet, environment, and many other factors may have contributed to produce these evolutionary distinctions; MST uses these species-specific distinctions to identify potential animal (including human) sources of fecal pollution. No MST method is 100 percent accurate, owing to variations (seasonal, ecological, and others) in occurrence and concentrations of the species-specific bacteria tested by most MST approaches. It has been documented that MST methods are most effective when they are part of a tiered approach that employs multiple lines of evidence (Santo Domingo and others, 2007).

Because the City of Wilmington, Del., uses Brandywine Creek as its primary source of drinking water, the City was in need of information about the occurrence and distribution of specific fecally derived pathogenic bacteria, their relations to commonly measured FIB, and the potential sources of the fecal pollution and pathogens in the basin. In cooperation with the City of Wilmington, the USGS did a 1-year study in the Brandywine Creek Basin to aid in understanding the occurrence and distribution of fecal-indicator bacteria, fecally derived pathogens, and potential fecal sources that contribute to microbial water-quality impairments. Information obtained in this study could be used by the City of Wilmington to develop more effective best management practices to control fecal contamination within the basin. The pathogen and fecal-source-tracking data collected during the study also support the USGS mission to provide scientific information to help manage the Nation's water resources by contributing to the nationwide water-quality data program.

Purpose and Scope

The purpose of this report was to document methodology and the results of analysis done to determine the patterns of occurrence of specific fecally derived pathogenic bacteria and their relations to commonly measured FIB and to provide information regarding the potential sources of the fecal pollution and pathogens in the Brandywine Creek Basin. FIB concentration, pathogen and MST marker occurrence, and MST marker concentration results were analyzed in the context of streamflow conditions both at and between sampling locations. The relation of pathogen and MST markers to recreational water quality criteria, and concurrent detection of pathogen and MST markers at upstream and downstream sampling locations was also analyzed. A detailed examination of the study results is provided to illustrate the implications of the study findings.

Description of Study Area

Brandywine Creek drains 327 mi^2 in southeastern Pennsylvania and northern Delaware. The headwaters of Brandywine Creek are in Chester County, Pa. The stream flows south into New Castle County, Del., where it is a tributary to the Christina River (fig. 1). The Christina River is a tributary to the Delaware River. Population centers in the upstream parts of the Brandywine Creek Basin in Pennsylvania include the city of Coatesville and the boroughs of Downingtown and West Chester. Wilmington, Del., is the largest population center and is in the downstream part of the basin.

Physical Setting

The Brandywine Creek Basin encompasses areas in the Piedmont Physiographic Province in southeastern Pennsylvania (Sevon, 2000) and the Piedmont and Coastal Plain Physiographic Provinces in northern Delaware. Land use in the Brandywine Creek Basin is predominantly agriculture (39 percent), forested (32 percent), and residential (17 percent), with lesser amounts of open (4 percent), urban (6 percent), and other land use (2 percent) including industrial and commercial uses (Senior and Koerkle, 2002).

The Brandywine Creek Basin has a moderate humid continental climate with mild to moderately cold winters and warm and humid summers. Mean annual air temperature at the National Oceanic and Atmospheric Administration (NOAA) weather station in West Chester, Pa. (fig. 1), for 1971–2001 is 52.2°F (11.2°C). Mean annual precipitation (1971–2000) at West Chester is 48.96 in. (National Oceanic and Atmospheric Administration, 2010). Precipitation is distributed evenly throughout the year.

Major water-withdrawal locations for drinking-water supplies are near Wilmington, Del., and Coatesville, Downingtown, and West Chester, Pa. Major wastewater discharges in the Brandywine Creek Basin are at Honey Brook, Pa., and below Coatesville, Downingtown, and West Chester, Pa. (fig. 1).

Methods

Water-Sample Collection

Stream-water samples were collected both routinely and for targeted hydrologic events between June 1, 2009, and May 30, 2010. Routine samples were collected monthly (on or about the 15th of each month) from five sites within the Brandywine Creek Basin (table 1, fig. 1). These samplings covered a range of seasonal and hydrologic conditions. Targeted samples were collected quarterly from the five routinely sampled sites plus three additional sites to provide more resolution for determining potentially important source areas (table 1, fig. 1). Two of the targeted sampling events were collected at high flow, and two other samplings were collected at normal flow. To isolate the influence of combined sewer overflows (CSOs), samples were collected at the Baynard Street Bridge, which is downstream of several CSO outfalls. All wading samples (12 of 16 samples) were collected proximal to the Baynard Street Bridge except during very high flows, when sampling from the stream itself would have been unsafe. On these occasions, the sampling location was moved to the Van Buren Street Bridge, which was upstream of most CSO outfalls (Colleen Arnold, Asst. Water Division Director, City of Wilmington, written commun., 2010). These storm and high-flow events occurred on October 19, 2009, December 9, 2009, December 15, 2009, and May 3, 2010, and were times when the CSOs may have been flowing.

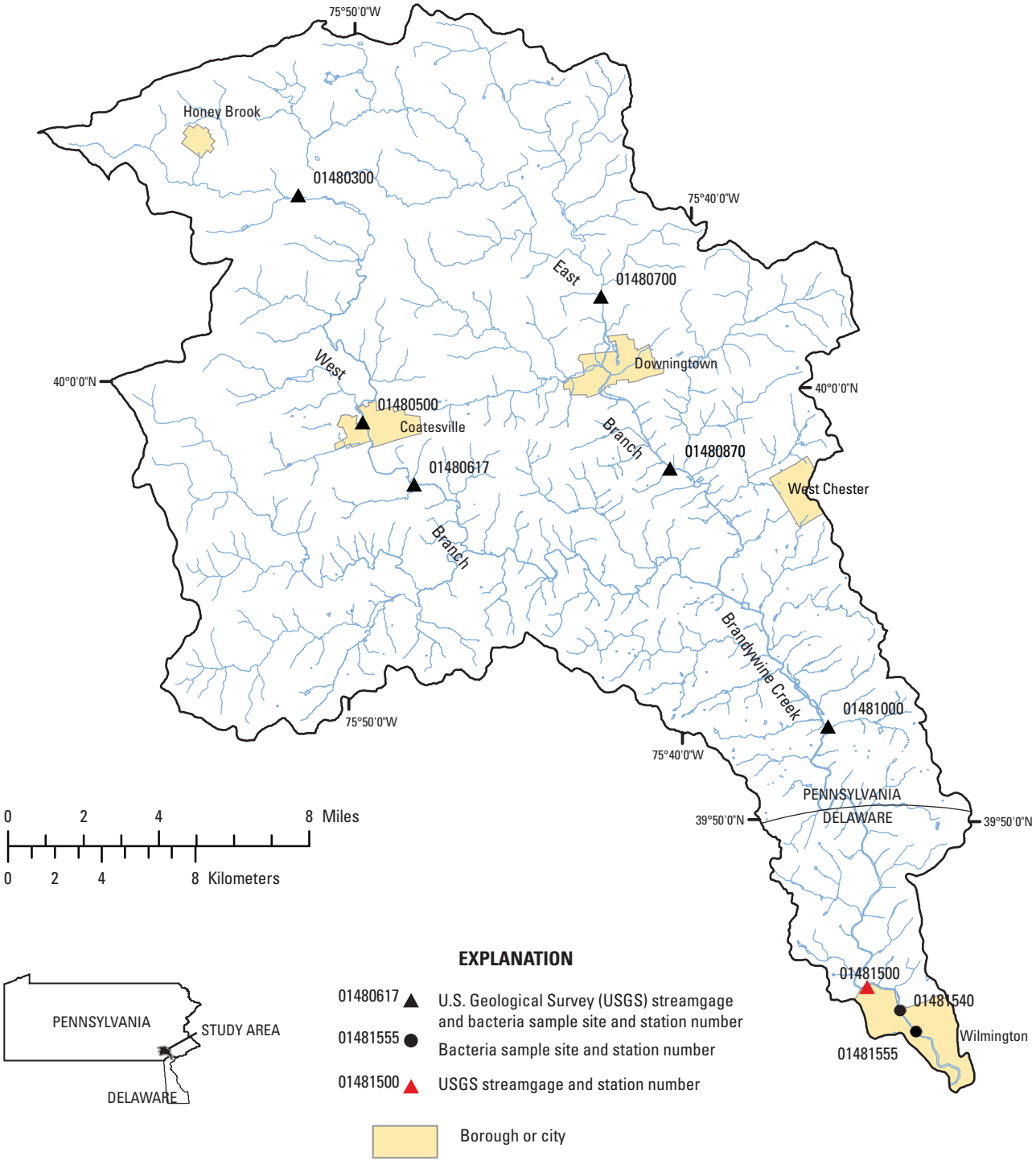


Figure 1. Location of sampling sites used in the Brandywine Creek Basin study, 2009–10.

Table 1. Sites sampled in the Brandywine Creek Basin study, 2009–10.

[USGS, U.S. Geological Survey; Pa., Pennsylvania; Del., Delaware; CSO, combined sewer overflow]

USGS station number	Field identification	Station name
Routinely monitored and targeted event survey stations[1]		
01480617	West Branch	West Branch Brandywine Creek at Modena, Pa.
01480870	East Branch	East Branch Brandywine Creek below Downingtown, Pa.
01481000	Main	Brandywine Creek at Chadds Ford, Pa.
01481540	Intake	Brandywine Creek near Wilmington, Del.
01481555	CSO	Brandywine Creek at Baynard Blvd near Wilmington, Del.
Additional stations for targeted event surveys only[2]		
01480300	Honey Brook	West Branch Brandywine Creek near Honey Brook, Pa.
01480500	Coatesville	West Branch Brandywine Creek at Coatesville, Pa.
01480700	Downingtown	East Branch Brandywine Creek near Downingtown, Pa.

[1]Routinely monitored stations were sampled monthly, plus four additional targeted event samples (16 total samples).

[2]Additional stations were sampled on four occasions only targeting flow events (4 total samples).

All samples were collected by USGS personnel using consistent protocols and procedures designed to obtain a sample representative of the stream waters; standard depth- and width-integrating techniques were employed (Shelton, 1994). At each site, raw composite water samples were collected from three to seven vertical profiles. The composites were subsequently split into appropriate containers for shipment to the USGS laboratory in Lansing, Mich. All samples were collected in sterilized plastic containers and stored on ice. A total of 100 samples (60 routine, 32 targeted, 4 field replicates, and 4 field blanks) were collected. All samples were shipped overnight on ice to the USGS laboratory in Lansing, Mich. Field measurements of discharge (using automated or manual measurements), pH, dissolved oxygen, specific conductance, temperature, and turbidity were made each time samples were collected (U.S. Geological Survey, 2010 and 2011).

Bacterial Enumeration, Enrichment, and Preservation

Samples were examined for the presence of FIB by use of standard membrane filtration and serial dilution methods as indicated in Britton and Greeson (1989). *E. coli* were enumerated according to EPA method 1603 (2006a), enterococci were enumerated according to EPA method 1600 (2006b), and fecal coliform bacteria were enumerated according to standard EPA methods (1989). In addition, for each sample, 50 mL of sample water was passed through one 0.45-µm nylon-membrane filter but was not enriched in a growth medium (non-enriched). The non-enriched filter, which served as the material for qPCR analysis for the quantitative MST part of the study, was folded in half four times and inserted open side down into a MoBio power soil DNA extraction bead beating tube and frozen at −70°C until all samples had been collected.

Selective growth enrichments for pathogenic bacteria were done by inoculating growth medium enrichments with a standard membrane filter through which 50 mL of sample water was passed. To enrich for *Campylobacter*, one filter was added to 14 mL of Bolton Broth with Preston supplement (Oxoid, Cambridge, United Kingdom) in a sterile 15-mL polypropylene tube (Baylis and others, 2000). To enrich for *Salmonella* species, a separate filter was added to 10 mL of Rappaport-Vassiliadis R10 media (BD, Franklin Lakes, N.J.) in a sterile 15-mL polypropylene tube (Zimbro and Power, 2003). Both enrichments were incubated for 4 hours at 37°C and then incubated for 48 hours at 41.5°C.

Preservation stocks were made from *E. coli*, enterococci, and fecal coliform bacteria growth from the enriched filter that received 50 mL of sample water, according to procedures in Duris and others (2009). Growth from the Bolton Broth and Rappaport-Vassiliadis R10 enrichments was collected after incubation, the filter was removed aseptically from the broth, and the culture was centrifuged to form a pellet. The supernatant was decanted, and the pellet was resuspended in 1 mL of phosphate buffered saline with a final concentration of 15 percent glycerol to create a freezer stock. These stocks were preserved at −70°C until further analyzed.

DNA Extraction

Stocks of enrichment cultures were removed from −70°C storage and allowed to thaw. The stocks were homogenized, and 50 µL of stock was added to 450 µL of alkaline polyethylene glycol reagent (pH ≈13.3) in a sterile cryotube and mixed (Chomczynski and Rymaszewski, 2006). The mixture was incubated at 70°C for 10 minutes and then stored at –20°C until analyzed by PCR.

The non-enriched filters were removed from −70°C storage and allowed to thaw. MoBio bead beating tubes were placed into a Mini Beadbeater-8 (Biospec Products, Bartlesville, Okla.) and homogenized at full speed for 2 minutes. After homogenization, DNA extraction was completed according to manufacturer's instructions.

Polymerase Chain Reaction to Detect Pathogens and Microbial-Source Tracking Markers

A volume of 1 µL of DNA solution, representing 1–100 ng of DNA, was isolated from the appropriate growth enrichment and used as a template to conduct PCR analysis for selected gene targets (table 2). Results from these analyses indicate only the presence or absence of the target genes; they do not indicate the quantity of the genes or the source (whether the target genes are all contained in the same organism), only that they were present in the original water sample.

The DNA from the modified mTEC stocks was analyzed for the genes required to confirm the presence of enterohemorrhagic *E. coli,* including the genes *eaeA*, stx_2, stx_1, and a gene common to all *E. coli*, (EC), which was used as an internal positive control to confirm the presence of *E. coli* (Duris and others, 2009). *E. coli* O157 was detected as indicated by targeting the rfb_{O157} gene that is responsible for the production of the specific antigen found on the surface of an *E. coli* O157 cell (Osek, 2003). The LTIIa gene, which is carried by some pathogenic *E. coli* strains common to bovine fecal sources, was analyzed according to methods of Jiang and others (2007). The STh gene that is carried by some pathogenic *E. coli* strains common to human fecal sources also was detected by using methods of Jiang and others (2007).

Table 2. Growth medium and gene targets for pathogens and microbial-source tracking organisms.

[*E. coli*, *Escherichia coli*]

Gene target	Gene product	Growth medium	Medium purpose	Organism	Reference
eaeA	Intimin protein	Modified mTEC agar	Enumeration/Enrichment	*E. coli*	Duris and others, 2009
stx_2	Shiga toxin 2	Modified mTEC agar	Enumeration/Enrichment	*E. coli*	Duris and others, 2009
stx_1	Shiga toxin 1	Modified mTEC agar	Enumeration/Enrichment	*E. coli*	Duris and others, 2009
EC	16s RNA (common to all *E. coli*)	Modified mTEC agar	Enumeration/Enrichment	*E. coli*	Duris and others, 2009
rfb_{O157}	O157 surface protein	Modified mTEC agar	Enumeration/Enrichment	*E. coli*	Osek, 2003
spvC	Salmonella plasmid of virulence	Rappaport-Vassilidais R10 broth	Enrichment only	*Salmonella*	Chiu and Ou, 1996
invA	Invasion determinant A	Rappaport-Vassiliadis R10 broth	Enrichment only	*Salmonella*	Chiu and Ou, 1996
ipaH	Invasion plasmid antigen H	mFC agar	Enumeration/Enrichment	*Shigella*	Islam and others, 1993
16s rDNA (Campy)	16s RNA (common to indicated organisms)	Bolton Broth	Enrichment only	*Campylobacter jejuni and coli*	Inglis and Kalischuk, 2003
esp	*Enterococcus* surface protein (human sources)	mEI agar	Enumeration/Enrichment	Enterococci	Haack and others, 2009
E. hirae	Putative helicase (bovine sources)	mEI agar	Enumeration/Enrichment	Enterococci	Soule and others, 2006
LTIIa	Heat labile toxin (bovine sources)	Modified mTEC agar	Enumeration/Enrichment	*E. coli*	Chern and others , 2004
STh	Heat stable toxin (human sources)	Modified mTEC agar	Enumeration/Enrichment	*E. coli*	Jiang and others, 2007

The DNA from the mEI stocks was analyzed for MST gene markers that indicate enterococci from a human source and enterococci from a bovine source. The *esp* gene is a commonly carried by pathogenic strains of *Enterococcus* that infect humans (Shankar and others, 1999) and as a result has since been found to be a representative indicator of *Enterococcus* from a human fecal source (Scott and others, 2005). A putative helicase gene in the organism *Enterococcus hirae* has been found to represent *Enterococcus* from a bovine fecal source (Soule and others, 2006).

The DNA from mFC agar enrichments was analyzed for a pathogenic marker of *Shigella sonnei. S. sonnei* was then detected by using PCR to target a specific variant of the *ipaH* gene, which codes for the invasion plasmid antigen of *Shigella* species (Islam and others, 1993).

The DNA from Rappaport-Vassiliadis R10 broth enrichments was analyzed for two genes from *Salmonella*. *Salmonella enterica* was detected by using PCR detection of the *invA* (invasion gene A) and the *spvC* (*Salmonella* virulence plasmid). These two genes have been demonstrated to be efficient at detection of a broad range of *Salmonella enterica* (Chiu and Ou, 1996).

The DNA from Bolton Broth enrichments was analyzed for a gene representing both *Campylobacter coli* and *Campylobacter jejuni*. Detection of *C. jejuni* and *C. coli* (Campy) was achieved by using PCR analysis for the 16S rDNA, which encodes the ribosomal RNA specific to *C. jejuni* and *C. coli* (Inglis and Kalischuk, 2003).

A summary of culture enrichments for detection of pathogen and MST genes can be found in table 2. All PCR assays followed standard protocols (U.S. Environmental Protection Agency, 2004). All methods included laboratory positive controls, consisting of DNA extracted from bacteria known to contain the target gene(s), and no-template (negative) controls.

Quantitative Polymerase Chain Reaction for Microbial-Source Tracking Markers

Quantitative polymerase chain reaction for AllBac (general feces-associated marker), BoBac (ruminant feces-associated marker) and qHF183 (human feces-associated marker) was done on an iCycler IQ5 thermal cycler and detector, with sample DNA placed in a semi-skirted, 96-well qPCR plate and sealed with an optically clear microseal B adhesive seal machine (Bio-Rad Laboratories, Inc., Hercules, Calif.). Reactions were carried out in 25 µL total volume, and the reaction mixture contained a final concentration of 1X Applied Biosystems Power SYBR Green PCR master mix (Life Technologies, Carlsbad, Calif.), 0.25 µM AllBac, BoBac, or HF183 forward and reverse primers (Seurinck and others, 2005; Layton and others, 2006), 2 µL of neat (undiluted) or 10-fold diluted DNA, representing <1 to 60 ng of DNA, with a balance of sterile ultrapure water. AllBac and BoBac PCR reactions were done by using a 50°C hold for 2 minutes, a 95°C hold for 10 minutes, and then 50 cycles of 95°C for 30 seconds and 57°C for 45 seconds (Layton and others, 2006). The HF183 qPCR was done by using a 50°C hold for 2 minutes, a 95°C hold for 10 minutes, and then 40 cycles of 95°C for 30 seconds, 53°C for 60 seconds, and 60°C for 60 seconds (Seurinck and others, 2005). All samples were run in triplicate, and a no-template (negative) control and standard curve (positive controls) were run for each reaction plate.

Quantitative PCR Data Analysis

For each run of the qPCR assay, a set of standards at known concentrations (from 10 copies to 10^9 copies of DNA) were run in triplicate concurrently with environmental samples. Raw fluorescence data from each qPCR assay were analyzed by using default data analysis on the iQ5 optical system software (Bio-Rad Laboratories, Inc., Hercules, Calif.). Default data processing consists of subtracting baseline fluorescence (determined by the iQ5 instrument) from total fluorescence detected by the instrument for each reaction well. A threshold value was set that accounted for fluorescence increases above baseline that fell within the exponential phase of the concurrently run standard curve. All thresholds were then checked manually after the automated data processing routine, and some were adjusted manually as needed. The iQ5 software calculated the cycle threshold (Ct) values for each sample in the reaction. The Ct value is the cycle number where the fluorescence emitted by the sample passes the threshold value (that is, it becomes greater than the background fluorescence). Higher concentrations of MST DNA markers in a sample will result in lower Ct values; lower concentrations of MST DNA markers in a sample will result in higher Ct values. Ct values were then converted to copies of DNA per microliter of extracted DNA by applying the standard curve relating Ct to known copy number of the target gene for each reaction. After conversion, Ct values are reported as copies of target DNA per 100 mL water (copies/100 mL).

Detection limits and data qualification were done in accordance with the methods of Bushon and others (2009). A limit of detection (LOD) for each reaction was established by using no-template negative control (NTNC) reactions. These reactions occasionally show fluorescence not related to target DNA late in the final cycles of thermal cycling. In the event that this occurred, the 99th-percentile confidence interval was calculated for NTNC for the specific reactions. To protect against false-positive results, the target concentration at the lower 99th percentile, transformed to a relevant concentration (copies/100 mL), was used as the limit of detection. Any reported concentration that was lower than this number was considered unreliable and was reported as a nondetect ("ND" in appendixes 1 and 2).

The lower and upper limits of quantification (LLQ and ULQ) for each reaction correspond to the lowest and highest concentration of positive-control DNA used to construct the standard curve. All DNA concentrations used to develop the standard curves fell within the linear range of that curve.

The LOD and LLQ were used to qualify low-concentration data from qPCR analysis. In all cases, the LOD was greater than the LLQ, therefore values that were above the LOD were not qualified, whereas values below the LOD were reported as nondetects. Quality-control information for all qPCR analysis is summarized in table 3.

Data Analysis

Instantaneous discharge measured or recorded from continuous discharge monitors during sample collection was compared to the daily mean flow values for the period of record at the corresponding USGS station. Flows at all main-stem sampling sites were compared to the flow statistics for USGS station 01481500. If the measured discharge was greater than the 75th percentile of mean daily flow for that station on that date, then the sample was considered to be a high-flow sample. If the measured discharge was between the 25th and 75th percentile of mean daily flow for that station on that date, the sample was categorized as a normal-flow sample. Samples with measured discharges less than the 25th percentile of mean daily flow for that station on that date were to have been categorized as low-flow samples; however, flows were higher than the 25th percentile at all sites and for all sampling dates during the study.

- For each site, differences in measured microbiological parameters were compared between samples collected at high flow and at normal flow. Differences in measured microbiological parameters were also compared between sites sampled at the same flow conditions. Statistical comparisons between sites were made only on samples from the five routinely sampled sites.
- To achieve the study objective, comparisons among all five routinely sampled sites were made to determine whether there were differences in measured parameters at these five locations within the basin. Refined comparisons were made between the routine-sampling sites on the East and West Branch, and separate comparisons were made between the three routine-sampling sites (Main, Intake, and CSO) on the main stem.
- Differences in median densities of fecal-indicator bacteria and median densities of MST markers were evaluated using the non-parametric Kruskal-Wallis test on log transformed values (Helsel and Hirsch, 2002). Frequencies of gene detections in water samples between different sampling sites, flow conditions, or in comparison to water-quality criteria were done using the Fisher Exact Test of independence (Helsel and Hirsch, 2002). All statistical comparisons were computed using TIBCO Spotfire S+ 8.1 for Windows statistics package (Tibco Software Inc., Somerville, Mass.) and Microsoft Excel 2007. A statistic was considered significant when the p-value was less than 0.05.

Microbiological Water Quality of Brandywine Creek

The three distinct methods used in this study provided multiple perspectives on the microbial quality of the Brandywine Creek Basin. The first method was traditional FIB quantification. FIB densities can yield a coarse differentiation of the microbial water quality across a range of sites and potentially help to identify "hot spot" areas where exogenous factors, such as point sources, could be contributing to fecal-indicator bacteria concentrations. The second method was the use of selective enrichment combined with presence/absence PCR assays for both common fecally derived pathogens and for selected MST markers for human and bovine source from both *E. coli* and enterococci. The third method was quantitative MST by means of quantitative PCR from direct (not enriched) samples. These three methods provide a multiple-lines-of-evidence approach that is suggested as a means to demonstrate real differences in the microbial quality of surface water (Santo Domingo and others, 2007).

Table 3. Standard-curve characteristics for AllBac, BoBac, and qHF183 microbial-source tracking (MST) markers used in the Brandywine Creek Basin study, 2009–10.

[R^2, coefficient of determination]

MST marker	Number of compiled curves	Dynamic range	R^2 range	Efficiency range (in percent)	Detection limit[1]
AllBac	6	4.31×101–1.00×109	0.966–0.997	78.9–105.4	160
BoBac	6	1.00×102–1.00×109	.951–.998	79.0–94.9	205
qHF183	4	1.00×102–1.00×109	.971–.986	81.5–92.3	274

[1] Gene copies per 100 milliliters of water.

Results of Indicator Organism Sampling

During the study, log *E. coli* densities ranged from 0.60 to 4.87 colony forming units per 100 mL (CFU/100 mL), with a log geometric mean of 2.37 CFU/100 mL. Log enterococci densities ranged from nondetection to 4.63 CFU/100 mL, with a log geometric mean of 2.12 CFU/100 mL. Log fecal coliform bacteria densities ranged from 0.60 to 4.72 CFU/100 mL, with a log geometric mean of 2.41 CFU/100 mL. Log FIB densities for each site for samples collected at high flow and normal flow are displayed by site in figure 2.

At routinely sampled sites on the West Branch and all sites on the main stem of Brandywine Creek (Main, Intake, CSO sites), median densities of FIB (*E. coli*, enterococci, and fecal coliform bacteria) were significantly higher in samples collected during high flow than in those collected during normal flow at the same site. However, no significant differences were found between FIB densities in high-flow and normal-flow samples collected in East Branch Brandywine Creek (table 4, fig. 2).

Median densities of FIB were not significantly different among all five routinely sampled sites for samples collected during normal flow. However, during high flow, the difference in median *E. coli* densities among all routinely sampled sites was statistically significant (Kruskal-Wallis test, $p<0.05$). Finer scale comparisons were made between the East and West Branches of Brandywine Creek and among the three sites on the main stem.

In comparisons of median densities of FIB between the West Branch and East Branch, no significant differences were observed at normal flows, but during high flows significantly higher counts of *E. coli* and enterococci (but not fecal coliform bacteria) were found on the West Branch than on the East Branch (table 4). No significant differences were observed in median densities of FIB among the three sites on the main stem of Brandywine Creek at either high or normal flow.

Because there are only two high-flow observations at the event-targeted sites, no statistical comparison of FIB densities can be made. However, the highest FIB counts were measured during event samples at West Branch Brandywine Creek near Honey Brook, Pa., site (USGS station number 01480300, appendix 1).

Table 4. Comparison of median fecal-indicator bacteria concentrations in East Branch and West Branch Brandywine Creek, 2009–10, by flow condition.

[CFU/100 mL, colony-forming units per 100 milliliters]

Fecal-indicator organism	Flow condition[1]	West Branch[2] Median concentration (CFU/100 mL)	East Branch[3] Median concentration (CFU/ 100 mL)	p-value[4]
Escherichia coli	High	4.60E+03	1.53E+02	p<0.05
	Normal	1.75E+02	3.75E+01	Not significant
p-value[4]		p<0.05	Not significant	
Enterococci	High	5.30E+02	7.50E+01	p<0.05
	Normal	5.00E+01	1.70E+01	Not significant
p-value[4]		p<0.05	Not significant	

[1]Flow condition in stream at time of sample.

[2]West Branch, U.S. Geological Survey (USGS) station number, 01480617.

[3]East Branch, USGS station number, 01480870.

[4]p-value of Kruskal-Wallis rank sum test on log-transformed bacteria concentrations, p-value <0.05 indicates a significant difference in the comparison.

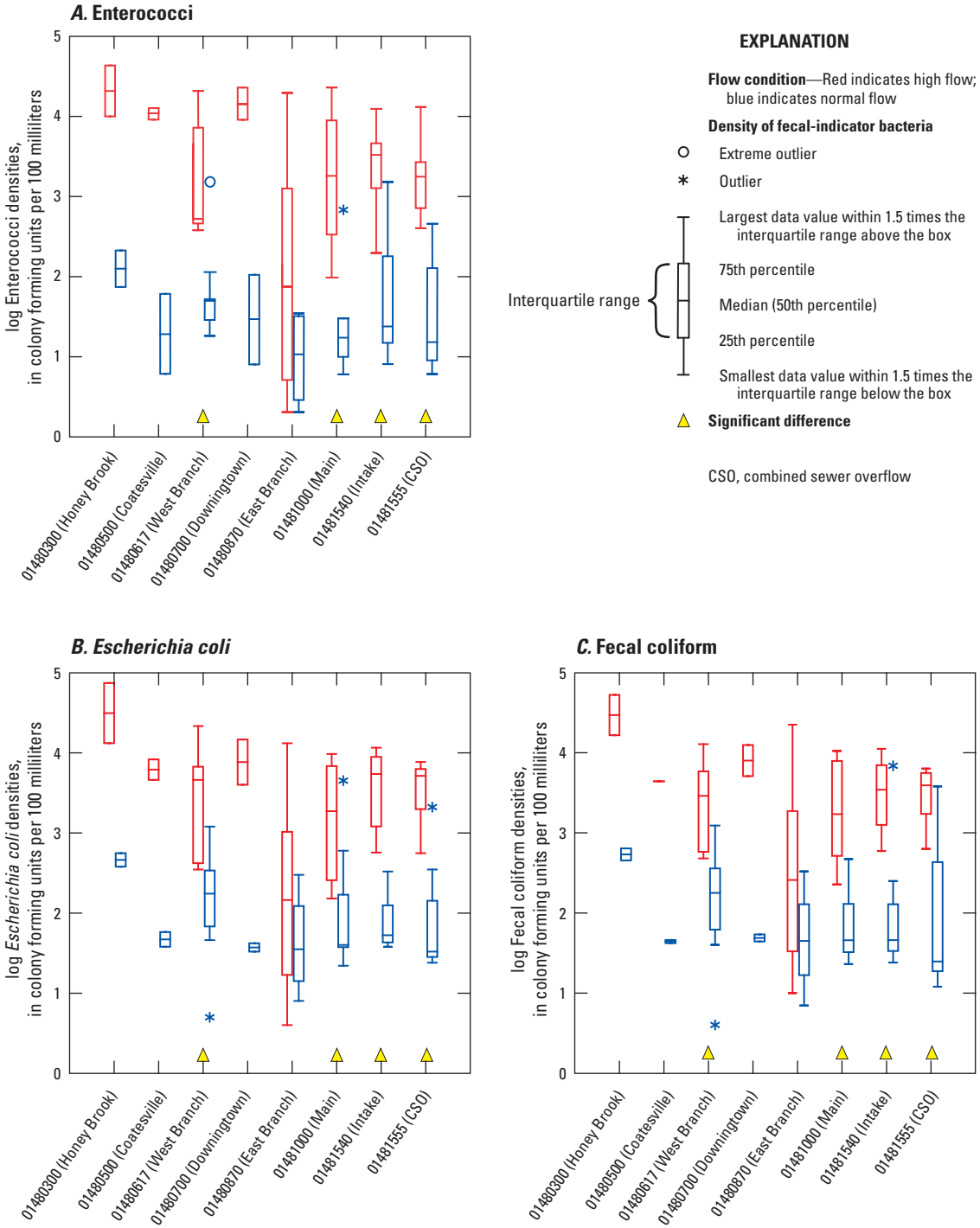


Figure 2. Concentrations of fecal-indicator bacteria, by sample location, from samples collected at high mean daily flow or normal mean daily flow during the Brandywine Creek Basin study, 2009–10. A yellow triangle above the x-axis label indicates a significant difference in median concentrations at that site between samples collected at high flow and normal flow.

Results of Pathogen Testing

All pathogen gene markers were detected on at least one occasion, from at least one site, during the course of the study. The internal positive control EC gene marker was detected in 98 percent of samples. It was not detected on two occasions when densities of *E. coli* were near detection limits (appendix 1). The overall frequency of pathogen gene markers ranged from 84 percent for the *eaeA* gene of pathogenic *E. coli* to 6.5 percent for the *spvC* gene marker of pathogenic *Salmonella*. A summary of the detection frequencies of pathogen and MST gene markers across all sites can be found in figure 3.

Table 5 shows the frequency of gene detections in samples meeting or exceeding the moderate full-body-contact single-sample recreational water-quality criteria (RWQC) for *E. coli* and enterococci FIB (Dufour and Ballantine, 1986). Of the 92 regular samples collected, 46 samples exceeded both the RWQC for *E. coli* and enterococci and 35 samples exceeded neither the RWQC for *E. coli* or enterococci. Four samples exceeded the RWQC for *E. coli* but not enterococci, and seven samples exceeded the RWQC for enterococci but not *E. coli*. Samples exceeding the *E. coli* RWQC of 298 CFU/100 mL more frequently contained the *eaeA*, stx_1, and rfb_{O157} pathogen marker genes. Pathogen marker frequencies also were compared in samples meeting or exceeding the enterococci RWQC of 78 CFU/100 mL; as with the *E. coli* RWQC, samples exceeding the enterococci RWQC also more frequently contained the *eaeA*, stx_1, and rfb_{O157} pathogen genes (table 5).

The frequency of pathogen marker detection in samples collected at high flow and normal flow at each routine site was compared (table 6). Detection of the stx_1 and rfb_{O157} pathogen markers were significantly more frequent in high-flow samples compared to normal-flow samples in the West Branch. In the East Branch, no difference was found in the detection frequencies of any pathogen marker between high and normal flow. Differences in the frequency of stx_1 and rfb_{O157} at the Main site were also observed between high and normal flow. The frequency of rfb_{O157} pathogen marker was also greater in high-flow samples than in normal-flow samples at the Intake and CSO sites, reflecting differences observed at the Main and West upstream sampling locations (table 6).

The frequency of pathogen gene occurrence specifically in the high-flow samples was compared among all five routinely sampled sites. Only the frequency of rfb_{O157} pathogen marker was significantly different among the five sites (Fisher Exact Test, $p<0.05$). The largest difference in rfb_{O157} frequency (when comparing all sites) was found to be between the East Branch and the Intake/CSO sites (fig. 4). When frequencies of gene detection from the same five sites were compared for samples collected at normal flow, no differences were found among the five sites.

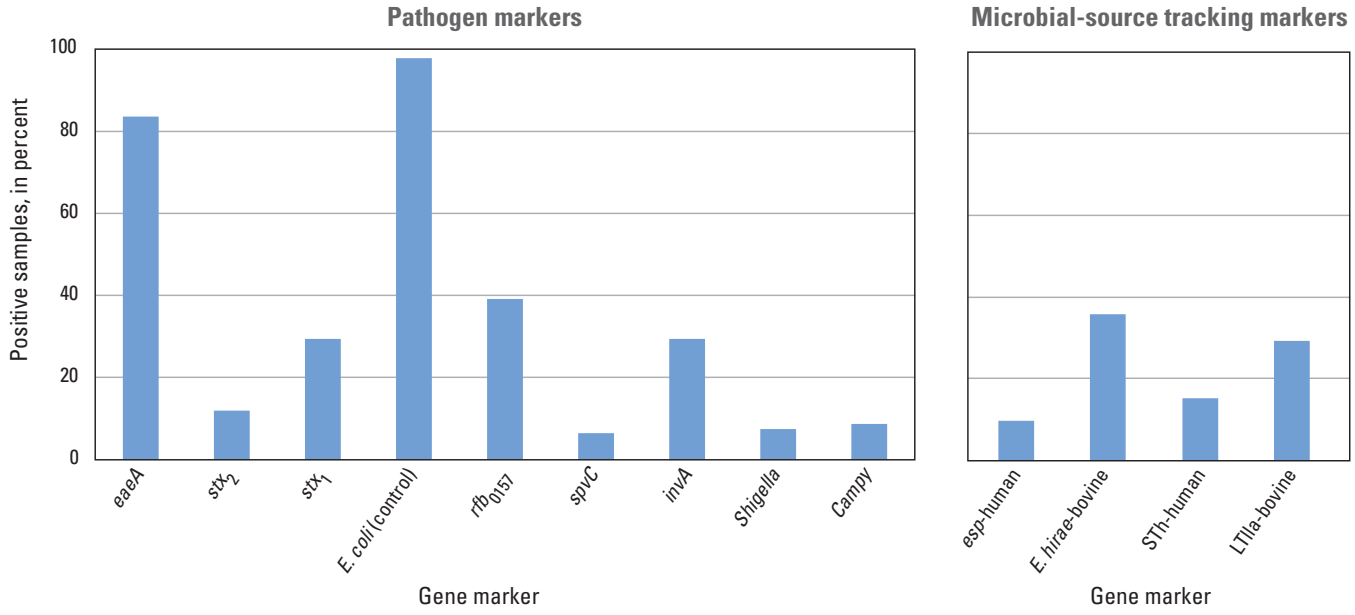
A comparison of the gene detection frequency that was restricted to the West Branch and East Branch samples collected at high flow revealed significantly more frequent detections of stx_1 marker in the West Branch but not $rfb_{O157,}$ for which significant differences were found in the comparison of all routine sites.

The stx_2 gene was found at the routinely sampled Main site on only two occasions (December 9, 2010, and March 15, 2010). Samples on both dates represented high flow for all sites, and on both occasions the gene was also found upstream only in West Branch Brandywine Creek, not in East Branch Brandywine Creek (table 7, appendix 1). The sample collected on December 9, 2010, was a targeted high-flow event sample, and in addition to the routinely sampled site being positive for the stx_2 gene, the Coatesville and Honey Brook sites on the West Branch also were positive on that occasion. The exclusive detection of this gene indicates that at high flow, West Branch Brandywine Creek is the most probable source for fecally derived pathogenic *E. coli* bacteria possessing the stx_2 gene marker that are being transported to the main stem. It should also be noted that other gene markers found at the main stem on December 9, 2010, including *eaeA* and stx_1 gene markers, were also found upstream in both the East and West Branches, indicating that pathogenic *E. coli* containing the *eae*A and stx_1 genes could be originating from either the West or East Branch subbasins.

When the frequency of gene detection was compared between the East and West Branches at normal flow, no significant differences were observed. However, these data show that at high flow, the stx_1 gene was more frequently detected in the West Branch than East Branch and that on selected sampling dates; the West Branch was acting as the only potential observed source for the stx_2 containing *E. coli* pathogens (table 7) at the Main site (Brandywine Creek at Chadds Ford, Pa., 01481000).

The frequency of pathogen markers was also specifically compared among the three main-stem sites (Main, Intake, and CSO), and no significant differences were found between high-flow and normal-flow samples. Four samples at the CSO site were collected from the Van Buren Street Bridge upstream of the CSO outfalls. In the four remaining samples collected at high flow that were downstream of the CSO outfalls (Baynard Street Bridge), pathogen markers detected at the CSO site were also detected upstream on the main stem, so no determination of CSO impact could be made.

Although frequency analysis is informative about the general condition of the surface-water system in the basin, it does not explain connections between sites or variation that is taking place over time. In an effort to address specific effects that upstream water quality had on downstream water quality, as indicated by frequency analysis, each pathogen gene marker was categorized on the basis of its relation with the upstream sample locations on the same date of sampling. For instance, if a pathogen marker was detected at the West Branch site and Main site on a particular sample date but not detected in the East Branch site on the same date, then that condition was noted as West and Main, and the number of samples meeting this condition was noted. The results from this categorization are displayed in table 7.



EXPLANATION

Gene marker	
eaeA	Intimin protein
stx_2	Shiga toxin 2
stx_1	Shiga toxin 1
E. coli (control)	*Escherichia coli*
rfb_{0157}	O157 surface protein
spvC	*Salmonella* plasmid of virulence
invA	Invasion determinant A
Shigella	*Shigella* invasion plasmid antigen H gene
Campy	16S ribosomal deoxyribonucleic acid gene of *Campylobacter coli* or *Campylobacter jejuni*
esp-human	Enterococcal surface protein (human sources)
E. hirae-bovine	*Enterococcus hirae* (bovine sources)
STh-human	Heat stable toxin (human sources)
LTIIa-bovine	Heat labile toxin (bovine sources)

Figure 3. Frequency of all samples testing positive for pathogen and microbial-source tracking markers in the Brandywine Creek Basin study, 2009–10.

Table 5. Detection frequency of pathogen and microbial-source tracking gene markers in the Brandywine Creek Basin study, 2009–10, based on relation to recreational water-quality criteria.

[RWQC, recreational water-quality criteria; n, number of samples; MST, microbial-source tracking; *E. hirae*, *Enterococcus hirae; E. coli*, *Escherichia col;*, --, not relevant; <, less than; ns, not significant; CFU/100 mL, colony-forming units per 100 milliliters]

		Pathogen gene markers									MST gene markers			
RWQC	n	*eaeA*	stx_2	stx_1	EC	rfb_{0157}	*spvC*	*invA*	*ipaH*	Campy	*esp* human	*E. hirae* bovine	STh human	LTIIa bovine
Meet *E. coli*[1]	42	0.64	0.12	0.048	0.95	0.095	0.071	0.24	0.024	0.048	0.024	0.048	0.19	0.02
Exceed *E. coli*[1]	50	1	.12	.5	1	.64	.06	.34	.12	.12	.16	.62	.12	.52
p-value[2]	--	<.05	ns	<.05	ns	<.05	ns	ns	ns	ns	ns	<.05	ns	<.05
Meet enterococci[3]	40	.62	.12	.05	.95	.075	.075	.2	.025	.075	0	.075	.22	.05
Exceed enterococci[3]	52	1	.12	.48	1	.63	.058	.37	.12	.096	.17	.58	.36	.48
p-value[2]	--	<.05	ns	<.05	ns	<.05	ns	ns	ns	ns	<.05	<.05	ns	<.05

[1]*E. coli* moderate full-body contact recreational water-quality criteria of 298 CFU/100 mL (Dufour and Ballantine, 1986).

[2]Fisher's exact test p-value, significant difference in frequency between groups when $p<0.05$.

[3]Enterococci moderate full-body contact recreational water quality criteria of 78 CFU/100 mL (Dufour and Ballantine, 1986).

Table 6. Detection-frequency comparison of pathogen and microbial-source tracking gene markers at routinely sampled sites during high and normal flow in the Brandywine Creek Basin study, 2009–10.

[USGS, U.S. Geological Survey; Field ID, station identifier from table 1; MST, microbial-source tracking; *E. hirae, Enterococcus hirae*]

USGS station number	Field ID	Pathogen gene markers									MST gene markers			
		eaeA	stx_2	stx_1	EC	rfb_{0157}	*spvC*	*invA*	*ipaH*	Campy	*esp* human	*E. hirae* bovine	STh human	LTIIa bovine
01480617	West	ns[1]	ns	high[2]	ns	high	ns	ns	ns	ns	ns	ns	ns	high
01480870	East	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
01481000	Main	ns	ns	high	ns	high	ns	ns	ns	ns	ns	high	ns	ns
01481540	Intake	ns	ns	ns	ns	high	ns	ns	ns	ns	ns	high	ns	ns
01481555	CSO	ns	ns	ns	ns	high	ns	ns	ns	ns	ns	high	ns	ns

[1]ns, no significant difference in frequency of marker detection between high- and normal-flow conditions.

[2]high, significantly higher frequency of marker detection under high-flow conditions when compared to normal-flow conditions.

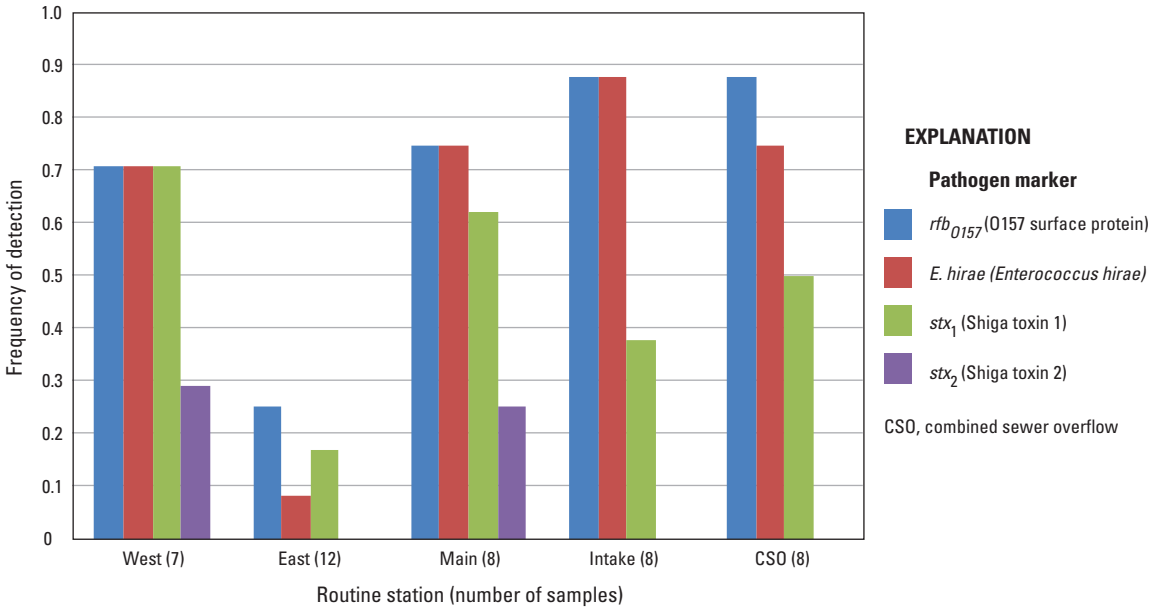


Figure 4. Detection frequency of selected gene markers at routinely sampled sites at high flow in the Brandywine Creek Basin study, 2009–10.

Table 7. Number of concurrently positive samples collected at selected sites in the Brandywine Creek Basin study, 2009–10.

[MST, microbial-source tracking; *E. hirae*, *Enterococcus hirae*; --, no concurrently positive samples]

Positive sample locations[1]	Pathogen gene markers								MST gene markers			
	eaeA	stx_2	stx_1	rfb_{0157}	*spvC*	*invA*	*ipaH*	Campy	*esp* human	*E. hirae* bovine	STh human	LTIIa bovine
High flow												
West and Main	1	2	2	2	--	--	1	--	--	4	--	1
East and Main	--	--	--	--	--	--	--	--	--	--	--	--
West, East, and Main	7	--	2	3	--	2	--	--	--	1	--	2
Main only	--	--	1	1	--	2	--	--	--	1	1	--
East or West, but not Main	--	--	--	--	1	2	2	--	--	--	1	2
Intake only	--	--	--	--	--	1	--	--	3	--	1	--
Intake and CSO	--	--	--	1	--	--	--	--	--	--	--	1
CSO, no other main stem	--	--	1	--	--	--	--	--	--	--	1	--
Normal flow												
West and Main	2	--	--	--	--	--	--	--	--	--	--	--
East and Main	1	--	--	1	--	--	--	--	--	--	1	--
West, East, and Main	3	--	--	--	--	--	--	1	--	--	--	--
Main only	--	--	--	--	1	2	1	--	--	--	1	1
East or West, but not Main	1	1	1	1	2	1	--	1	1	3	2	1
Intake site only	--	--	--	2	--	1	--	1	--	--	--	1
Intake and CSO	--	--	--	--	--	--	--	--	1	--	--	--
CSO, no other main stem	--	2	2	--	--	1	--	--	--	--	1	--

[1]Sample location names refer to field names in table 1.

At high flow, there were eight separate occasions when five different pathogen markers (*eaeA*, stx_2, stx_1, rfb_{O157}, and *ipaH*) were detected in the West Branch samples but not the East Branch samples, and these same genes were also detected in the Main samples on the same day; this pattern indicates a likely connection between the West Branch and Main sites (table 7). These samples were all collected from October through March (appendix 1). At no time under high-flow sampling conditions did an East Branch sample exclusively contain a gene that was concurrently found in the Main sample, indicating the East Branch was an unlikely sole source of increased FIB densities in the main stem on those occasions. However, it should be noted that there were 14 other occasions covering 4 pathogen gene markers (*eaeA*, stx_1, rfb_{O157}, and *invA*) when the marker was found at both the East and West sites and also at the Main site. Therefore on select occasions at high flow the West Branch was the only measured source of stx_2, stx_1, rfb_{O157}, and *ipaH* to the Main site. The East Branch was never observed to be the sole contributor of pathogen genes to the Main site at high flow. However, on select occasions, both the East and West Branch sites at high flow could have been contributing pathogen gene markers to the Main site, making determination of the probable subbasin delivering markers and FIB difficult on those occasions.

There were several occasions where *Salmonella* markers (*invA* and *spvC*) and the *Shigella* marker (*ipaH*) were detected upstream at East and West Branch but not at the Main site downstream. Flow at the time of these samples was greater than the 75th percentile of daily mean flow at each corresponding USGS site, which should have been sufficient to transport these markers downstream. The markers were possibly present in low (but detectable) concentrations in the East and West Branches but were diluted to nondetectable amounts as they were moved downstream.

The stx_1 pathogen marker was detected at the CSO site on one high-flow occasion when it was not concurrently detected upstream at either the Main or the Intake site. On this occasion (December 15, 2009) the site was sampled at the Van Buren Street Bridge, which is upstream of the CSOs (Colleen Arnold, Asst. Water Division Director, City of Wilmington, written commun., 2010). So, the occurrence of the marker on that date was likely unrelated to the CSOs. The stx_1, *invA*, and stx_2 genes also were found under normal flow conditions at the CSO site (but not at the Main or Intake sites) at normal flow (table 7). Finding elevated pathogens and FIB downstream of CSOs that are not actively discharging has been reported by Donovan and others (2008) in a study of the Lower Passaic River, and could explain gene detections downstream of the CSO during normal flow in this study.

At normal flow, there were two instances when the *eaeA* pathogen marker was detected in the West Branch and concurrently detected at the Main site (table 7). Also at normal flow, there were two occasions, one each for *eaeA* and rfb_{O157}, when pathogen markers were detected in the East Branch and concurrently at the Main site. However, on three occasions, the *eaeA* pathogen marker was detected at the Main site when it was detected concurrently in both the East and West Branches, a pattern that indicates that both subbasins are contributing to the movement of the *eaeA* gene to the main stem. There were several normal and high flow samples when genes were detected in the East or the West Branch but were not detected downstream at the Main site. This result could be indicative of specific point sources affecting the occurrence of these genes on the East and West Branches.

The combined pathogen analysis indicates that certain of the pathogen markers are significantly more likely to occur when densities of FIB exceed RWQC. The stx_2 gene on two occasions was exclusively detected at upstream locations in the West Branch and was also observed on the same dates at the Main site downstream. The stx_2 gene was never detected on the East Branch, indicating that the West Branch is serving as the only measured source of stx_2-carrying pathogens at high flows in the Brandywine Creek Basin. In addition, there were six other observations when pathogen genes were measured at the West Branch and Main sites but not at the East Branch site at high flow. This finding also supports the conclusion that the West Branch is a likely source for fecally derived pathogens at high flows in the Brandywine Creek Basin. However, several observations of matching pathogen gene markers at the East Branch, West Branch, and Main sites were also made. Determining the source of the markers at the Main site is difficult on every occasion, but these findings indicate that there are most likely specific conditions and times when the West Branch is the exclusive contributor of pathogen genes to the main stem of Brandywine Creek. This study found the West Branch to be the only potential measured contributor on eight occasions. These samples were collected at high flow between October and March.

Results of Microbial-Source Tracking

All MST gene markers were detected on at least one occasion, from at least one site, during the course of the study. The overall frequency of MST gene markers ranged from 35.9 percent for the *E. hirae* bovine MST marker to 9.8 percent for the *esp* human MST marker. A summary of the detection frequencies can be found in figure 3.

Quantitative analyses for general (AllBac), bovine (BoBac), and human (qHF183) *Bacteroides* MST markers were also done. The AllBac marker ranged in detection from a maximum of 4,210,000 copies/100 mL to a minimum of nondetection, with a mean concentration of 162,000 copies/100 mL, and a median concentration of 800 copies/100 mL. The BoBac marker ranged from a maximum of 3,650,000 copies/100 mL to minimum of nondetection, with a mean concentration of 89,900 copies/100 mL and a median of 103 copies/100 mL (nondetection). The qHF183 marker ranged from a maximum concentration of 171,000 copies/100 mL to a minimum of nondetection, with a mean concentration of 16,200 copies/100 mL, and a median concentration of 2,500 copies/100 mL.

Results of Qualitative Microbial-Source Tracking

Table 5 shows the frequency of MST marker detections in samples meeting or exceeding the moderate-full-body-contact single-sample recreational water-quality criteria (RWQC) for *E. coli* and enterococci FIB (Dufour and Ballantine, 1986). Samples exceeding the *E. coli* RWQC more frequently contained the *E. hirae* and LTIIa MST gene markers that represent enterococci and *E. coli* from a bovine source. MST marker frequencies were also compared in samples meeting or exceeding the enterococci RWQC of 78 CFU/100 mL. Samples exceeding this criterion more frequently contained the *esp* (human sources), *E. hirae* (bovine sources), and LTIIa (bovine sources) MST markers (table 5). Results indicate a relation between samples exceeding the *E. coli* RWQC and bovine fecal pollution, but exceedance of the enterococci RWQC was related to both human and bovine fecal sources.

The frequency of MST marker detection in samples collected during high flow and normal flow at each routine site was compared (table 6). Significantly more frequent detections of LTIIa bovine MST marker were found in high-flow samples than in normal-flow samples in the West Branch. In the East Branch, there was no significant difference in the frequencies of any MST marker between high and normal flow. Interestingly, the *E. hirae* bovine MST marker was more frequent at the Main site, in contrast to the LTIIa bovine MST marker, which was more frequent in the West Branch. Differences in frequency of *E. hirae* bovine MST marker between high and normal flow were also observed at the Intake and CSO sites, again reflecting differences observed at upstream sampling locations; however, the occurrences of *E. hirae* at the main stem sites appear unrelated to those on the East or West Branch.

The frequency of MST marker occurrence during high flow was compared among all five routinely sampled sites. Only the frequency of the *E. hirae* bovine MST marker was different among the five sites (fig 4), with the largest difference occurring between the Intake site and the East Branch site. When the frequency of gene detection from the same five sites was compared for normal flow, the frequency of *E. hirae* marker detection was again found to be significantly different among the sites (not shown). At normal flow, the *E. hirae* MST marker was detected on one of four occasions in the East Branch and three of nine occasions in West Branch Brandywine Creek. This difference between the East and West Branch samples was never translated downstream because the *E. hirae* marker was never found on the main stem at normal flow, even when found upstream.

A comparison of the MST marker detection frequency only between the routinely monitored sites in West Branch and East Branch Brandywine Creek revealed that at high flow, detections of the *E. hirae* bovine MST marker were more frequent in the West Branch, a result that parallels observations when simultaneously comparing all five routinely monitored sites at high flow (fig. 4). When the frequency of gene detection was compared between the East and West Branches at normal flow, no significant differences in the frequency of gene detection were observed. These results indicate that West Branch of Brandywine Creek is the most likely source of increased frequencies of the *E. hirae* MST bovine marker at high flow in the basin.

The frequency of MST markers was also specifically compared among the three main-stem sites (Main, Intake, and CSO). No differences were found in the frequency of MST marker detection among the main-stem sites at high flow (fig. 4) or normal flow (not shown). Four samples at the CSO site were collected from the Van Buren Street Bridge upstream of the CSO outfalls. In the four other samples collected at high flow downstream of the CSO outfalls (Baynard Street Bridge), only one sample (June 15, 2009) was found to contain a MST marker (STh) that was not also found upstream. Although this indicates a potential effect on the microbial water quality of the stream from the CSO outfalls, the lack of downstream samples during the most appropriate conditions to observe a CSO effect substantially limits interpretation.

In an effort to understand the specific relations that upstream water quality had on downstream water quality on each sample date, each MST marker was categorized on the basis of its relation with the upstream sample locations, as was done for pathogen gene markers (table 7). On a total of five sampling occasions at high flow, *E. hirae* (four occasions) and LTIIa (one occasion) were detected upstream in the West Branch and concurrently at the Main site (table 7). These five occasions were spread throughout the year (June, October, December, and March) (appendix 1). The East Branch was never the exclusive contributor of either *E. hirae* or LTIIa gene at high flow, but there were three occasions when the genes were observed at both the East and West Branches and downstream at Main, making determination of the subbasin delivering these markers to the main stem difficult on those occasions. On other sampling occasions at high flow, MST markers were detected at the Main site but not upstream at the East or West Branch sites. This pattern could indicate that, under certain conditions, there may be sources that are closer to the Main site that also are contributing the fecal load (and thus MST markers) to the stream. There were few occasions when MST markers (two LTIIa, one STh) were detected upstream in the East and West Branches but not downstream at the Main site, suggesting that the markers may have been diluted or reduced past detectable densities at the Main site.

On the main stem of Brandywine Creek at high flows, the *esp* human MST marker was detected exclusively on three occasions at the Intake site. On a fourth occasion (independent of the *esp* detections), another human marker—the STh marker—was found at the Intake site but not upstream or downstream on the main stem of Brandywine Creek. Neither marker was found at normal flow at the Intake site; however, the rfb_{O157}, *invA*, and Campy pathogen markers were found exclusively at the Intake site at normal flow on separate occasions (table 7). These four separate detections of human MST

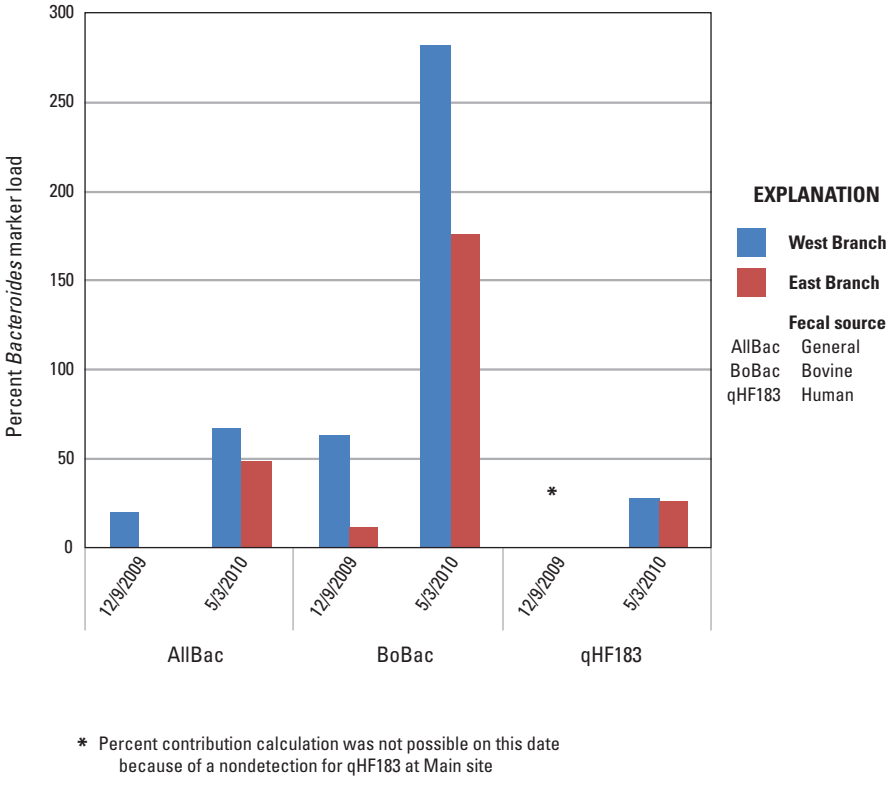
markers at high flow in the absence of immediate upstream or downstream detection of the observed genes, and the exclusive detection of selected pathogen genes at normal flow, likely indicate a potential proximal human source that is contributing human fecal markers as well as *Salmonella* and pathogenic *E. coli*. Storm-water runoff from non-sanitary storm-water drainage systems has been implicated as a source of pathogens in urban environments and could explain detection of human MST markers at high flow (Arnone and Walling, 2007).

Results of Quantitative Microbial-Source Tracking

Analysis for selected MST markers from the fecal anaerobe *Bacteroides* also was done for general (AllBac), human (qHF183), and bovine (BoBac) fecal sources. The median of log transformed *Bacteroides* concentrations were compared among all routinely sampled sites. No significant differences were found between samples collected at high and normal flows. Comparisons were also made between median *Bacteroides* concentrations in the East and West Branches for high and normal flows. During high flow, the median AllBac concentration was significantly greater in the West Branch, which parallels the finding, that median FIB densities were also greater in the West Branch during high flow. No differences were observed between median MST marker concentrations in samples collected at the West and East Branches at normal flow. Comparison of samples collected from the main-stem sites revealed no significant differences in median concentrations for high and normal flows.

The highest AllBac, BoBac, and qHF183 concentrations were measured in samples collected during high-flow event sampling. Instantaneous loads were calculated for the three MST markers and the percent contribution of load from the West and East Branch was computed at the Main site. Percent contribution data are presented in figure 5. Instantaneous loads of qHF183 were excluded for the December 9, 2009, sample because no qHF183 markers were detected at the Main site on this day, even though there were high concentrations in the contributing tributaries. In some samples collected during the study, the measured qHF183 and BoBac concentrations exceed the concentration of the AllBac measurement. This phenomenon has been observed in previous studies, and it is typically attributed to differences in the performance of qPCR assay primers (Layton and others, 2006).

During both high-flow event samples, the West Branch was found to contribute a greater percentage of the load of AllBac and BoBac markers to the Main site than the East Branch did. The qHF183 contributions were found to be roughly similar for the high-flow sample where the analysis was possible. It is important to note that differences in the quantity of MST markers in different fecal sources (even from individual animals) can vary greatly, making these types of direct comparisons useful guides—but not necessarily indications of absolute differences—in the amount of fecal contamination (Field and Samadpour, 2007; Stoeckel and Harwood, 2007).



* Percent contribution calculation was not possible on this date because of a nondetection for qHF183 at Main site

Figure 5. Percent contribution of AllBac, BoBac, and qHF183 microbial-source tracking marker load from East and West Branches of Brandywine Creek during targeted high-flow event sampling, 2009–10. The asterisk indicates that the percent contribution calculation was not possible on this date because of a nondetect for qHF183 at the Main site.

Quality Control

Four field blanks and four field replicates were collected and run in the same manner as regular field samples. A routine laboratory no-template (negative) control was processed for every 25 samples analyzed for each assay, and positive controls were run at 2–3 concentrations ranging from near the detection limit of the assay to three orders of magnitude above the detection limit. All no-template controls were found to be negative as expected and all positive assay controls yielded appropriate responses (data not shown).

All field blanks were negative (below detection limits) for all FIB, for all qualitative pathogen markers and MST markers, and for the AllBac and BoBac analysis. However, on two of four occasions, the qHF183 marker was found at concentrations just above the qHF183 method limit of detection of 272 copies/100 mL. This result suggests that a very low level of field contamination was possible but, because of the low magnitude of the contamination, the impact on data interpretation was judged to be negligible. All laboratory blanks were found to be negative.

Results from field replicates were assessed in two ways. For quantitative FIB assays, a relative standard deviation was calculated and a mean was computed. In all cases, the average RSD fell below the recommended 35 percent (U.S. Environmental Protection Agency, 2006a). For quantitative MST assays, a relative standard deviation was also calculated and a mean computed. There is no currently recommended RSD standard for field replicates for *Bacteroides* MST qPCR methods. A summary of the RSD replicate analysis can be found in table 8.

For qualitative pathogen and MST assays, which yield only a presence or absence result, the frequency of agreement between the replicates was determined by dividing the number of paired replicates with identical values by the total number of replicates. Only two assays showed any variation: the *eaeA*, and stx_1 gene assays both showed variable results in the same replicate. This result is suggestive of a quantity of target DNA very near the detection limit and was considered to have a negligible effect on data interpretation. A summary of the frequency of agreement field replicate analysis can be found in table 9.

Table 8. Relative standard deviation of field replicates for quantitative microbial methods in the Brandywine Creek Basin study, 2009–10.

[*E. coli*, *Escherichia coli*; RSD, relative standard deviation]

Statistic	*E. coli*	Enterococci	Fecal coliform bacteria	AllBac	BoBac[1]	HuBac
Maximum RSD, in percent	29	70	6	141	0	141
Minimum RSD, in percent	18	10	33	16	0	18
Average RSD, in percent	24	28	19	110	0	72
Number of field replicates	4	4	4	4	4	4

[1]All four field replicate samples fell below the BoBac assay limit of detection.

Table 9. Frequency of agreement between field replicates for qualitative microbial methods in the Brandywine Creek Basin study, 2009–10.

[MST, microbial-source tracking; *E. hirae, Enterococcus hirae*]

	Pathogen gene markers									MST gene markers			
Statistic	*eaeA*	stx_2	stx_1	EC	rfb_{0157}	*spvC*	*invA*	*ipaH*	Campy	*esp* human	*E. hirae* bovine	STh human	LTIIa bovine
Percent agreement	75	100	75	100	100	100	100	100	100	100	100	100	100
Number of field replicates	4	4	4	4	4	4	4	4	4	4	4	4	4

Summary and Conclusions

The City of Wilmington, Delaware, is in the downstream part of the Brandywine Creek Basin, on the main stem of Brandywine Creek. Wilmington uses this stream, which drains a mixed land-use area upstream, for its main drinking-water supply. Because the stream is used as a drinking-water source, Wilmington is in need of information about the occurrence of specific fecally derived pathogenic bacteria (disease-causing organisms) and their relations to commonly measured fecal-indicator bacteria (FIB), as well as information regarding the potential sources of the fecal pollution and pathogens in the basin. Since 2005, Wilmington has been investing in best-management-practices (BMP) projects in the Brandywine Creek headwaters to control sediments and pollutants, including bacteria that could affect the drinking-water supply. In cooperation with the City of Wilmington, the U.S. Geological Survey did a 1-year study in the Brandywine Creek Basin to aid in understanding the occurrence and distribution of FIB, fecally derived pathogens, and potential fecal sources that contribute to microbial water-quality impairments. Information obtained in this study could be used by the City of Wilmington to target BMP strategies within the Brandywine Creek Basin.

The study focused on five routinely sampled sites within the basin, one each on the West Branch and the East Branch and three on the main stem of Brandywine Creek below the confluence of the West and East Branches. These sites were sampled monthly for 1 year. Targeted event samples were collected on two occasions during high flow (greater than the 75th percentile of mean daily flow at the site) and two occasions during normal flow (between the 25th and 75th percentiles of mean daily flow). During these targeted events, three additional sites were sampled upstream of the routine sampling sites on the West and East Branches. In addition, four field blanks and four field replicates were collected throughout the study period.

The collected samples were analyzed for the densities of the FIB, *Escherichia coli* (*E. coli*), enterococci, and fecal coliform bacteria. Samples were enriched for growth of the fecally derived pathogens *Campylobacter* and *Salmonella,* and total DNA was extracted from each water sample. FIB cultures, plus enrichments originating from each water sample, were analyzed for the presence of the selected genes that indicated the presence of pathogenic types of *E. coli*, *Salmonella, Shigella,* and *Campylobacter* bacteria, as well as the presence of specific microbial-source tracking (MST) genes indicating bovine or human fecal pollution. DNA extracted directly from each water sample was analyzed for the quantity of each of three types of *Bacteroides* MST markers indicating general, bovine, and human fecal pollution. In addition, routine chemical measurements, turbidity measurements, and stream-discharge measurements were made with each sample.

Median concentrations of FIB in Brandywine Creek Basin were more elevated at the West Branch, Main, Intake, and combined sewer overflow sites (the latter three on the main stem) in samples collected during high flow than in those collected at normal flow. There was no difference in median FIB density in samples collected during high and normal flow in the East Branch. Concentrations of *E. coli* and enterococci were higher in samples collected from the West Branch than from the East Branch during high flow. No differences in FIB densities were observed among the three sites on the main stem under any flow condition. The highest concentrations of FIB were found in the West Branch samples during high flow.

Samples that exceeded the U.S. Environmental Protection Agency's recreational water quality criteria (RWQC) for *E. coli* and enterococci FIB were found to more frequently contain the *eaeA*, stx_1, and rfb_{O157} pathogen markers than those meeting RWQC. However, there was no difference in the frequency of the stx_2 *E. coli* pathogen marker or any measured marker for *Salmonella*, *Shigella*, or *Campylobacter* when comparing samples that met, versus exceeded, the RWQC for either *E. coli* or enterococci FIB. Water samples exceeding the FIB RWQC were more likely to contain selected *E. coli* pathogens, but the occurrence of other *E. coli* pathogens (with stx_2), *Salmonella*, *Shigella*, and *Campylobacter* was unrelated to the FIB RWQC in this study.

The data clearly demonstrate that there are specific instances during high flow where the West Branch is the only observed source of selected pathogenic *E. coli* and *Shigella* to the main stem, whereas the East Branch was never found to be the exclusive contributing source of any measured marker. During high flow, the stx_1 marker was more frequently detected in the West Branch than the East Branch. On two occasions during high flow, the West Branch was the only observed source of the stx_2 gene to the main stem. On other occasions during high flow, the West Branch was the only observed source of the *eaeA*, stx_1, rfb_{O157}, *and ipaH* genes to the main stem. There were occasions when both the East Branch and West Branch were likely delivering markers to the main stem; however, results from this study indicate that increased discharge was a likely factor related to pathogen delivery from the West Branch to main stem. There are likely seasonal, spatial, ecological, and hydrological factors not measured during this study that would help refine the understanding of fecally derived pathogens in the basin. Indeed, on some occasions at normal flow, we observed markers of pathogenic *E. coli* and *Salmonella* near the Intake site on the main stem but did not observe these markers immediately upstream; this pattern suggests diverse transport pathways and alternative sources.

Samples exceeding the *E. coli* RWQC more frequently contained markers of bovine feces, suggesting a relation between high *E. coli* densities and bovine fecal pollution. Samples exceeding the enterococci RWQC were found to more frequently contain markers for both bovine and human feces, suggesting that high enterococci densities are related to both bovine and human fecal pollution. The *E. hirae* bovine fecal marker was found more frequently in the West Branch than the East Branch during high flow, indicating that elevated FIB densities in the West Branch are more frequently related to bovine sources than in the East Branch. Matching bovine markers were found in the West Branch and main stem on four sample dates, indicating the most likely source of FIB in the main stem on those dates was water from the West Branch that was carrying FIB of bovine origin. The finding of the West Branch as the most likely source of FIB was supported by the similar increases in frequency of stx_1 and rfb_{O157} markers during high flows in the West Branch as well as in the main stem, whereas there was no parallel increase in the frequency of pathogen or MST marker detection during high flows in the East Branch. In addition to having similar patterns of detection frequency (which do not account for temporal differences in detection), the West Branch and main stem had concurrently matching pathogen and MST markers on several occasions, whereas the markers were absent from the concurrent East Branch samples. It should be noted that there were other occasions at high flow when the markers found in East Branch samples (both MST and pathogen markers) matched those in main-stem samples; but on those occasions, the markers were also found in the West Branch, so no clear determination of source can be made. There was never an occasion at high flow when the East Branch markers exclusively matched main-stem markers, indicating that the East Branch is less likely to be a source for FIB in the main stem.

Quantitative analysis of MST markers revealed that during targeted high-flow event samples, the West Branch accounted for a larger proportion of the load of both general and bovine source markers at the main stem when compared to the East Branch, but care should be taken in direct comparisons of MST quantities because these data are often highly variable.

On the basis of this study, high flow in the Brandywine Creek Basin was related to increases in FIB densities and increases in the detection frequency of selected pathogens and MST gene markers in West Branch Brandywine Creek and at sites on the main stem of Brandywine Creek, but not in East Branch Brandywine Creek. Water exceeding the RWQC for *E. coli* was more likely to contain the selected markers for pathogenic *E. coli* and bovine fecal sources, whereas samples exceeding the enterococci RWQC were more likely to contain the same pathogenic markers but also carried indications of human sources.

On four sampling dates, during high flow between October and March, the West Branch was the only observed contributor of selected pathogen and bovine source markers to the main stem of Brandywine Creek. Indeed, the stx_2 marker, which indicates a highly virulent type of pathogenic *E. coli,* was found only in the West Branch and main stem at high flow—never in the East Branch. However, it must be noted that throughout the entire year of sampling there were occasions, during both high and normal flows, when both the East and West Branches were potential contributors of pathogen and MST markers to the main stem. The major conclusion from this study is that under selected conditions (high flow, October through March), West Branch Brandywine Creek is the most likely source of elevated FIB densities in the main stem. These elevated densities are associated with more frequent detection of the stx_1 pathogenic *E. coli* marker and are associated with the *E. hirae* MST marker of bovine source. However, during other times of the year, both the West Branch and East Branch are likely acting as potential sources of FIB and fecally derived pathogens on the main stem.

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Appendix 1. Concentrations of fecal-indicator bacteria, detections of pathogen and microbial-source tracking markers, and concentrations of microbial-source tracking markers from water collected in the Brandywine Creek Basin in Pennsylvania and Delaware, 2009–10.

[USGS, U.S. Geological Survey; EC, *Escherichia coli*; ENT, enterococci; FC, fecal coliform bacteria; *E. coli*; *Escherichia coli* marker; *E. hirae*, *Enterococcus hirae* marker; CFU/100 mL, colony-forming units per 100 milliliters; +, marker detected; -, marker not detected; ND, marker concentration below detection limit; E, estimated concentration; k, concentration based on calculated value; <, less than]

USGS station number	Sample date	EC	ENT	FC	*eaeA*	*stx*$_2$	*stx*$_1$	*E. coli*	*rfb*	*spvC*	*invA*	*ipaH*	Campy	*esp*	*E. hirae*	LTIIa	STh	AllBac	BoBac	qHF183
		CFU/100 mL			Presence/absence													Copies/100 mL		
01480300	10/6/2009	380	210	453Ek	+	-	+	+	-	-	-	-	-	-	-	-	-	2,460	912	1,150
01480300	12/9/2009	13,225Ek	10,025Ek	16,550Ek	+	+	+	+	+	-	+	-	-	-	+	+	-	4,210,000	3,650,000	11,100
01480300	4/21/2010	560	74	640Ek	+	-	-	+	-	-	-	-	+	-	-	-	-	ND	ND	ND
01480300	5/3/2010	74,400Ek	42,800Ek	52,800Ek	+	-	+	+	+	-	+	+	-	-	+	+	-	2,480,000	655,000	6,100
01480500	10/6/2009	38Ek	60	46	-	+	-	+	-	-	-	-	-	-	-	-	-	ND	ND	ND
01480500	12/9/2009	4,600	12,700Ek	4,400	+	+	+	+	-	-	-	-	-	+	+	+	-	836,000	849,000	13,900
01480500	4/21/2010	58	6Ek	42	+	-	-	+	-	-	-	-	-	-	-	-	-	8,030	ND	ND
01480500	5/3/2010	8,300Ek	9,175Ek	4,400Ek	+	-	-	+	-	+	+	-	-	-	-	-	-	413,000	54,000	13,100
01480617	6/15/2009	4,600	3,700	5,800	+	-	+	+	+	-	-	-	-	-	+	+	-	234	ND	ND
01480617	7/15/2009	577	52	1,233	+	-	-	+	-	-	-	-	-	-	-	-	+	821	ND	ND
01480617	8/17/2009	340	112	360	+	-	-	+	-	-	+	-	-	-	-	-	-	ND	ND	ND
01480617	9/15/2009	410	470	700	+	-	-	+	-	-	-	-	-	-	-	+	+	1,920	ND	1,090
01480617	10/6/2009	68	50	66	+	+	-	+	-	-	-	+	-	-	-	-	-	333	ND	2,470
01480617	10/19/2009	7,800	530	2,900	+	-	+	+	+	-	-	-	-	-	+	+	-	209	5,830	ND
01480617	11/16/2009	175Ek	40	178Ek	-	-	-	+	-	-	-	-	-	-	+	-	-	ND	ND	61,300
01480617	12/9/2009	5,800	14,100Ek	5,925Ek	+	+	+	+	+	-	-	+	-	-	+	+	-	562,000	860,000	58,300
01480617	12/15/2009	430	450	480	+	-	+	+	+	-	-	-	-	-	+	-	-	ND	ND	9,070
01480617	1/13/2010	82	52	62	+	-	-	+	-	-	-	-	-	-	-	-	-	34,500	ND	ND
01480617	2/16/2010	5Ek	18Ek	4Ek	-	-	-	-	-	-	-	-	-	-	+	-	+	16,800	ND	3,110
01480617	3/15/2010	350	390	480	+	+	+	+	-	-	-	+	-	-	+	-	-	365,000	ND	14,300
01480617	4/12/2010	46	20Ek	40	+	-	+	+	-	-	-	-	-	-	-	-	-	4,210	ND	14,000
01480617	4/21/2010	290	29Ek	310	+	-	-	+	-	+	+	-	+	-	-	-	-	ND	ND	945
01480617	5/3/2010	21,600Ek	20,800Ek	12,800Ek	+	-	-	+	+	+	+	-	-	-	-	+	-	1,140,000	98,900	85,400
01480617	5/12/2010	1,200	1,500	1,000	+	-	-	+	-	-	-	-	+	+	+	+	-	9,310	ND	2,610
01480700	10/6/2009	42	104	54	+	-	-	+	-	-	-	-	-	-	-	-	-	565	ND	ND
01480700	12/9/2009	4,000	8,975Ek	5,100	+	-	+	+	+	-	-	-	-	+	+	+	-	419,000	148,000	10,600
01480700	4/21/2010	33Ek	8Ek	44	+	-	-	+	-	-	+	-	-	-	-	-	-	735	ND	1,900
01480700	5/3/2010	14,700Ek	22,800Ek	12,500Ek	+	-	-	+	+	-	+	+	-	-	-	+	-	322,000	2,680	14,100
01480870	6/15/2009	800	353Ek	1,167	+	-	+	+	+	-	-	-	-	-	-	+	-	ND	ND	ND
01480870	7/15/2009	300	34Ek	330	+	-	-	+	-	-	-	-	-	-	+	-	+	1,060	ND	ND
01480870	8/17/2009	200	58	290	+	-	-	+	-	-	+	-	-	-	-	-	+	ND	ND	4,840

Appendix 1. Concentrations of fecal-indicator bacteria, detections of pathogen and microbial-source tracking markers, and concentrations of microbial-source tracking markers from water collected in the Brandywine Creek Basin in Pennsylvania and Delaware, 2009–10.—Continued

[USGS, U.S. Geological Survey; EC, *Escherichia coli*; ENT, enterococci; FC, fecal coliform bacteria; *E. coli*; *Escherichia coli* marker; *E. hirae*, *Enterococcus hirae* marker; CFU/100 mL, colony-forming units per 100 milliliters; +, marker detected; -, marker not detected; ND, marker concentration below detection limit; E, estimated concentration; k, concentration based on calculated value; <, less than]

USGS station number	Sample date	EC	ENT	FC	*eaeA*	stx_2	stx_1	*E. coli*	*rfb*	*spvC*	*invA*	*ipaH*	Campy	*esp*	*E. hirae*	LTIIa	STh	AllBac	BoBac	qHF183
		CFU/100 mL			Presence/absence													Copies/100 mL		
01480870	9/15/2009	230	280	400	+	-	-	+	-	-	-	-	-	-	-	-	+	371	2,030	1,400
01480870	10/6/2009	50	30Ek	40	-	+	-	+	+	-	-	-	-	-	-	-	-	ND	ND	1,030
01480870	10/19/2009	4Ek	2<	10Ek	-	-	-	-	-	-	-	-	-	-	-	-	-	780	ND	1,200
01480870	11/16/2009	22Ek	4Ek	48	+	-	-	+	-	-	-	-	-	-	-	-	-	ND	ND	ND
01480870	12/9/2009	3,600	10,650Ek	5,900	+	-	+	+	-	-	+	-	-	-	+	+	-	ND	58,600	ND
01480870	12/15/2009	106	92	230	+	-	-	+	-	-	-	-	-	-	-	-	-	1,220	ND	2,450
01480870	1/13/2010	5Ek	2<	10Ek	+	-	-	+	-	-	-	-	-	-	-	-	+	ND	ND	2,950
01480870	2/16/2010	8Ek	2<	7Ek	-	-	-	+	-	-	-	-	-	-	-	-	+	ND	ND	ND
01480870	3/15/2010	84	42	90	+	-	-	+	-	+	+	-	-	-	-	+	-	ND	ND	30,300
01480870	4/12/2010	13Ek	6Ek	23Ek	-	-	-	+	-	-	-	-	-	-	-	-	-	ND	ND	2,590
01480870	4/21/2010	25Ek	4Ek	50	-	-	-	+	-	-	-	-	-	-	-	-	-	ND	ND	7,950
01480870	5/3/2010	13,200Ek	19,600Ek	22,400Ek	+	-	-	+	+	-	+	+	-	-	-	+	-	276,000	20,500	27,300
01480870	5/12/2010	1,333	4,300	3,000	+	-	-	+	+	+	-	-	+	+	-	+	-	466	ND	6,810
01481000	6/15/2009	6,800	2,000	6,000	+	-	+	+	+	-	-	-	-	-	+	-	-	ND	ND	ND
01481000	7/15/2009	4,500	30Ek	290	+	-	-	+	-	-	-	-	-	-	-	+	-	906	ND	ND
01481000	8/17/2009	152	100	227Ek	+	-	-	+	-	-	+	-	-	-	-	-	-	ND	ND	137,000
01481000	9/15/2009	200	98	484Ek	+	-	-	+	-	-	+	-	-	-	-	-	-	ND	ND	ND
01481000	10/6/2009	38Ek	29Ek	30Ek	+	-	-	+	+	-	-	-	-	-	-	-	+	ND	ND	1,500
01481000	10/19/2009	6,900	5,325Ek	10,525Ek	+	-	+	+	+	-	+	-	-	-	+	+	-	62,700	ND	ND
01481000	11/16/2009	37Ek	12Ek	35Ek	+	-	-	+	-	-	-	-	-	-	-	-	-	679	ND	2,000
01481000	12/9/2009	3,500	22,900Ek	4,200	+	+	+	+	+	-	-	-	-	-	+	+	+	721,000	352,000	ND
01481000	12/15/2009	1,000	1,600	698Ek	+	-	-	+	+	-	-	-	-	-	+	-	-	268,000	71,300	ND
01481000	1/13/2010	22Ek	21Ek	23Ek	+	-	-	+	-	-	-	-	-	-	-	-	-	345	ND	1,470
01481000	2/16/2010	40	8Ek	40	-	-	-	+	-	+	+	-	-	-	-	-	+	9,890	ND	7,370
01481000	3/15/2010	330	1,079Ek	550	+	+	+	+	+	-	-	+	-	-	+	-	-	595,000	ND	171,000
01481000	4/12/2010	40	14Ek	58	-	-	-	+	-	-	-	-	-	-	-	-	-	2,700	ND	2,590
01481000	4/21/2010	48	6Ek	52	+	-	-	+	-	-	-	-	-	-	-	-	-	1,540	ND	2,710
01481000	5/3/2010	9,700Ek	14,900Ek	10,400Ek	+	-	+	+	+	-	+	-	-	-	+	+	-	303,000	6,240	55,300
01481000	5/12/2010	600	667	470	+	-	-	+	-	-	+	-	+	-	-	-	-	5,920	ND	3,640
01481540	6/15/2009	5,500	2,900	6,650Ek	+	-	+	+	+	-	-	-	-	-	+	+	-	ND	ND	ND
01481540	7/15/2009	230	1,500	6,875Ek	+	-	-	+	+	-	-	-	-	+	-	-	-	22,900	ND	6,400

Appendix 1. Concentrations of fecal-indicator bacteria, detections of pathogen and microbial-source tracking markers, and concentrations of microbial-source tracking markers from water collected in the Brandywine Creek Basin in Pennsylvania and Delaware, 2009–10.—Continued

[USGS, U.S. Geological Survey; EC, *Escherichia coli*; ENT, enterococci; FC, fecal coliform bacteria; *E. coli*; *Escherichia coli* marker; *E. hirae*, *Enterococcus hirae* marker; CFU/100 mL, colony-forming units per 100 milliliters; +, marker detected; -, marker not detected; ND, marker concentration below detection limit; E, estimated concentration; k, concentration based on calculated value; <, less than]

USGS station number	Sample date	EC	ENT	FC	*eaeA*	*stx*$_2$	*stx*$_1$	*E. coli*	*rfb*	*spvC*	*invA*	*ipaH*	Campy	*esp*	*E. hirae*	LTIIa	STh	AllBac	BoBac	qHF183
		CFU/100 mL			Presence/absence													Copies/100 mL		
01481540	8/17/2009	10,625Ek	3,900	7,375Ek	+	-	-	+	+	-	-	-	-	-	+	-	+	1,820	ND	59,400
01481540	9/15/2009	11,600Ek	2,921Ek	11,200Ek	+	-	-	+	+	-	+	-	-	-	-	-	-	9,940	ND	79,200
01481540	10/6/2009	68	22Ek	66	+	-	-	+	-	-	-	-	-	-	-	-	-	ND	ND	ND
01481540	10/19/2009	7,475Ek	5,300	2,000	+	-	+	+	+	-	-	-	-	-	+	+	-	ND	ND	ND
01481540	11/16/2009	56	22Ek	50	+	-	-	+	-	-	-	-	+	-	-	-	-	ND	ND	ND
01481540	12/9/2009	5,400	12,175Ek	5,975Ek	+	-	+	+	+	-	-	-	-	-	+	+	-	897,000	557,000	34,600
01481540	12/15/2009	1,833	3,700	1,867	+	-	-	+	+	-	+	-	-	+	+	-	-	610,000	292,000	138,000
01481540	1/13/2010	50	78	28Ek	+	-	-	+	-	-	-	-	-	-	-	-	-	5,870	ND	ND
01481540	2/16/2010	42Ek	8Ek	40	-	-	-	+	-	-	-	-	-	-	-	-	-	16,200	ND	9,700
01481540	3/15/2010	570	543Ek	845Ek	+	-	-	+	+	-	-	-	-	+	+	-	-	ND	ND	56,300
01481540	4/12/2010	44	25	42	-	-	-	+	-	-	-	-	-	-	-	-	-	1,090	ND	ND
01481540	4/21/2010	38Ek	10Ek	24Ek	+	-	-	+	+	-	+	-	-	-	-	-	-	319	ND	1,780
01481540	5/3/2010	792	200	593Ek	+	-	-	+	-	-	+	-	-	+	+	-	-	54,600	ND	ND
01481540	5/12/2010	330	410	250	+	-	-	+	-	-	-	-	+	-	-	+	-	ND	ND	12,600
01481555	6/15/2009	6,300	1,900	5,300Ek	+	-	+	+	+	-	-	-	-	-	+	+	+	ND	ND	ND
01481555	7/15/2009	2,100	450	3,800	+	-	-	+	-	-	-	-	-	+	-	-	-	3,500	16,200	4,050
01481555	8/17/2009	7,700	1,667	6,350Ek	+	-	-	+	+	-	+	-	-	-	-	-	-	ND	ND	90,000
01481555	9/15/2009	4,600	867	5,800	+	-	-	+	+	-	+	-	-	-	-	-	-	8,530	ND	62,200
01481555	10/6/2009	24Ek	12Ek	28Ek	+	+	+	+	-	-	-	-	-	-	-	-	-	ND	ND	ND
01481555	10/19/2009	6,300	3,400	2,900	+	-	+	+	+	-	+	-	-	-	+	+	-	ND	3,370	ND
01481555	11/16/2009	58	19Ek	62	+	-	-	+	-	-	+	-	-	-	-	-	-	ND	ND	1,240
01481555	12/9/2009	5,800	13,350Ek	5,400	+	-	+	+	+	-	-	-	-	-	+	+	-	ND	561,000	33,400
01481555	12/15/2009	2,000	2,200	1,567	+	-	+	+	+	-	-	-	-	-	+	-	-	532	ND	3,780
01481555	1/13/2010	25Ek	54	12Ek	+	-	-	+	-	-	-	-	-	-	-	-	-	30,100	1,160	4,300
01481555	2/16/2010	32Ek	8Ek	22Ek	-	+	-	+	-	-	+	-	-	-	-	-	+	20,700	ND	8,460
01481555	3/15/2010	560	400	631	+	-	-	+	+	-	-	-	-	-	+	-	-	1,420	5,260	71,100
01481555	4/12/2010	34Ek	10Ek	22Ek	-	-	-	+	-	-	-	-	-	-	-	-	-	ND	ND	1,940
01481555	4/21/2010	32Ek	6Ek	16Ek	-	-	-	+	-	-	-	-	-	-	-	-	-	1,020	ND	2,880
01481555	5/3/2010	1,967	590	1,900	+	-	-	+	-	-	-	-	-	-	+	-	-	136,000	ND	ND
01481555	5/12/2010	350	300	3,000	+	-	+	+	-	-	-	-	+	-	-	-	-	ND	ND	9,220

Appendix 2. Concentrations of fecal-indicator bacteria, detections of pathogen and microbial-source tracking markers, and concentrations of microbial-source tracking markers from quality-control samples collected in the Brandywine Creek Basin in Pennsylvania and Delaware, 2009–10.

[USGS, U.S. Geological Survey; QC, quality control; EC, *Escherichia coli*; ENT, enterococci; FC, fecal coliform bacteria; *E. coli*; *Escherichia coli* marker; *E. hirae*, *Enterococcus hirae* marker; CFU/100 mL, colony-forming units per 100 milliliters; +, marker detected; -, marker not detected; ND, marker concentration below detection limit; E, estimated concentration; k, concentration based on calculated value; <, less than]

USGS station number	Sample date	QC type	EC	ENT	FC	*eaeA*	stx_2	stx_1	*E. coli*	*rfb*	*spvC*	*invA*	*ipaH*	Campy	*esp*	*E. hirae*	LTIIa	STh	AllBac	BoBac	qHF183
			CFU/100 mL			Presence/absence													Copies/100 mL		
01480617	4/12/2010	Replicate	70	17Ek	64	-	-	-	+	-	-	-	-	-	-	-	-	-	837	ND	818
01480700	4/21/2010	Replicate	24Ek	24Ek	48	+	-	-	+	-	-	-	-	-	-	-	-	-	ND	ND	370
01481555	5/3/2010	Replicate	1,533	450	1,500	+	-	-	+	-	-	+	-	+	-	-	-	-	ND	ND	939
01481000	5/12/2010	Replicate	420	767	350	+	-	-	+	-	+	-	-	+	-	-	-	-	ND	ND	1,670
01481540	4/12/2010	Blank	2<	2<	2<	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	ND	ND
01480617	4/21/2010	Blank	2<	2<	2<	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	ND	ND
01480500	5/3/2010	Blank	2<	2<	2<	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	ND	663
01481540	5/12/2010	Blank	2<	2<	2<	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	ND	308

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