LFUCG MOU Final Report and Executive Summary

Date of Final Report: April 15th, 2011

Title: A PLAN FOR IDENTIFYING HOT-SPOTS AND AFFIRMING REMEDIATION IMPACTS ON SURFACE WATER QUALITY: PHASE I

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Institution: University of Kentucky

Research Category:

Project Period: 3-23-2010 4-14-2011

Description and Objective of Research:
To use a model blending signals from indicators representative of fecal load, source, and age to identify leaking sewers from other, stormwater related inputs of human sewage into Wolf Run Watershed.

Synopsis: Wolf Run is under the influence of human fecal materials from different types of events that can be distinguished from each other by measuring multiple water quality indicators that capture information about fecal load, source, and age. Vaughn's Branch was predominantly influenced by leaking sewers during the time of study. Wolf Run was predominantly influenced by sewer overflows during rain events, but may have a smaller sewer leak near one site adding sewage into the watershed. Cardinal Run is the cleanest creek in the watershed. A simple modeling system based on multiple water quality indicators was created that can rank regions within the watershed and prioritize areas for remediation. This system can be applied in the future to validate effectiveness of remediation undertaken since the end of this study.

Executive Summary:

Wolf Run Database. In concert with the volunteer group, Friends of Wolf Run, an intensive sampling program was initiated at 19 sites selected jointly in Wolf Run Watershed during the period of April 6th through August 5th 2010. Grab samples from these sites, along with inlet domestic sewage and manhole overflows, were analyzed for indicators of fecal load (E. coli, and a non-host specific *Bacteroides* DNA marker), fecal source (two human host specific *Bacteroides* DNA marker), and fecal age (AC/TC ratio). A final database of indicator concentrations on the 10 days at the 19 sites was created.

The resultant database was analyzed for the purpose of ranking the average water quality at each site under all conditions. Results were also split and analyzed with respects to weather conditions, rainy and dry, to determine the presence and impact of sewer overflows and leaking infrastructure. The indicator values were compared to conditions found in domestic sewage using a simple, categorical model (Model II). Model II calculated a Sanitary Category Value (SCV) between 0 and 3 for each location and observation. The SCV calculated by the

model was comprised of a summation of values between 0 and 1 for each of the three indicator classes selected: 1) fecal load (E. coli), 2) fecal age (AC/TC), and 3) relative strength of human fecal source (log_{10}HuBac/log10HuBacMax). Low SCVs (<1.3) related to conditions associated with cleaner surface waters (low fecal load, little human signal, and old fecal age). High SCVs (>1.5) were associated with high values of fecal load, a greater than reliably detectable value for human specific qPCR markers, and a low fecal age. Inlet sewage SCVs were used as a referent to compare the average water quality at each site against. SCVs were used to rank water quality at the sites relative to sewage, and to each other.

Common statistical analyses were done to determine if there were differences between the calculated SCVs at each site and sewage SCVs under all conditions, rainy conditions, and dry conditions. The purpose of the analysis of average SCVs found during dry and wet conditions was to highlight and separate leaking sewers from combined and sanitary sewer overflows. One site (D10) located along Vaughn's Branch was found to have SCVs indistinguishable (not significantly different by Repeat Measure ANOVA) from sewage during dry conditions, which is indicative of leaking sewer lines. A sanitary survey confirmed an active sewage link impacting this site during the time of study. The D10 site also did not show a great difference in average SCV values comparing dry and rainfall conditions, which is indicative of a consistent input of fecal contamination, again a signature of water quality impacted by leaking sewers. Only two sites (D04 and D18) were significantly different under dry conditions from D10, with average SCVs less than 0.5 indicating cleaner water quality. Under wet conditions, the watershed quality declined. All but eight sites (D01, D02, D04, D06, D07, D15, D20, and D22) average SCVs were indistinguishable from sewage under rainy conditions. While this does not mean that these sites were as loaded with human fecal materials as sewage due to the structure of the model truncating the *E. coli* signal at 24,000 MPN/100mL, the SCVs indicate that the water quality at these sites was unacceptable. This is indicative of precipitation associated overflows of human sewage into the watershed. Cardinal Run appears to be the least sewage impacted tributary in the Wolf Run Watershed under wet and dry conditions. Vaughn's Branch is the most impacted during dry conditions, and is heavily impacted under rain conditions as well. Wolf Run is inundated with human sewage primarily during rainfall events, yet Site D16 on Wolf Run has a high SCV score during dry conditions that is potentially indicative of leaking human sewers.

Conclusions: The presence of human sewage in the urban Wolf Run Watershed is undeniable, but it can be linked to different types of events that require different detection and remediation schemes. Using a multivariate modeling approach, combined with domestic sewage as a referent, it is clear that there were consistent sources of leaking sewage impacting Vaughn's Branch, and intermittent, precipitation-linked sources impacting Vaughn's Branch, Wolf Run, and Town Branch. The cleanest tributary in the watershed was Cardinal Run. The ability to separate sites from each other using average SCVs has been proven, which can provide information to LFUCG to support effective planning and investigation for remediation of the "hottest spots" within the watershed.

Supplemental Keywords: water quality, pathogens, indicators, modeling, *Bacteroides*

Relevant Web Sites: N/A

Introduction:

The purpose of this project was to identify regions within Wolf Run Watershed that are impacted by domestic human sewage during the time of study using a multivariate indicator approach and establish baseline conditions of fecal load, fecal source, and fecal age under dry and rainy conditions.

Objectives of the project:

- Define the unique pattern of indicators that define regions of local urban streams contaminated with proportionally great amounts of human/domestic sewage (hotspots) from those contaminated by other, less hazardous fecal sources.

- Establish baseline values for the indicators in these urban streams, and relative risk categorizations, to be used to evaluate future data against to illuminate water quality improvements or changes.

Approach:

An initial Quality Assurance Program Plan (QAPP) was written and submitted to all involved parties for review and revision. A copy of the plan is appended to this document. Detailed information on methods and approach can be found in the appended QAPP. What follows here is a brief summary.

Nineteen sites within Wolf Run Watershed were selected for study in conjunction with officials of LFUCG and volunteers from the Friends of Wolf Run group (Table 1, Figure 1). Influent sewage, or direct sewer overflow, samples were added to the 19 watershed sample sites to provide referent against which to compare water quality measurements. These 20 sites were assigned numeric identifications and were sampled a total of 10 times during the period from April through August of 2010. Sites were measured under dry conditions 4 times and under wet conditions 6 times. Dry and wet weather conditions were determined from records of gauge heights and rainfall data during the sample day, and 48 hours prior to the sample day. Dry days (4/6, 4/13, 7/31, 8/5) had gauge heights <1.1 ft. and insignificant precipitation for the prior 24 and 48 hours. Wet days (5/2, 6/3, 6/9, 6/14, 7/10, 7/21) had rainfall amounts >0.39 inches recorded for the prior 24 to 48 hour period and gauge heights >1.25 ft. (Table 2). The gauge station was located at the lower reaches of Wolf Run Before the confluence with Town Branch (Figure 1)

Every effort was made to get samples at each site on each of the 10 sampling events. However, site D20 was not safely accessible on 5/2/2010 due to extreme precipitation events (3.89 inches in 24 hours). On this extreme wet day, a sample was taken at an overflowing manhole (D26). Bacterial samples for site D10 were not available due to matrix contamination (presumed disinfectants) from a leaking sewer that prevented bacterial growth even after dilution from a sample taken on 7/10/2010. Samples at the inlet to the Town Branch Sewage Treatment facility were taken only on the last 7 sampling events. The first 2 sampling events, mixed liquor suspended solids were collected and on 5/2/2010, inlet sewage was not taken

due to extreme rain preventing timely sampling response of treatment plant personnel. Other than these noted occasions, the sampling and analysis plan originally planned was completed resulting in a large, multivariable database for analysis and modeling.

Table 1: Site IDs and GPS Coordinates

Site ID	Latitude	Longitude	Number of Times Sampled
D01	38.048916	-84.551207	10
D02	38.042167	-84.524331	10
D03	38.055278	-84.518889	10
D04	38.057336	-84.542167	10
D06	38.037167	-84.522667	10
D07	38.022336	-84.512000	10
D09	38.032551	-84.526524	10
D10	38.044997	-84.536003	10
D12	38.051667	-84.545831	10
D13	38.054833	-84.549667	10
D14	38.023000	-84.528503	10
D15	38.030167	-84.537169	10
D16	38.034667	-84.543167	10
D18	38.053664	-84.550500	10
D19	38.066833	-84.554336	10
D20	38.030000	-84.537169	9
D22	38.040466	-84.559753	10
D23	38.044982	-84.549966	10
D24	38.015015	-84.522393	10
D26 Manhole	38.042304	-84.549044	1
STP Influent	NA	NA	7
STP Outfall	38.063653	-84.533960	0

Figure 1: Map of Sample Sites and Other Features in Wolf Run Watershed

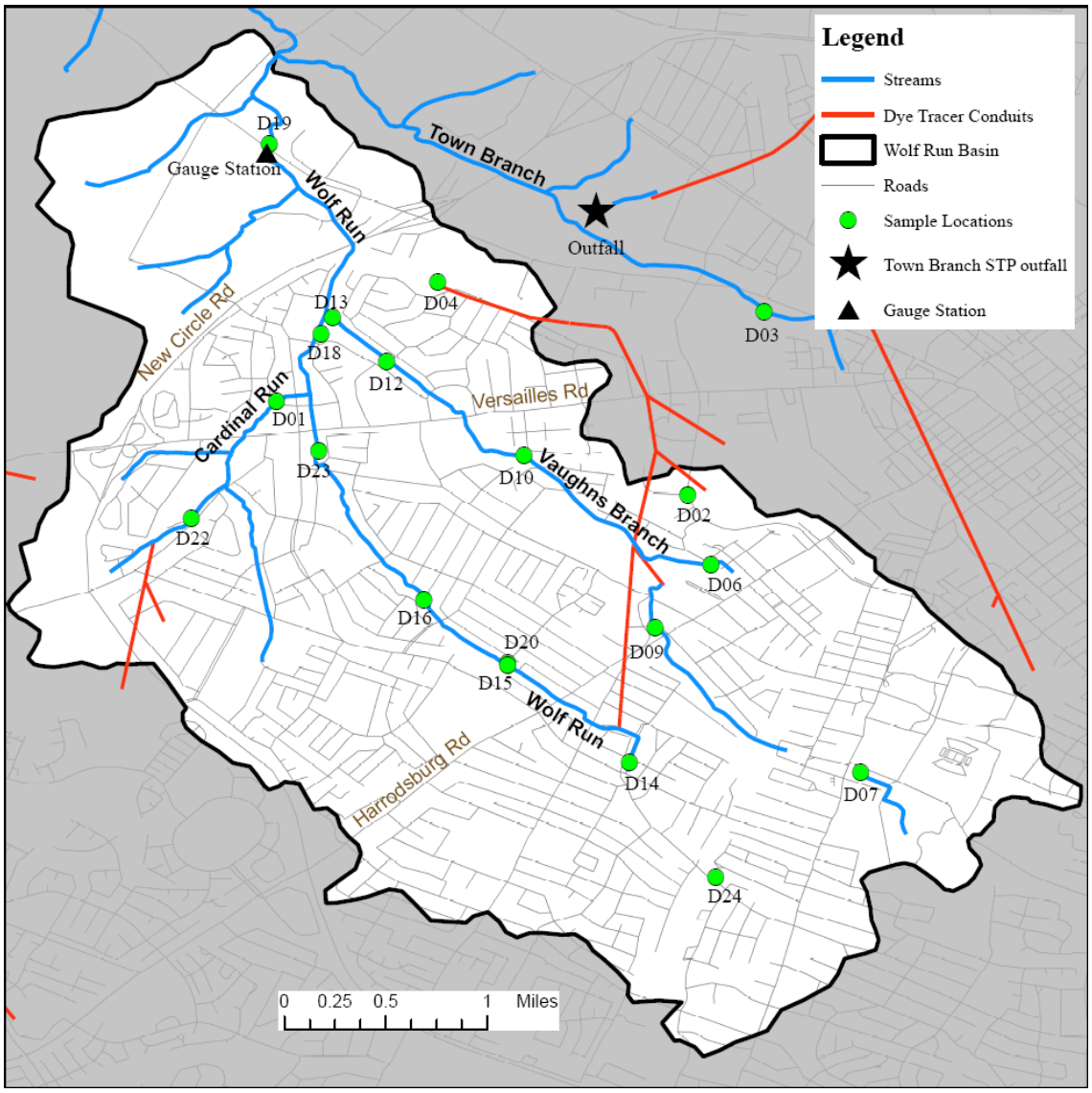


Table 2. Stream Flow and Rain Data for Sampling Days

Sampling Date	Daily Average Gauge Height, (ft.)	Daily Average Discharge, (ft3/s)	24 Hour Rainfall, (in.)	48 Hour Rainfall, (in.)
4/6/2010	0.98	2	0.00	0.00
4/13/2010	0.97	2	0.00	0.00
5/2/2010	4.57	816	3.89	4.86
6/3/2010	1.25	10	0.44	0.44
6/9/2010	1.58	36	0.39	0.39
6/14/2010	2.01	86	1.08	1.08
7/10/2010	1.48	23	1.68	1.79
7/21/2010	2.32	179	1.86	2.69
7/31/2010	1.09	5	0.02	0.03
8/5/2010	0.96	2	0.00	0.00

Grab samples collected by the volunteers from the Friends of Wolf Run citizen group on the sampling days were gathered at a central point and then transported to the Environmental Research and Training Laboratories (ERTL), which are under the direction of the PI, Dr.Gail Brion. Tricia Coakley, Microbial Laboratory Manager of ERTL, received the samples and directed the assay of the samples for *E. coli* bacteria (by IDEXX-Quantitray with Colilert media), the AC/TC ratio (by membrane filtration for Total Coliforms using m-Endo media), and filtered and archived for quantitative PCR assay (qPCR). Human-specific and non-specific DNA markers for *Bacteroides* were analyzed. The HuBac marker (developed by Layton, *et.al.* 2006) and the qHF183 (developed by Haugland, *et.al.* 2010) were measured by qPCR for the assessment of human-specific fecal contributions. The non-host specific fecal contributions were measured by qPCR for the AllBac marker (developed by Layton, *et.al.* 2006). All assays were run utilizing current standard, and evolving quality control standards.

Results from these assays were combined into a final dataset (see Appendix) and analyzed for mean values, standard variation, and relationships between the indicators. Several types and varieties of models were created to explore the ability of the multivariate approach to distinguish hotspots contaminated by human sewage in the watershed. The simplest and most plausible model created is presented herein (Model II). This model is simple to understand and can be run in an Excel spreadsheet, or by hand calculation when a “back-of-the-envelope” calculation is required by the layperson, or in the field.

Model II assigned a Sanitary Category Value (SCV) based upon the simple summation of values from 0 to 1.0 assigned for observed concentrations of indicators for fecal load (*E. coli*), fecal source (qPCR markers), and fecal age (AC/TC). Each of the three indicator classes could be assigned a value of 0 to 1.0 based on measured concentrations, with small values (<0.5) in each indicator class representative of low fecal loads, low proportion of human-specific qPCR signal, and high fecal age. High values (>0.5) represented high fecal loads, high proportion of

human specific qPCR signal, and low fecal ages. The midpoint values (0.5) for each indicator class were set with respects to threshold values of concern, so that any sample that met or exceeded midpoint values for all three input classes would have a summary SCV score of 1.5 or higher. Sewage was set as the top SCV referent with values near 3.0. The input values to the SCV assigned for the indicator class ranges for *E. coli* and AC/TC are listed in Table 3. The input value for fecal source was created by proportioning the qPCR human sourced signal in any sample with the maximum signal found in sewage after log-transformation of the data. The equation for this model is as follows:

SCV = Categorical Value *E. coli* + Categorical Value AC/TC + Calculated Value $\log_{10}$HuBac/$\log_{10}$HuBacMax

Table 3: Indicator Class Categorical Values Assigned for Input to Model II SCV

E. coli MPN/100mL	= Value	AC/TC	= Value	Log_{10}HuBac:Log_{10}HuBacMax
<235	= 0	>20	= 0	
>235, <576	= 0.17	<20, >15	= 0.25	Not Categorized Value, but
>576, <1,000	= 0.33	<15, >10	= 0.50	Directly Calculated Value
>1,000, <2,000	= 0.50	<10	= 1.00	
>2,000, < 10,000	= 0.67			
>10,000, <24,000	= 0.83			
>24,000	= 1.00			

The midpoint (0.5) categorical value for *E. coli* and the AC/TC class values were set at a breakpoint for level of concern from state and federal water quality recommendations and past research experience, respectively. For example, while it is not expected that the water quality in Wolf Run meet the EPA recommended levels for full body immersion at a designated beach area at all times (<235 *E. coli*/100mL for any single sample), the Kentucky standards for secondary recreational contact state that fecal coliforms shall not exceed 1,000 colonies/100 ml as a thirty (30) day geometric mean based on not less than five (5) samples; nor exceed 2,000 colonies/100 ml in twenty (20) percent or more of all samples taken during a thirty (30) day period. This study did not measure fecal coliforms, but the subgroup *E. coli* that is generally present in numbers less than the total number of fecal coliforms. The proportion in freshwater of *E. coli* to fecal coliforms has been reported to range from 0.5 to 0.95, with higher proportions found in influent sewage. For the purposes of our model, we assumed the proportion to be equal to 1.0; that if fecal coliforms had been measured, they would have equal to the numbers of *E. coli*. This was reflected in the categorical assignation for any single sample containing 1,000 to 2,000 *E. coli* MPN/100mL to be assigned value of 0.5 for input into the SCV model.

Setting the top value of 1.0 for the *E. coli* class was done with the common application of the analytical test method to stormwater in mind. The IDEXX-Quantitray method has a top range of about 2,400 *E. coli*/100 mL on an undiluted sample. Since stormwater measurements from combined and sanitary sewer overflows in surrounding cities have reported minimum values 10 times higher than the analytical range of the test, it is common to run a 10-fold dilution resulting in a maximum level of detection of about 24,000 *E. coli* MPN/100mL. Any sample with values found to be greater than 24,000 *E. coli* MPN/100mL was assigned the highest value of 1.0 for the fecal load category. Although individual sample analyses measured amounts greater than 24,000 *E. coli* MPN/100mL, this truncation of the database was not seen as detrimental to the SCV model prediction because these observations are already elevated to concentrations 10-times higher than the mid-point level of concern.

The AC/TC fecal age indicator categorization scheme set 15 as the breakpoint or level of concern. In prior studies, it has been seen that levels below 15 are associated with fresh fecal inputs from cattle and other warm-blooded mammals. AC/TC values above 20 are associated with aged fecal materials, such as those found in water hazards at local golf courses. Prior studies have shown that surface waters with AC/TC values below the level of 10 are associated with significant, raw sewage inputs into local creeks, and the appearance of detectable human enteric viruses in the Kentucky River, so observations where the ratio was <10 were assigned the highest level of concern (1.0). Inlet sewage had a consistent AC/TC value below 5, with averages recorded at wastewater treatment plants between 1.5 and 3.0 depending on the time of study and plant. The results of this study are consistent with previous studies and the levels of concern are set reflective of this prior knowledge.

There is insufficient data available at present to establish a level of concern for the proportion of human marker signal in environmental waters. Therefore, a unitless value was calculated for each sample by taking the $\log_{10}$ transformed value for HuBac divided by the $\log_{10}$ transformed value for the maximum amount of HuBac detected during the study (4,750,000 DNA copies/uL of extract analyzed from sewage sample on 7/31/2010). This proportion is referred to as $\log_{10}$HuBac/$\log_{10}$HuBacMax and is representative of the relative strength of human host associated signal found in a water sample with respects to that found in sewage. It is a proportional value that provides for the inspection of large differences into a small scale. This unitless value, which varied from 0.1 to 1.0, was used directly in the calculation of the SCV value by the model. The midpoint 0.5 value for this indicator represents 2,178 DNA copies HuBac/uL, or a direct proportion of about 0.05% to the strongest signal found in sewage. As the lower level of detection for this study was set at 100 DNA copies/uL, this value is 200 times higher than the established level of detection.

As an example of how Model II would assign a SCV value to an individual observation, the inlet sewage sample taken on 6-14-2010 had recorded values of 1,112,000 *E.coli* MPN/100mL, AC/TC of 2.2, and a calculated $\log_{10}$HuBac/$\log_{10}$HuBacMax of 0.97. This sewage sample observation would be assigned a SCV value of 2.97. The SCV for this sample was calculated as the sum of 1, 1, and 0.97 for the categorized input values for *E. coli* and AC/TC indicator classes, and the calculated $\log_{10}$HuBac/$\log_{10}$HuBacMax value, respectively.

The most contaminated sites sampled in this study were sewage and an overflowing manhole with SCVs of 2.98 and 2.88 respectively denoting high fecal loads, low fecal ages, and a high proportion of human-specific qPCR marker relative to the maximum human-specific qPCR value found in sewage. The model appropriately assigned high SCV values reflecting the degree of contamination with pathogen carrying raw sewage.

Standard Repeat Measures ANOVAs, with All Pairwise Multiple Comparison Procedures (Holms-Sidak method with significance =0.05) were applied to illuminate differences between the sites under dry and wet conditions, and against sewage as a reference. SCVs for sites under wet and dry conditions were mapped with GPS and were color coded to show which sites had statistically different SCVs from that of sewage versus those that did not. Sites not significantly different from sewage are placed on high priority for investigation and remediation.

Methods:

Laboratory Assays. All assays applied were agreed upon and are referenced in the signed Quality Assurance Project Plan (QAPP). The membrane filter and broth culture methods used were standardized (SM9222b (1.) for the AC/TC ratio obtained from the m-endo broth based, membrane filter analysis for total coliforms, IDEXX Quanti-Tray 2000 with Colilert media for *E. coli* (2.)). The IDEXX analysis was done per published procedural manuals from IDEXX. Basically, 100 mL samples of water, or diluted water samples, were mixed with pre-packaged amounts of media, and then distributed into a sterile multiple well Quanti-Tray and incubated at 35 degrees C for 24 hours ± 2 hours before counting the number of wells with blue florescence under UV light. The numbers of large and small positive wells are used to provide a statistical estimate of the most probable number of bacteria per 100 mL of sample to be read from a chart provided by IDEXX. The AC/TC analysis required colony counts for two types of bacterial colonies grown on m-endo fed membrane filters. Three dilutions were analyzed, plated in duplicate. Those colonies presenting as total coliform colonies (dark red with sheen) and those presenting as atypical colonies (pink to red, no sheen) were counted and an average count value established and calculated per 100mL of sample. The AC/TC value reported was produced by dividing the number of atypical colonies per 100 mL by the number of typical coliform colonies per 100 mL. The AC/TC ratio reported is unitless.

Filter extractions for qPCR analyses were done using commercially available, pre-packaged kits following guidance from USEPA and published literature. Briefly, 100 mL samples were filtered through 0.45um cellulose membranes and the resultant filters stored at -20°C until extraction. DNA extractions were done using the method described in the 2010 USEPA document, “Method B: *Bacteroidales* in Water by TaqMan(R) Quantitative Polymerase Chain Reaction (qPCR) Assay” (3.). The EPA method applied used AE buffer with a known concentration of Salmon sperm DNA added as an internal standard. The Salmon DNA was subsequently measured by real-time PCR to check for PCR inhibition.

The amplification and quantification of AllBac and HuBac genetic markers was performed according to published protocols (4.) which include a TaqMan fluorescently labeled probe, a 60°C annealing temperature and 50 PCR cycles. The qHF183 marker was analyzed according to protocols recently published by the USEPA (5.) with a couple of adaptations to

template addition and PCR cycles. IAC template was not added to samples and Salmon internal standard was analyzed to determine inhibition. The PCR was run for 50 cycles rather than 40. Threshold cycles from samples were compared with calibration curves to determine concentration of target in copies per uL and reported as DNA copies per uL of extract.

Establishing Reportable Levels of Detection (LOD). For the cultured bacterial analysis, the lower LOD was obtained by assuming 1 bacterium per volume of sample tested. For the IDEXX Quantitray, where samples were analyzed with a 10 fold dilution this value was recorded as <10 MPN/100mL. The maximum LOD for the Quantitray with a 10 fold dilution was >24,192 MPN/100mL. For the membrane filtration, an assumption of 1 bacterium per volume of sample assayed on the lowest dilution sample was assumed. For the qPCR marker analyses, the lower LOD was established to be 100 DNA copies per uL of filter extract.

Data Infilling. There were many samples assayed by qPCR where the copies of DNA/uL filter extract fell below the reportable LOD of 100 DNA copies/uL. To allow arithmetic calculations from these observations, data infilling was done for the HuBac and AllBac observations that fell below the reportable LOD of 100 copies of DNA/uL in two ways. If the HuBac signal was below the LOD, but the AllBac signal was above the LOD, then a value of 50 copies/uL (1/2 the analytical LOD) was assigned for HuBac. If both the HuBac and AllBac signals were below the LOD, then a value of 5 copies/uL was assigned to that HuBac observation. If the level of AllBac found was below the LOD, a value of 50 copies/uL was assigned for the AllBac observation

Statistical Analyses. All statistical analyses were done utilizing the statistical program embedded in Sigma Plot 11 with standard settings for significance. Data (raw and transformed) was checked for normality and equal variance for regressions and other procedures sensitive to these qualities. Where indicated, non-parametric tests were used when the data failed normality and equal variance testing.

Model II. A simple model was developed to classify the water quality at each site with respects to inlet sewage and other sites. The model utilized three input values from three indicator classes assigned equal weights and summarized. The model blended two categorization scales for fecal load and fecal age classes with a directly computed proportion of human specific qPCR marker at each site relative to sewage for fecal source. Sewage was set as the maximum value (3.0) that could be computed. Water meeting ambient quality criteria standards was set as the lowest value (0.0). The value obtained from the model for individual observations was named the Sanitary Category Value (SCV) and used to compute averages, rank, and statistical significance between sites.

Results:

Summary of Indicator Values. The Wolf Run Watershed is under significant fecal loading as indicated by direct measures of *E. coli* concentrations per 100 mL. As can be seen in Table 4 below, all sites have average geometric mean *E. coli* values (expressed as $\log_{10}$ transformed

values to allow for ease of comparison) higher than what is recommended by the EPA for either a geometric mean value for full body contact (log_{10} *E. coli* /100mL $\leq$2.10), or a single sample of ambient water where people are expected to have infrequent full body contact (log_{10} *E. coli* per 100mL $\leq$ 2.76) (6.). Both recommendations are set to limit swimming-associated gastroenteritis rates to an acceptable 8/1000. The geometric mean for all sites over the entire period of study is greater than 1,000 *E. coli* /100mL (log_{10} *E. coli* /100mL=3.0). It is evident that except for a few places in Wolf Run Watershed (D02, D04), under dry conditions, that indicator bacteria levels are high and following EPA recommendations, contact with creek water should be limited to prevent excess disease in the community.

As is expected, inlet sewage *E. coli* values are significantly different from all other sample sites under summary, dry, and wet conditions by several orders of magnitude. However, due to the large confidence intervals associated with the statistically computed averages obtained from the MPN assay method, and the variance found in concentrations on different days at the same sites, it is difficult to find statistically significant differences when comparing between the sample sites within the watershed, even under similar conditions. As an example, D10 appears to be a heavily contaminated site during dry weather sampling events with an average of 1,863 *E. coli* MPN/100mL. However, this average *E. coli* value at D10 is only marginally different from the average found at D02 (50 *E. coli* MPN/100mL) in the same dry weather period when applying common statistical techniques (Holm-Sidak, p=.002). The inherent variability surrounding the assay of bacterial indicators, the variability found between different days, and the random distribution of bacteria in the environment, make statistically significant differences difficult to demonstrate. That is why it is important not to rely upon levels of *E. coli* alone when trying to define differences between areas within a watershed. There is simply too much variability in the distribution and measurement of this load indicator for it to be applied in a snapshot approach with limited sampling.

There is even more variability in *E. coli* values when looking at sites under differing weather conditions. As indicated by the large changes in average concentrations between dry and wet conditions, the fecal load in this watershed undergoes significant fluxes at many of the sites. As an example of this, Site D02, located north of Vaughn's Branch, averaged only 50 *E. coli* per 100 mL when dry weather predominated, but after rain events, jumps to a significantly different average of 15,849 *E. coli* per 100 mL ($t=5.7$, $P<0.001$). It is important to consider the conditions that impact the measurement

Inspection of the value in Table 4 shows the extreme variability in all of the indicators measured at all sites, with the exception of inlet sewage. Sewage is a very consistent signal. The log transformed *E. coli* concentrations in sewage had a standard deviation of only 0.21 around a mean value of 6.401. The average sewage AC/TC values were 2.7 with an accompanying standard deviation of 1.4. Inlet sewage qPCR HuBac values averaged 3,601,428 $\pm$ 1,151,888 DNA copies/uL. To create a more scaled signal for the HuBac marker, it was decided to log transform each qPCR value and divide it by the maximum log transformed HuBac concentration seen in sewage. The resulting average value (log_{10}HuBac/ log_{10}HuBacMax) in sewage is quite high (0.979) with minimal standard deviation 0.024. Sewage was quite consistent and provided a referent against which to compare the water quality at other sampling sites.

Table 4: Summary Results for All Sites, All Weather Conditions

Site ID#	Log_{10} *E. coli* MPN/100mL (Ave. Dry-Ave. Wet)[1]	AC:TC (Ave. Dry-Ave. Wet)	HuBac Copies/1uL (Ave. Dry-Ave. Wet)[2]	AllBac Copies/1uL (Ave. Dry-Ave. Wet)[2]	Log_{10}HuBac/ Log_{10}HuBacMax (Ave. Dry-Ave. Wet)[2]
D 01	3.369 (2.43-3.99)	27.5 (13-37)	2,233 (1,022-3,041)	13,983 (3,525-20,955)	0.394 (0.372-0.408)
D 02	3.201 (1.70-4.20)	19.3 (15-22)	278 (63-421)	7,325 (5,992-8,213)	0.281 (0.235-0.311)
D 03	3.263 (2.15-3.96)	11.7 (12-12)	375 (28-607)	6,451 (1,540-9,725)	0.284 (0.180-0.353)
D 04	3.225 (2.02-4.03)	28.3 (34-25)	229 (28-363)	2,872 (223-4,638)	0.264 (0.180-0.321)
D 06	3.005 (2.28-3.49)	20.3 (23-18)	462 (260-596)	3,671 (4,270-3,273)	0.344 (0.355-0.366)
D 07	3.309 (2.92-3.57)	35.0 (28-39)	269 (146-351)	4,636 (2,751-5,892)	0.318 (0.271-0.349)
D 09	3.802 (3.27-4.16)	14.9 (15-14)	3,497 (231-5,675)	26,802 (23,888-28,746)	0.418 (0.351-0.463
D 10	3.785 (3.56-3.93)	16.1 (5-24)	23,627 (14,948-29,414)	157,393 (170,700-148,521)	0.567 (0.610-0.539)
D 12	3.740 (2.93-4.28)	16.6 (12-20)	1,356 (775-1,744)	14,681 (19,390-11,542)	0.417 (0.401-0.427)
D 13	3.730 (3.73-4.25)	13.9 (16-13)	15,743 (166-26,128)	84,100 (1,442-139,206)	0.382 (0.292-0.442)
D 14	3.541 (2.66-4.13)	16.5 (22-12)	5,119 (69-8,487)	21,187 (3,414-33,036)	0.383 (0.269-0.459)
D 15	3.703 (3.01-4.16)	15.4 (17-14)	2,451 (78-4,034)	9,568 (1,428-14,995)	0.347 (0.273-0.397)
D 16	3.775 (3.36-4.05)	21.1 (9-29)	4,999 (130-8,244)	20,078 (2,514-31,788)	0.373 (0.373-0.429)
D 18	3.354 (2.25-4.09)	78.2 (175-13)	2,799 (114-4,590)	12,978 (1,315-20,753)	0.379 (0.284-0.442)
D 19	3.485 (2.25-4.30)	13.8 (11-15)	2,641 (77-4,351)	104,161 (1,170-172,822)	0.377 (0.377-0.447)
D 20	3.202 (2.60-3.68)	24.1 (12-34)	105 (39-158)	1,017 (596-1,354)	0.265 (0.217-0.303)
D 22	3.631 (2.76-4.21)	18.0 (14-20)	286 (97-413)	6,684 (1,017-10,463)	0.348 (0.289-0.388)
D 23	3.618 (2.75-4.20)	18.9 (22-17)	5,015 (91-8,298)	20,471 (2,070-32,738)	0.395 (0.286-0.468)
D 24	3.603 (0.77)	32.3 (48-22)	1,788 (112-1,788)	13,626 (4,559-19,670)	0.364 (0.301-0.407)
Sewage	6.401	2.7	3,601,428	22,098,571	0.979
Manhole Overflow	4.384	0.8	796,000	3,000,000	0.884

[1] = Geometric mean, [2] = Database in-filled before calculation

Interestingly enough, some sites have very little difference in average *E. coli* values, as is indicated by inspection of D10 averages under wet versus dry conditions. Comparison by Mann-Whitney Rank Sum test on D10 under dry conditions versus wet did not show significant difference in average *E. coli* values, and D10 had on average the highest number of *E. coli*, indicating that this site is under constant and significant contamination, presumably from human sewage. However, the high levels of *E. coli* found at most sites within the watershed varied widely over time and with rain events, which makes statistical distinction difficult. Examining the summary *E. coli* values for D10 with Repeat Measures ANOVAs, it is found that these average *E. coli* values are significantly different from only two sites, D02 and D04, under dry conditions. Under wet conditions, statistical distinctions between *E. coli* values at D10 and other sites within Wolf Run is not possible. Only inlet sewage is significantly different in fecal load from D10 and all other sites.

E. coli values alone cannot determine the potential risk inherent in urban watersheds as there are many sources of fecal materials with differing degrees of hazard. Further complicating single, load indicator analysis is the fact that *E. coli* can come from many different places in the environment with widely varying potential for disease. *E. coli* is even known to grow to high numbers in nutrient enriched waters during warm weather, or be resuspended from sediments into the water column during storm events. The link between *E. coli* and water related disease is complicated. More information is needed to classify the relative cleanliness of these sites in the watershed. The predominate fecal age and fecal sources need to be considered along with fecal load information in order to obtain a more complete understanding of the potential risk indicated by *E. coli* values. Hence our use of another indicator class, one for fecal age.

The AC/TC value changes with the average age of fecal contamination, rising as fecal material ages in the environment from initial values less than 1 in freshly deposited fecal materials to values in the hundreds in water held in golf ponds. When applying the AC/TC age indicator, one looks for two criteria: Sites that have average values below expected values found in Kentucky watersheds, and those that drop significantly in average values after a rain. Low fecal age in inlet sewage samples was consistently measured in this study, and prior studies, by an AC/TC value below 5 for any individual sample and an average of 2.7. Under wet weather, the AC/TC ratio in sewage rose slightly (average = 3), but not significantly, as aged fecal material was swept into the treatment plant from the urban environment. This is consistent with previous studies that have seen a slight increase in the AC/TC value in sewage under rainfall events. Under dry conditions, the individual values observed were consistently below 3.

The AC/TC value modifies and supports the information obtained on fecal loads as measured *E. coli* concentrations. Prior study has shown the AC/TC value does not change appreciably in the time it takes wastewater to be treated in an activated sludge system. These studies at the Town Branch Sewage Treatment Plant have shown the AC/TC value in plant effluent before chlorination to be indistinguishable from inlet values. Therefore, creeks that receive a significant portion of their flow from sewage treatment plant effluents will present a low AC/TC value, while the *E. coli* levels can be quite low. In contrast, creeks that receive significant influxes of untreated sewage will have low AC/TC values, but high *E. coli* concentrations.

These relationships between fecal load and age allow one to begin to sort the most risky conditions in a watershed from those with less potential disease risk.

In prior studies on flowing creeks and rivers in the Bluegrass Region, when the AC/TC value fell below 15, it was indicative of sites that were impacted by human sewage, or fresh and copious quantities of agricultural fecal materials (cows in the creek). Sites in the urban Wolf Run Watershed with average AC/TC ratios below 15 for any of the three conditions (summary, wet, or dry) were of interest and required further inspection to confirm the presence of sewage (D03, D09, D10, D12, D13, D14, D15, D16, D18, D19, D20, and D22). D10 had the lowest recorded AC/TC ratio under dry conditions (5), and high *E. coli* values, which was indicative of fresh sewage impacting the water quality at this site, presumably from leaking sewers. Indeed, a leaking sewer was confirmed to be impacting this site in July, but it could not be determined how long it might have been leaking. Our data suggest it was leaking the entire time of study. The rise in AC/TC values at D10 under rainfall is expected as aged fecal materials are added into the stream from overland scour, as is the rise under late summer conditions where flow is stilled to pools. As mentioned prior, even sewage has a slight rise in AC/TC values during rain events. Site D09 hovered near an AC/TC value of 15 under wet and dry conditions, suggestive of another site under continuous influence of sewage, but at lesser volumes than D10. D12 has lowered AC/TC values, especially during dry periods, and this may be partly due to its position downstream of D09 and D10.

However, because of the wide variability around the AC/TC indicator, it is difficult to show statistical significance when comparing sites against each other. Only the much higher average value for AC/TC at D18 under dry conditions was found to be significantly different from the average AC/TC value found in sewage (Holmes-Sidak test $P<0.001$). The average AC/TC ratio at site D18 under dry conditions was also found to be significantly different from all sites except for site D24 under wet conditions (Holmes-Sidak test $P<0.001$).

D03 (McConnell Springs), while not in the channel flow of Wolf Run, is of particular interest to the local community. Because the AC/TC value is often low (average=11.7 $\pm$ 5.4) and does not change much with varying weather conditions, and the *E. coli* concentrations are high, it is indicative of fresh fecal impact to this site's water quality. However, this indication may be due to D03 being a karst upwelling spring where the underground flow is kept cold, which retards fecal aging. Under cold, dark conditions indigenous bacterial growth (AC) is repressed while introduced coliforms (TC) survival is enhanced. Under these conditions, the distance and time between the original fecal input and the sampling site can be large with little difference in the AC/TC or *E. coli* values. The spring is contaminated by fecal material, but it is not clear that this contamination is local. As is true with the interpretation of fecal load levels, the fecal age indicator should not be used alone, nor applied without knowledge of the factors that can impact it as this spring site has demonstrated.

The next indicator class to investigate and add into the multivariate modeling approach is for fecal source. To indicate human host-specific fecal sources, the proportion of human-specific genetic material from select portions of the 16S rRNA region of the genome for the strictly anaerobic group of bacteria known as *Bacteroides* were utilized. There were three markers selected for use: 1) the non-host specific marker AllBac, 2) the mostly human specific marker

HuBac, and 3) the more human specific qHF183 marker. While the community at large is united at this time in the use of the AllBac marker to indicate overall load of *Bacteroides*, there are differences of opinion on which human specific marker to use. There is a trade-off between sensitivity and specificity, and which marker to use is dependent upon which questions you intend to answer. In the Wolf Run watershed, previous studies have used the HuBac marker, which had been found to come from human sources in large quantities, and from several animal sources in smaller quantities. For the purpose of our study, to find significant sources of human sewage, and to maintain continuity with past studies, we included the less specific, but more sensitive qPCR marker HuBac to look for human contamination.

This decision was fortuitous as HuBac was detected more frequently above the LOD, and at greater concentrations, than the qHF183 human specific marker at all sites. Inlet sewage had significantly less qHF183 marker signal than HuBac on average (5.11×10^5 versus 3.6×10^6 DNA copies/uL respectively). While this increased signal could be due to HuBac detecting non-human sources in addition to human, a log-log linear relationship between the concentrations of the two markers was found, suggesting that either could be used to indicate human sewage presence above the LOD. Since the approach was to relate the proportion of human signal at each site relative to the human signal in sewage, the frequency of non-detects for qHF183 became problematic. Values for HuBac were below the LOD 28.5% of the time whereas values for qHF183 were below the LOD 74.5% of the time. However, when qHF183 marker was detected, it was meaningful. In support of the suspicions raised about leaking sewers impacting site D10 above, qHF183 marker was consistently detected above the LOD in all dry weather samples. At D10, the qHF183 signal was diluted to extinction 33% of the time under wet conditions, and on two of the more moderate rainfall events when the sample should have been more concentrated than rainfall events with greater potential dilution. All markers were detected in significantly lower concentrations in mixed liquor than inlet sewage, denoting loss of signal through treatment.

Prior studies in the Wolf Run watershed have looked at the proportion of human specific to non-host specific qPCR marker concentrations for *Bacteroides* to pinpoint hot-spots. While this was a defensible approach at the time, with only one sample per site available for analysis and no data on inlet sewage concentrations, as more data was available for study it became apparent that another approach was needed to more accurately reflect sewage intrusions. Two factors motivated this change in approach: 1) that the proportion of human specific to non-host specific markers for *Bacteroides* in sewage was low, and 2) the discovery of a directly proportional relationship between the log transformed values for human specific and non-host specific markers.

In the inlet sewage samples from this study, the average proportion of log transformed concentrations of HuBac to AllBac markers was 0.175, a much lower value than expected from reported proportions found in human fecal samples. The average proportion of transformed values for qHF183 to AllBac markers in sewage was even less (0.026). As well, the human specific marker concentration was not independent from the concentration of the non-host specific marker for *Bacteroides*. A linear relationship was found between the $\log_{10}$ transformed values of HuBac and qHF183 ($\log_{10}\text{HuBac} = 1.0239(\log_{10}\text{qHF183}) + 0.6117$, $R^2 = 0.9061$), and

between similarly transformed marker concentration values of HuBac and AllBac (log_{10} HuBac= 0.974(log_{10}AllBac) - 0.943, R^2 = 0.748).

These inter-correlations may well be reflective of the fact that the Wolf Run watershed is significantly impacted by human sewage, which had a fairly stable ratio during the time of this study. Since the proportion of human to total signal were log-log linearly related, it was decided to relate the log_{10} transformed concentration of human specific marker to the maximum log_{10} transformed concentration of human specific marker found in inlet sewage (log_{10}HuBac/log_{10}HuBacMax) avoiding colinearity in the model inputs. The fecal source indicator log_{10}HuBac/log_{10}HuBacMax was calculated for all observations and average values presented in Table 4 for all, dry, and wet conditions.

Wolf Run is under the influence of human fecal materials. No site in the watershed consistently lacked the presence of either human specific marker. D01 and D22, sites along Cardinal Run, did not show the presence of the more specific, but found to be less abundant, qHF183 marker during the entire time of study, but both sites on this creek had detectable amounts of the HuBac marker, especially during rainfall events. Site D20, a small tributary feeding into Wolf Run near site D15, also did not have qHF183 detected, but had quantifiable amounts of HuBac detected. Human contamination cannot be ruled out at these sites based on the absence of qHF183 due to its lower abundance in sewage and the low levels of HuBac marker found. It may well be that the qHF183 marker is present, but with the 100 mL volume of sample filtered for extraction, not detectable.

The presence of human fecal materials is verified at other sites by the presence, and abundance of both human markers. As found prior, the Vaughn's Branch sample sites (D09, D10, and D12) had the highest average proportion of HuBac signal relative to the maximum found in inlet sewage, supportive of the presence of leaking sewers. One Way Repeat Measures ANOVA utilizing an All Pairwise Multiple Comparison Holm-Sidak method found the average sewage proportioned values to be different from all averaged sample site values under all conditions, but not different from the proportion found in the sample from the overflowing manhole. This is valuable information as it demonstrates that storm diluted sewage has a different signal from storm overflow in the creeks. The average proportional value calculated for all conditions for site D10 was different from all other sites except for D09, D12, and the manhole overflow. This higher proportion links these sites numerically closer to human sewage. The proportion value changed when comparing dry events to wet event averages, with the exception of site D10 whose proportion decreased slightly, presumably due to other fecal sources being swept in by storm scour. The overall increase in the proportion of human signal at the Wolf Run sites is indicative of the addition of more human fecal materials under times of rain, presumably from sewage overflows.

Sanitary Category Value Modeling Results: While the previous analysis of the data is informative, the results of the modeling are the most significant findings with respects to the original objectives and purpose of this project. One of the objectives of this project was to create a scheme that can rank sites within the watershed relative to each other for the purpose of identifying priority areas for remediation, and for verifying the impact of remediation undertaken in the future. The rankings and values will provide a baseline against which future

studies can compare and a way to prioritize regions within the watershed for further investigations. This scoring and ranking was done as described prior by calculating a summary Sanitary Category Value (SCV) between 0 and 3 from each indicator class and input values between 0 to 1.0 for fecal load, source, and age. At this time, each indicator class is given equal weight. Table 5 shows the calculated average SCVs for each site under all, dry, and wet weather conditions. While there are many combinations that could result in an elevated SCV, only average SCVs less than 2.0 were found to be significantly different from sewage SCV under all weather conditions. The overflowing manhole sampled under the rain event of 5/2/2010 was not significantly different from sewage with an SCV value of 2.88. The average SCV at D10 is close to being indistinguishable from sewage ($t= 3.864$, $P<0.001$). The average SCVs for D10 are significantly different from those at D04, D07, and D20 indicating a greater degree of contamination at this site than at these others.

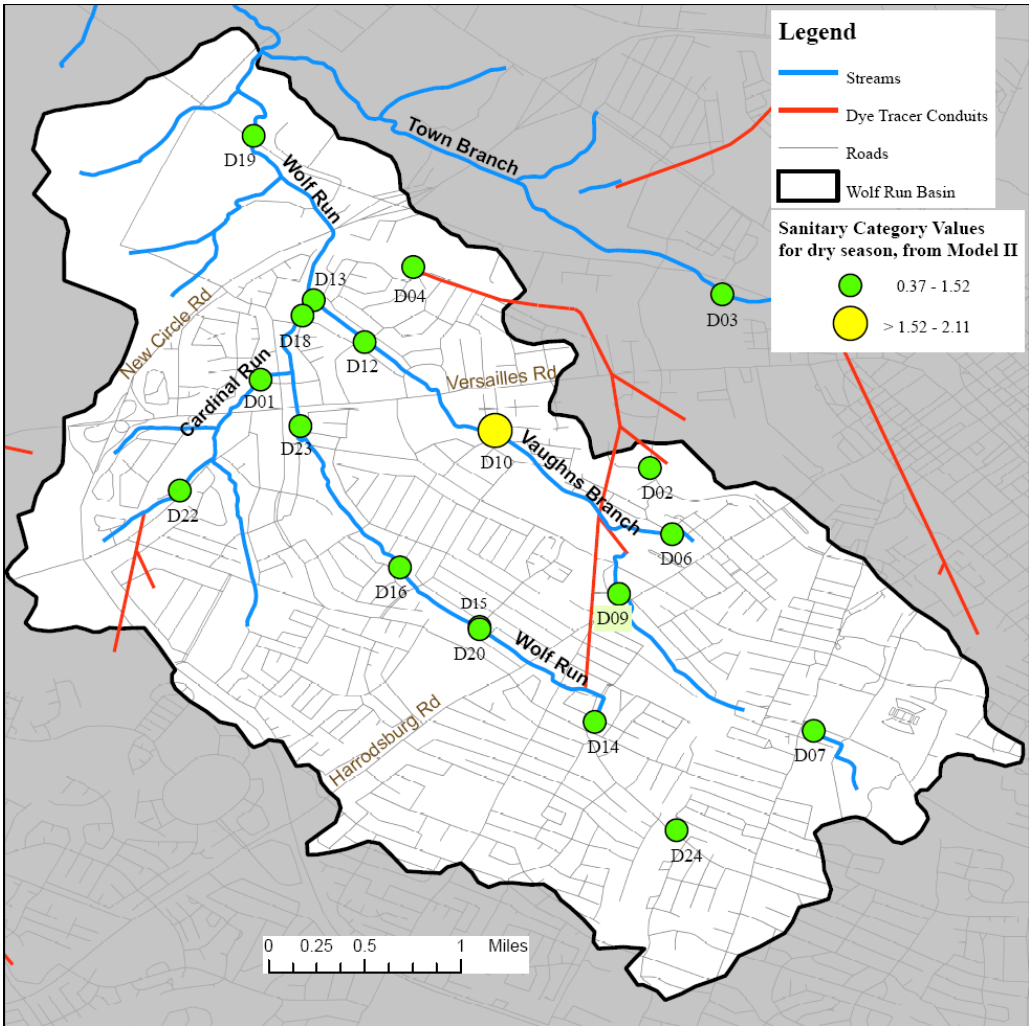
As it happened, the suspicions of leaking sewage, indicated by low AC/TC values and High E. coli values at site D10, were confirmed by a sanitary survey performed by the Friends of Wolf Run on 7/12/2010, two days after a rainfall associated sampling event for this study. Photos of a broken sewer collection pipe that connected to several facilities, one of which was an assisted living center, were sent to LFUCG. Conductivity readings at the site on the day of discovery were high and there was a chemical smell to the water. This chemical smell had been noted two days prior when processing the samples from the latest sampling event of 7/10/2010. Bacterial results for 7/10/2010 sampling event had suppressed growth signals impacting the ability to obtain measures for fecal load and fecal age. The lack of data for these two model inputs prevented preventing calculation of an SCV for D10 for 7-10-2010, but the levels of human specific markers on this sampling day were detectable (HuBac= 1.60×10^5, qHF183= 2.56×10^4 copies DNA/uL extract) and well above the 100 copies/uL extract level of detection. The proportion of human signal to the maximum sewage ($\log_{10}$HuBac/$\log_{10}$HuBacMax) value was 0.78, the highest value recorded for that site during the entire time of study. The leak was not resolved until after the time of this study, and both SCVs and proportion of human marker remained high. This event points out a pitfall of utilizing growth dependent indicator analyses; the results from these assays can be misleading. A multi-indicator approach, with non-growth dependent assays, provides multiple barriers to failure. That is why we continue to stress the importance of developing and proving multiple indicator systems, rather than seeking the “silver bullet” for monitoring water quality.

Table 5: Model II Calculated SCV

Site ID#	Average SCV All Days (SD)	Average SCV Wet Days (SD)	Average SCV Dry Days (SD)	Difference in SCVs Wet minus Dry
D 01	1.31 (0.62)	1.56 (0.59)	0.94 (0.50)	0.62
D 02	1.28 (0.60)	1.48 (0.62)	0.99 (0.50)	0.49
D 03	1.44 (0.58)	1.80 (0.37)	0.89 (0.36)	0.91
D 04	0.94 (0.54)	1.25 (0.35)	0.47 (0.44)	0.78
D 06	1.30 (0.57)	1.48 (0.55)	1.02 (0.53)	0.46
D 07	1.05 (0.50)	1.23 (0.44)	0.79 (0.53)	0.44
D 09	1.66 (0.57)	1.76 (0.52)	1.52 (0.69)	0.24
D 10	1.99 (0.55)	1.89 (0.41)	2.11 (0.73)	-0.22
D 12	1.52 (.050)	1.65 (0.58)	1.34 (0.34)	0.31
D 13	1.54 (0.82)	1.83 (0.67)	1.10 (0.92)	0.73
D 14	1.55 (0.67)	1.90 (0.62)	1.02 (0.31)	0.88
D 15	1.56 (0.58)	1.72 (0.58)	1.32 (0.58)	0.40
D 16	1.69 (0.66)	1.85 (0.66)	1.45 (0.67)	0.40
D 18	1.21 (0.86)	1.78 (0.62)	0.37 (0.08)	1.41
D 19	1.48 (0.61)	1.82 (0.45)	0.96 (0.42)	0.86
D 20	1.02 (0.36)	1.12 (0.36)	0.89 (0.37)	0.23
D 22	1.37 (0.69)	1.58 (0.65)	1.04 (0.69)	0.54
D 23	1.59 (0.71)	1.91 (0.62)	1.10 (0.57)	0.81
D 24	1.36 (0.76)	1.68 (0.54)	0.88 (0.84)	0.80
Sewage	2.98 (0.02)			
Overflow		2.88		

The summary of the study's significant findings can be easily visualized in the following figures. Figure 2 shows that under dry weather conditions, the average SCV is significantly different from that of sewage for all sites in the Wolf Run Watershed except site D10. Initial feedback on depressed AC/TC values and elevated *E. coli* loadings to LFUCG and the Friends of Wolf Run had pointed to D10 as a potential hotspot of leaking sewage. This was confirmed by independent sanitary survey conducted by Friends of Wolf Run on 7/12/2010 that a sewer was leaking significant amounts of fresh human sewage into the watershed near D10, negatively impacting water quality conditions. The SCV model had indicated a condition that was confirmed. Clearly, D10 was a priority site for remediation, and LFUCG has reportedly completed repairs to the leaking sewer at the time of this report. So, the SCV, especially when applied under dry conditions, can be used to pinpoint hot-spots of human sewage leaking into the environment.

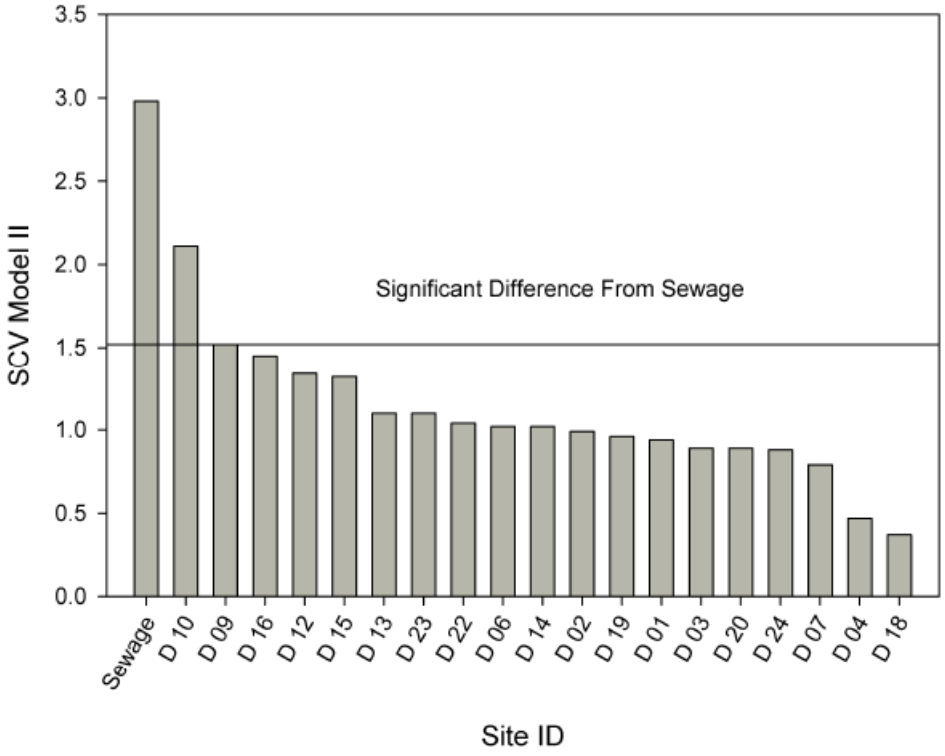
Figure 2: Average Sanitary Category Values in Wolf Run under Dry Weather Conditions



The second objective was to create a way to rank the sites relative to each other. Figure 3 is a graph of the average dry weather SCVs for each individual site. A clear ranking is visible in

Figure 3 with D10 being the most contaminated site in the watershed during dry conditions, followed by sites D09, D16, and D12. D09 is upstream of D10 and is thought to be impacted by more leaking sewers on Vaughn's Branch. D12 is downstream of both D09 and D10 and may be reflecting the contamination from the upstream sites with minimal dilution. D16 however, is on another creek within Wolf Run and needs to be investigated further for potential sewage intrusion. The rankings under dry weather conditions clearly show that sewers near D16 warrant additional inspection.

Figure 3: Average Sanitary Category Value (SCV)
For Sample Sites in Wolf Run Watershed
Under Dry Conditions



Information on rain-linked sewage overflow events is obtained looking at a similar map for average SCVs under rainy conditions. As can be seen in Figure 4, during rainy weather conditions, Wolf Run became severely impacted. A number of sites previously found to be significantly different from sewage under dry weather, are no longer significantly different under wet weather conditions. With the exception of Cardinal Run, major branches of the Wolf Run watershed had average SCVs that were indistinguishable from that of sewage. Clearly, wet weather influxes of both aged and fresh fecal material, much of which is human in origin, negatively impacted water quality.

Figure 4: Average Sanitary Category Values in Wolf Run under Wet Weather Conditions

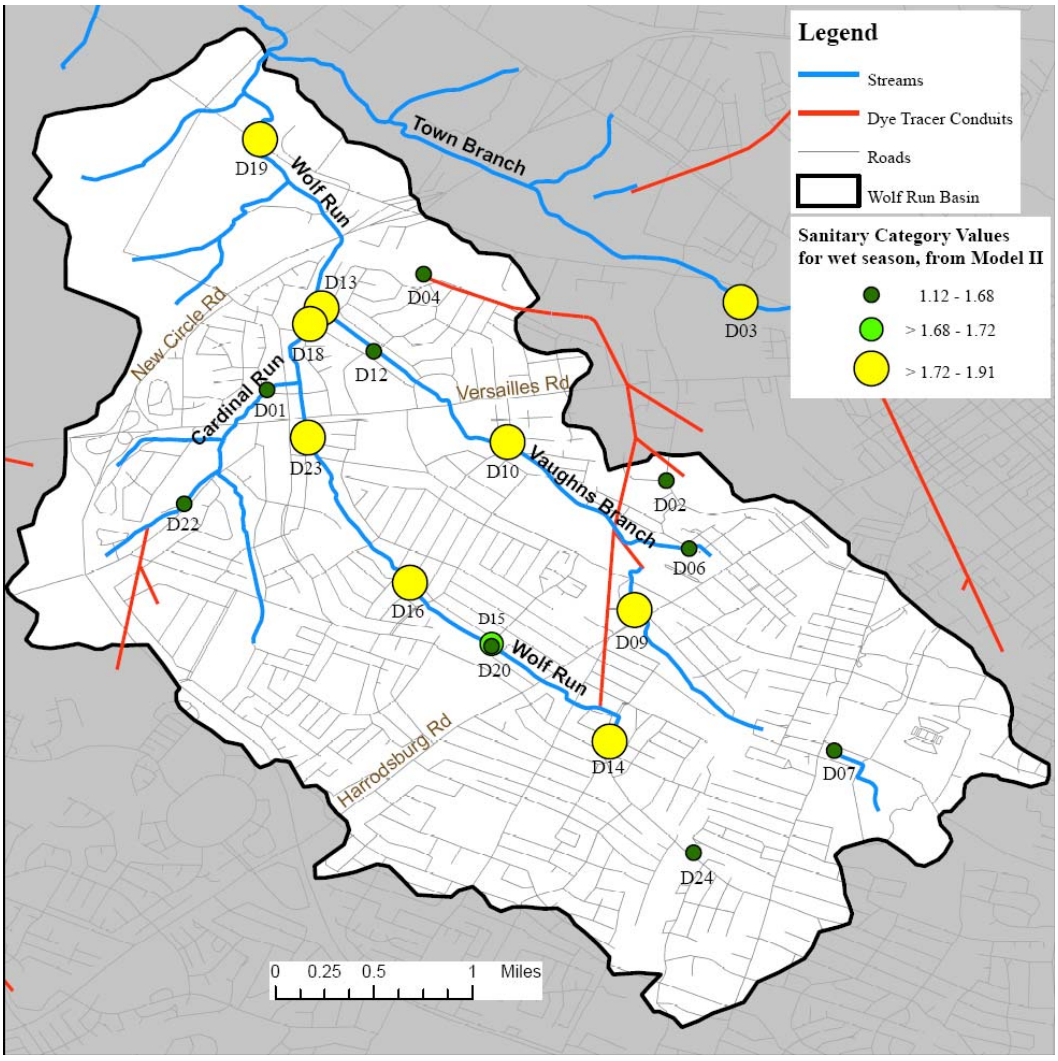


Figure 5 shows the relative ranking of the sites with respects to sewage and each other by average SCV. Clearly, fewer sites were significantly different from sewage as is indicated by falling above the reference line in the graph. Site D18, once the cleanest site in the watershed under dry conditions, has moved in rank to the 8th most contaminated site when collecting flow from the more contaminated upper branches. Further investigation of wet weather sources of

sewage needs to be undertaken, and effective remediation planned and completed. A resampling after the remediation efforts are completed is recommended to see if there have been demonstrable changes in the SCVs. It is also recommended that signs be posted advising people to limit water contact during wet weather events, or in areas known to have leaking sewers along Vaughn's Branch, until the contamination can be controlled to acceptable levels.

Figure 5: Average Sanitary Condition Value (SCV) for Sample Sites in Wolf Run Watershed Under Wet Conditions

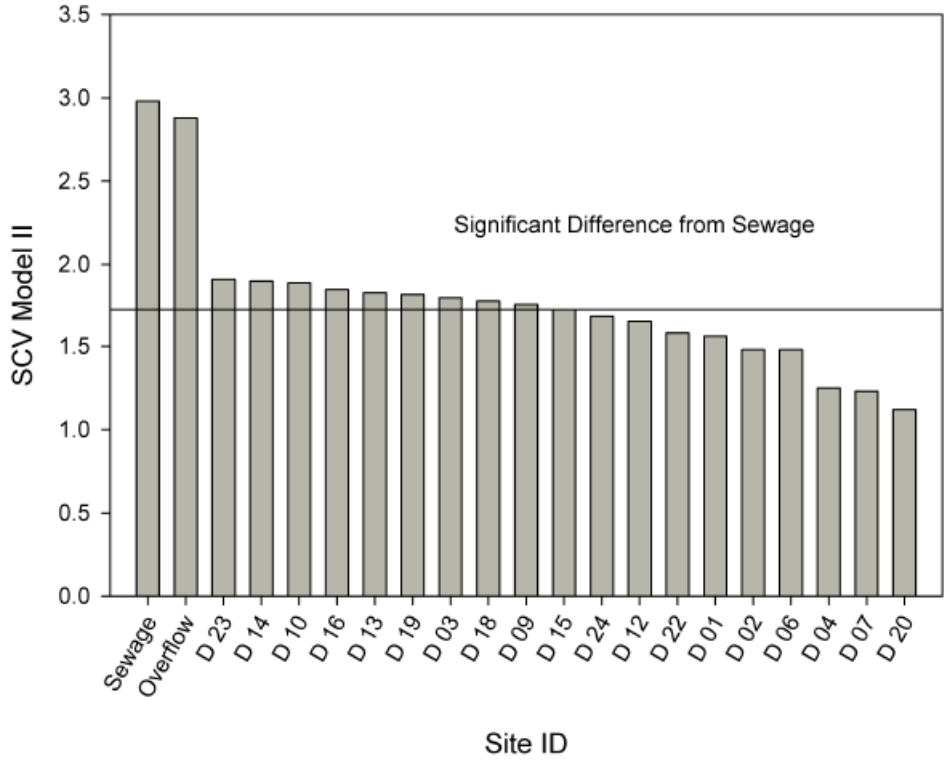
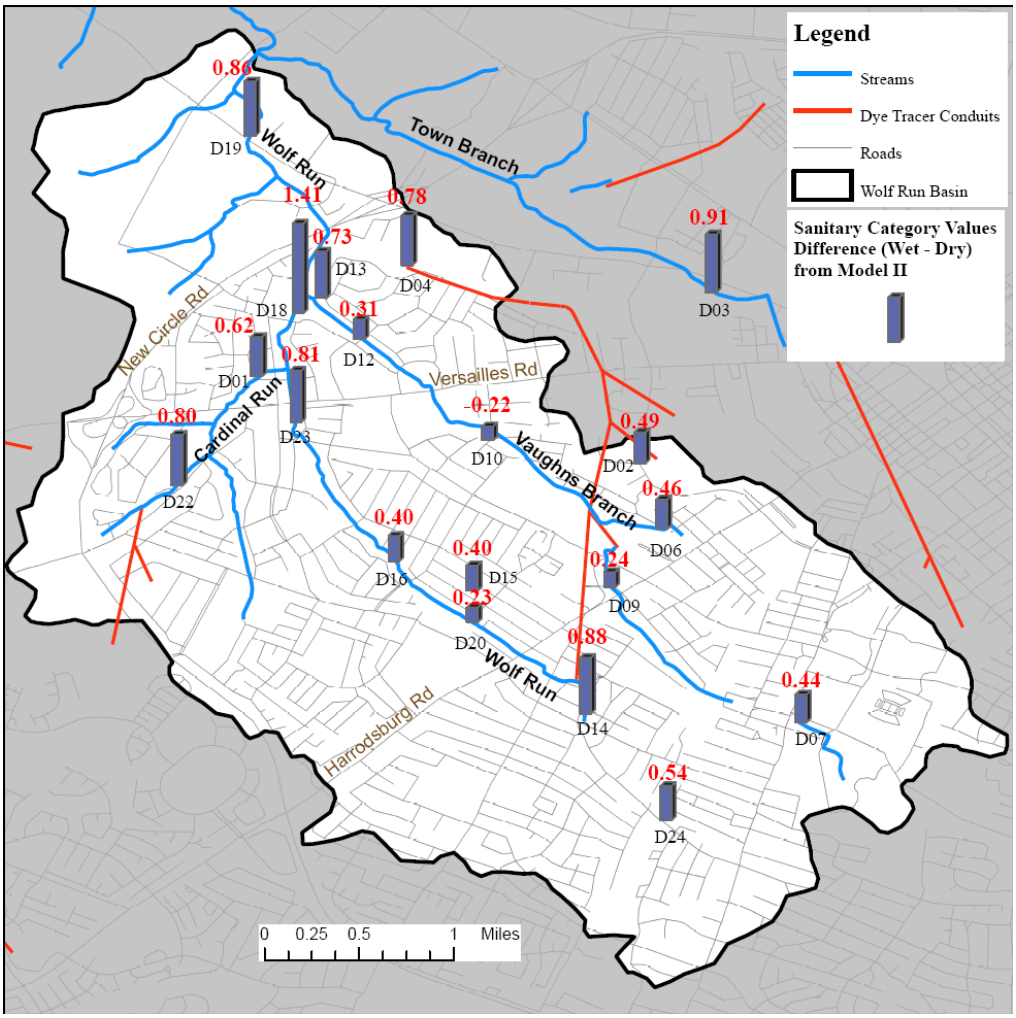


Figure 6 shows the degree of change in average SCV under dry versus wet conditions. This information is useful in several ways. First, noting sites with the greatest difference between wet and dry condition highlights the total impact of weather related sewer overflows on the watershed. The increase in SCV at D18 shows the total impact of wet weather overflow events primarily along the upper reaches of Wolf Run and Cardinal Run. Secondly, the lack of change in SCV along Vaughn's Branch indicates the consistent presence of sewage leakage that masked and overpowered the impact of rain-swept inputs of fecal materials. At site D10, the dilution from rainfall actually improved the SCV as it provided greater flow and diluted the consistent input from a leaking sewer pipe. This negative change is a key signature that differentiates consistent sewer leaks from periodic storm-linked inputs. It is important to sample under both rainy and dry conditions, to illuminate different types of problems and rank the hazards for remediation. Clearly, it must be of the highest priority to fix all leaking sewers and consistent cross connections. This must happen before replacing inadequately sized sewers, even if they are the ones that are broken, to protect the populace from exposure to contaminants they cannot see, or be advised about, and avoid.

Figure 6: Impact of Weather Conditions on Average Sanitary Category Values in Wolf Run



Summary Conclusions:

It is quite evident from this study that Wolf Run is under the influence of leaking and overflowing sewers. The multi-indicator approach used in this study has refined information gathered on fecal load with information about fecal age and source. This combined approach, with sampling under different weather conditions, allowed for the separation of different types of sewage introducing events that significantly impacted the water quality. The creation of a system to rank the sites relative to sewage, and to each other, with a Sanitary Category Value (SCV) is key to future planning decisions on appropriate remediation to obtain the best improvement for the investment. The approach has pinpointed areas in the watershed where camera inspection of the lines is required and reduced priority for camera inspection in areas with little leaking sewage impact. The categorized Model II created for analysis of the data produced by this study has demonstrated sensitivity in sorting sites from each other, and from sewage. The average SCVs documented in this report have established a baseline against which to measure improvements in future watershed quality. It is highly recommended that this approach be applied to the watershed after remediation has occurred, as well as to other urban watersheds.

Literature Cited:

(1.) Standard Methods for the Examination of Water and Wastewater, *American Public Health Association*. Method 9222B. 20th, 19th, and 18th Editions. 1998, 1995, 1992.

(2.) IDEXX Laboratories, Inc. Westbrook, Maine 04092 USA. www.idexx.com.

(3.) U.S. Environmental Protection Agency, 2010. Method B: Bacteroidales in Water by TaqMan(R) Quantitative Polymerase Chain Reaction (qPCR) Assay. *Office of Water*. EPA-822-R-10-003. www.epa.gov/waterscience/methods/method/biological/

(4.) Layton, A., L. McKay, D. Williams, V. Garrett, R. Gentry, and G. Sayler. 2006. Development of Bacteroides 16S rRNA Gene TaqMan-Based Real-Time PCR Assays for Estimation of Total, Human, and Bovine Fecal Pollution in Water. *Applied & Environmental Microbiology* 72:11p.

(5.) Haugland,R.A.;Varma,M.;Kelty,C.A.; Sivaganesan,M.; Shanks, O. C. 2010. Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selectedBacteroidales species and human fecal waste by real-time PCR. *Syst. Appl. Microbiol* 33:6 p348-357.

(6.) U.S. Environmental Protection Agency, 1986. Ambient Water Quality Criteria for Bacteria – 1986. *Office of Water* .EPA440/5-84-002.

Appendices:

Summary Results for Analytical Measurements

Quality Assurance Program Plan

Presentation Notes from 2010 KWRRI Symposium, Lexington, KY

E.COLI										
MPN/100mL	4/6/2010	4/13/2010	5/2/2010	6/3/2010	6/9/2010	6/14/2010	7/10/2010	7/21/2010	7/31/2010	8/5/2010
D 01	119	253	14136	3076	17329	19863	3310	18600	364	487
D 02	10	142	1374	4611	24192	24192	241920	17890	110	41
D 03	20	472	5172	24192	269	24192	41060	17440	134	313
D 04	20	187	5475	24192	1553	11199	54750	11450	298	110
D 06	74	480	426	504	24192	24192	410	16580	1222	30
D 07	262	780	3255	2063	6488	14136	1890	2280	10462	215
D 09	341	1470	12033	2851	17329	24192	9060	64880	2382	10462
D 10	132	3654	8164	14136	24192	24192	100	57940	15531	24192
D 12	231	354	14136	17329	24192	24192	17220	19180	2014	3255
D 13	116	201	24192	7270	24192	19863	14210	24810	1178	24192
D 14	96	228	24192	3654	24192	19863	3500	41060	3076	620
D 15	63	959	24192	14136	19863	24192	4255	13540	5475	3448
D 16	801	862	24192	4611	19863	24192	2920	13130	24192	1624
D 18	134	52	19863	5635	24192	24192	3790	13740	350	414
D 19	135	20	19863	24192	14136	24192	30760	13405	413	933
D 20	197	547	N/A	1430	4884	11248	4390	7540	960	246
D 22	379	1565	6488	24192	24192	17329	4880	57940	521	354
D 23	399	839	24192	17329	18596	24192	5290	14830	733	419
D 24	269	712	19863	8164	15531	15531	3450	16580	11616	216
Sewage	20490	14830	N/A	2010000	1842000	1112000	3911000	2696500	3513000	4237000
D26			24192							

> values, but sign removed for calculations
< values, but sign removed for calculations
potential matrix interference
MLSS not raw influent, not used for calculations
underlined value= average of duplicates

AC/TC										
unitless	4/6/2010	4/13/2010	5/2/2010	6/3/2010	6/9/2010	6/14/2010	7/10/2010	7/21/2010	7/31/2010	8/5/2010
D 01	20.2	2.5	4.4	19.2	8.2	43.8	20.7	123.9	19.7	12.1
D 02	8.9	1.3	26.2	32.5	0.4	33.9	3.7	34.2	43.5	8.0
D 03	11.6	7.7	8.6	14.1	2.5	12.4	23.1	9.1	13.8	14.0
D 04	42.3	1.4	32.5	16.8	41.5	26.7	15.3	15.3	28.9	62.0
D 06	4.0	1.0	12.2	5.4	7.4	49.3	4.3	30.7	37.7	51.5
D 07	10.6	20.2	28.4	45.2	3.8	62.7	16.7	79.4	15.3	67.9
D 09	41.5	8.5	16.7	16.1	6.6	9.6	15.3	21.1	3.1	10.2
D 10	12.8	2.4	16.7	9.6	3.7	15.2	M/C	77.5	2.5	4.1
D 12	14.1	5.3	28.4	20.4	3.0	32.7	5.8	27.7	17.6	10.6
D 13	24.1	13.2	2.0	26.4	3.1	13.6	10.0	21.7	18.4	6.5
D 14	8.0	9.1	4.4	27.7	4.2	14.3	13.1	11.3	43.7	29.0
D 15	14.0	0.2	6.6	17.1	5.7	23.6	15.7	18.0	4.6	48.6
D 16	10.0	0.5	4.9	93.2	8.1	14.0	7.1	47.1	3.0	23.4
D 18	521.0	110.0	8.0	20.7	7.2	12.4	16.1	15.5	20.3	51.2
D 19	0.6	1.0	5.2	15.2	8.0	17.9	19.7	26.7	24.7	19.3
D 20	12.7	1.0	N/A	41.3	10.8	18.4	20.5	78.9	21.0	12.3
D 22	9.9	0.1	34.3	5.6	1.8	23.8	36.2	21.2	26.7	21.1
D 23	5.1	0.4	4.7	49.5	4.7	10.6	8.8	23.2	15.8	66.7
D 24	62.9	38.7	8.6	55.8	4.9	26.8	9.5	24.4	4.2	86.7
Sewage	1.7	0.8	N/A	5.4	1.7	2.2	2.1	3.7	1.4	2.6
D26 overflowing manhole			0.8							

<1 total coliform detected, used LOD

M/C= matrix contamination

MLSS not raw influent, not used for calculations

N/A sample site unavailable

HuBac-Human Specific										
DNA copies/uL extract	4/6/2010	4/13/2010	5/2/2010	6/3/2010	6/9/2010	6/14/2010	7/10/2010	7/21/2010	7/31/2010	8/5/2010
D01	**2.73E+03**	1.26E+03	1.60E+04	1.16E+02	**1.42E+02**	1.51E+03	2.59E+02	**2.19E+02**	BDL	BDL
D02	1.49E+02	BDL	1.59E+03	2.53E+02	5.76E+02	BDL	BDL	BDL	BDL	BDL
D03	BDL	BDL	1.22E+03	1.53E+03	BDL	**1.58E+02**	5.66E+02	1.64E+02	BDL	BDL
D04	BDL	BDL	8.36E+02	8.04E+02	BDL	1.17E+02	3.69E+02	BDL	BDL	BDL
D06	2.81E+02	1.63E+02	BDL	BDL	2.84E+03	3.53E+02	2.36E+02	BDL	4.44E+02	1.53E+02
D07	3.17E+02	2.12E+02	**5.96E+02**	BDL	7.85E+02	4.37E+02	BDL	1.88E+02	BDL	BDL
D09	1.72E+02	3.52E+02	1.23E+04	BDL	4.85E+02	2.66E+03	**2.53E+02**	1.83E+04	**2.31E+02**	**1.68E+02**
D10	3.19E+03	**1.81E+04**	4.86E+03	3.26E+02	5.18E+02	5.84E+03	1.60E+05	**4.94E+03**	2.52E+04	1.33E+04
D12	3.73E+02	2.04E+02	6.68E+03	6.53E+02	8.88E+02	3.44E+02	1.85E+03	BDL	3.14E+02	2.21E+03
D13	BDL	BDL	1.54E+05	2.31E+02	5.84E+02	2.98E+02	1.61E+03	BDL	BDL	5.12E+02
D14	BDL	BDL	3.30E+04	**1.31E+02**	3.66E+02	5.52E+02	1.72E+02	1.67E+04	1.26E+02	BDL
D15	BDL	**BDL**	**2.26E+04**	1.43E+02	3.35E+02	9.21E+02	1.58E+02	BDL	1.61E+02	BDL
D16	BDL	3.72E+02	3.67E+04	BDL	3.84E+02	1.19E+04	BDL	3.82E+02	BDL	BDL
D18	3.07E+02	BDL	2.22E+04	1.24E+02	4.38E+02	4.10E+03	4.25E+02	2.52E+02	BDL	**BDL**
D19	1.58E+02	BDL	2.27E+04	3.60E+02	3.19E+02	1.92E+03	4.57E+02	3.50E+02	BDL	BDL
D20	**BDL**	BDL	N/A	BDL	3.33E+02	3.07E+02	**BDL**	BDL	BDL	BDL
D22	BDL	1.41E+02	3.40E+02	4.61E+02	5.66E+02	4.64E+02	1.88E+02	4.60E+02	BDL	1.47E+02
D23	1.30E+02	1.35E+02	3.58E+04	1.02E+02	3.11E+02	1.15E+04	1.84E+03	2.32E+02	**BDL**	BDL
D24	1.05E+02	1.75E+02	9.69E+03	5.55E+02	**BDL**	2.12E+02	BDL	6.88E+03	1.16E+02	BDL
D25(sewage)	6.23E+03	4.60E+03	N/A	**2.30E+06**	4.42E+06	**3.19E+06**	4.44E+06	1.87E+06	4.75E+06	4.24E+06
D26(manhole)			7.96E+05							
	N/A sample site unavailable									
	MLSS not influent, not used for calculations									
	Underlined=duplicate average									
	BDL = less than 100									

AllBac: Non-specific										
DNA copies/uL extract	4/6/2010	4/13/2010	5/2/2010	6/3/2010	6/9/2010	6/14/2010	7/10/2010	7/21/2010	7/31/2010	8/5/2010
D01	7.43E+03	4.93E+03	5.37E+04	2.31E+03	5.63E+03	4.65E+04	3.59E+03	1.40E+04	1.18E+03	5.59E+02
D02	1.25E+04	8.27E+03	8.64E+03	7.46E+03	2.78E+04	4.61E+03	BDL	7.20E+02	3.15E+03	BDL
D03	2.23E+03	3.83E+03	2.69E+04	2.46E+04	BDL	1.77E+03	2.70E+03	2.33E+03	BDL	BDL
D04	5.14E+02	BDL	9.49E+03	1.42E+04	5.23E+02	1.78E+03	1.71E+03	1.25E+02	2.78E+02	BDL
D06	5.57E+03	3.59E+03	2.29E+02	8.94E+02	1.40E+04	3.18E+03	7.78E+02	5.57E+02	6.38E+03	1.54E+03
D07	7.58E+03	2.78E+03	3.13E+03	1.63E+03	2.39E+04	3.31E+03	3.67E+02	3.02E+03	5.96E+02	BDL
D09	1.66E+04	6.66E+04	5.46E+04	4.52E+02	5.23E+03	9.37E+03	8.22E+02	1.02E+05	5.98E+03	6.37E+03
D10	2.18E+04	1.46E+05	2.28E+04	7.12E+03	6.91E+03	3.70E+04	7.88E+05	2.93E+04	3.71E+05	1.44E+05
D12	2.90E+03	4.54E+03	3.52E+04	5.19E+03	1.23E+04	8.19E+03	7.73E+03	6.40E+02	3.42E+03	6.67E+04
D13	1.01E+03	1.14E+03	8.02E+05	2.11E+03	8.14E+03	1.32E+04	9.02E+03	7.68E+02	1.20E+03	2.42E+03
D14	2.66E+03	4.98E+03	1.11E+05	9.31E+02	4.41E+03	4.00E+03	7.75E+02	7.71E+04	5.73E+03	2.87E+02
D15	7.74E+02	8.19E+02	7.51E+04	1.14E+03	6.33E+03	5.96E+03	6.93E+02	7.45E+02	2.63E+03	1.49E+03
D16	6.57E+02	4.73E+03	1.33E+05	8.35E+02	5.50E+03	4.78E+04	3.34E+02	3.26E+03	2.40E+03	2.27E+03
D18	1.54E+03	1.17E+03	7.70E+04	2.92E+03	9.54E+03	2.83E+04	2.00E+03	4.76E+03	1.58E+03	9.71E+02
D19	1.28E+03	9.39E+02	9.98E+05	5.25E+03	7.00E+03	2.06E+04	2.19E+03	3.89E+03	6.43E+02	1.82E+03
D20	8.93E+02	4.36E+02	N/A	7.38E+02	1.94E+03	2.29E+03	4.30E+02	1.37E+03	9.48E+02	1.08E+02
D22	6.40E+02	2.24E+03	7.06E+03	3.53E+03	2.96E+03	9.60E+03	1.63E+03	3.80E+04	4.09E+02	7.79E+02
D23	1.99E+03	1.71E+03	1.37E+05	2.17E+03	9.47E+03	3.92E+04	5.12E+03	3.47E+03	1.36E+03	3.22E+03
D24	1.21E+04	3.59E+03	6.71E+04	2.61E+03	4.75E+02	2.07E+03	4.69E+02	4.53E+04	1.84E+03	7.07E+02
D25(sewage)	9.39E+03	2.92E+04	N/A	9.32E+06	2.12E+07	1.73E+07	2.01E+07	2.30E+07	3.15E+07	3.23E+07
D26(manhole)			3.00E+06							
	N/A =missing sample									
	MLSS not influent:not used for calculations									
	Underlined=duplicate average									
	BDL = less than LOD of 100 DNA copies/uL									

qHF183-Human Specific										
DNA copies/uL extract	4/6/2010	4/13/2010	5/2/2010	6/3/2010	6/9/2010	6/14/2010	7/10/2010	7/21/2010	7/31/2010	8/5/2010
D01	BDL	BDL	3.24E+03	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D02	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D03	BDL	BDL	1.02E+02	2.44E+02	BDL	BDL	BDL	BDL	BDL	BDL
D04	BDL	BDL	1.72E+02	1.99E+02	BDL	BDL	BDL	BDL	BDL	BDL
D06	BDL	BDL	BDL	BDL	2.25E+02	BDL	BDL	BDL	BDL	BDL
D07	BDL	BDL	2.62E+02	BDL	1.38E+02	BDL	BDL	BDL	BDL	BDL
D09	BDL	BDL	5.00E+03	BDL	1.18E+02	3.05E+02	BDL	4.64E+03	BDL	BDL
D10	3.91E+02	2.84E+03	2.30E+03	BDL	BDL	1.39E+03	2.56E+04	1.40E+03	3.40E+03	1.58E+02
D12	BDL	BDL	1.87E+03	1.20E+02	BDL	BDL	2.63E+02	BDL	BDL	BDL
D13	BDL	BDL	3.10E+04	BDL	BDL	BDL	4.01E+02	BDL	BDL	BDL
D14	BDL	BDL	7.36E+03	5.75E+02	BDL	BDL	BDL	2.63E+03	BDL	BDL
D15	BDL	BDL	3.19E+03	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D16	BDL	BDL	7.40E+03	BDL	BDL	1.94E+03	BDL	1.26E+02	BDL	BDL
D18	BDL	BDL	5.18E+03	BDL	BDL	4.31E+02	BDL	BDL	BDL	BDL
D19	BDL	BDL	4.60E+03	BDL	BDL	2.03E+02	BDL	BDL	BDL	BDL
D20	BDL	BDL	N/A	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D22	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D23	BDL	BDL	6.50E+03	BDL	BDL	1.28E+03	3.35E+02	BDL	BDL	BDL
D24	BDL	BDL	2.33E+03	1.47E+03	BDL	BDL	BDL	2.56E+03	BDL	BDL
D25 (sewage)	BDL	2.93E+02		4.53E+05	3.74E+05	4.89E+05	4.36E+05	2.93E+05	7.90E+05	7.44E+05
D26 (manhole)			1.26E+05							

N/A sample site unavailable

MLSS not influent: Not used for calculations

Underlined=duplicate average

BDL = less than 100

Lexington Fayette Urban County Government

A PLAN FOR IDENTIFYING HOT-SPOTS AND AFFIRMING REMEDIATION IMPACTS ON SURFACE WATER QUALITY: PHASE I

Quality Assurance Project Plan

University of Kentucky

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Abstract: This document details a quality assurance plan to guide the successful implementation of a pilot project entitled A PLAN FOR IDENTIFYING HOT-SPOTS AND AFFIRMING REMEDIATION IMPACTS ON SURFACE WATER QUALITY: PHASE I. Lexington Kentucky has impaired water quality with respects to indicators of pathogens in the watersheds. Some of this impairment is due to leaking sanitary sewers, cross-connections, and stormwater overflows. A watershed management plan for identifying the areas most impacted by inputs of sanitary sewage is needed to identify the most appropriate sites for remediation and as is a method for determining the relative impact of any remediation taken in these highly impacted regions of local streams and waterways. Over the past 15 years, Dr. Gail Brion has developed a systematic approach to sampling and data analysis that identifies hot-spots of human fecal wastes in local streams and rivers using a triad of water quality indicators for fecal age, load, and source. This system has been proven in other watersheds (Eagle Creek, Georgetown, Frankfort, Lexington) and is published in the scientific literature. It relies upon utilizing multiple indicators for fecal load, source, and age to create a relative risk classification rubric to categorize the relative impairment in surface waters. This provides a systematic way to prioritize selected sections of a stream or creek for further investigation and remediation of significant sources. For this project, fecal load will be measured by enumeration of *E. coli* with Colilert media by IDEXX, fecal age by the AC/TC ratio obtained from membrane filtration testing for Total coliforms, and fecal source identified by quantifying three types of fecal source specific genetic markers for the strictly anaerobic bacterial group known as *Bacteriodes*, one of which is human specific. Dr. Brion proposes to the LFUCG that this system be applied to watersheds of concern for the purpose of identifying hot-spots of human fecal waste intrusion and validating the effectiveness of any remedial actions taken in these watersheds.

A PROJECT MANAGEMENT

A1. Approval Sheet

Gail Brion Professor of Civil Engineering University of Kentucky	Date
David Price Quality Assurance Officer Lexington-Fayette Urban County Government (LFUCG)	Date
Charlie Martin Director Division of Water Quality Lexington-Fayette Urban County Government (LFUCG)	Date Title
Ken Cooke Volunteer Coordinator Friends of Wolf Creek	Date

A2. Table of Contents

List of Tables

List of Tables

A3. Distribution List

Each person listed on the approval sheet and each person listed under Project/Task Organization will receive a copy of this Quality Assurance Project Plan (QAPP). Individuals taking part in the project may request additional copies of the QAPP from personnel listed under Section A4.

This document has been prepared according to the United States Environmental Protection Agency publication *EPA Requirements for Quality Assurance Project Plans* dated March 2001 (QA/R-5).

A4. Project/Task Organization

Personnel involved in project implementation are listed in Table 1, and shown as an organization chart in Figure 1.

Table 1: Project Implementation Personnel

Individual	Role in Project	Organizational Affiliation
Gail Brion, Ph.D.	Project Manager	University of Kentucky (UK)
Charlie Martin, P.E.	Director Division of Water Quality	Director Division of Water Quality Lexington-Fayette Urban County Government (LFUCG)
Ken Cooke	Volunteer Coordinator and Technical Resource	Friends of Wolf Run
Tricia Coakley	Lab Manager	UK ERTL Labs
David Price, Ph.D.	Lab Supervisor Town Branch WWTP	Lexington-Fayette Urban County Government

The University of Kentucky Project Manager will be responsible for the following QAPP/project related activities:

- Overall design, implementation, and management of pilot project.
- Outreach with regulated municipality and internal/external stakeholders for selection of sampling sites and pilot target areas.
- Serve as nexus of communications between all parties.
- Provide direct oversight to ERTL laboratories and UK personnel.

- Maintain official, approved QAPP.
- Develop amended QAPP as required.
- Data analysis and interpretation.
- Issue final report to LFUCG.
- Arranging for technology transfer of findings to interested industry and internal/external stakeholders.

Charlie Martin of the Lexington Fayette Urban County government will be responsible for the following activities:

- Approval of initial QAPP and all amended documents.
- Approval of final report.
- Archival of final report and datafile.
- Coordination of technology transfer with appropriate parties.

Ken Cooke of the Friends of Wolf Run will be responsible for the following activities:

- Coordination of volunteer sampling efforts.
- Training of volunteers in QA/QC procedures according to the practices and procedures set forth at http://kywater.net/01-Watershed%20Watch/06_Sampling/2005-QAPP/!Readme.htm.
- Collaboration in pilot sampling design as required.
- Communication with local volunteers.

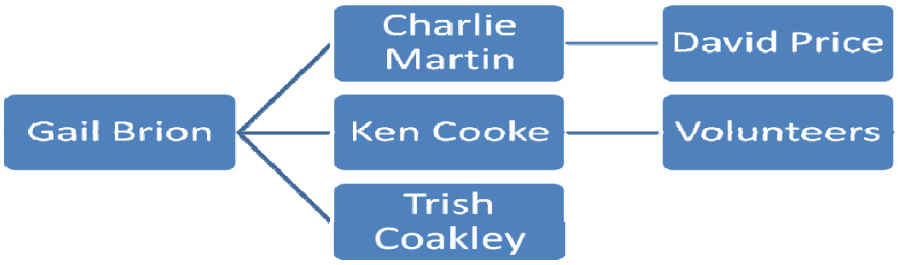
Tricia Coakley of the ERTL labs at the University of Kentucky will be responsible for the following activities:

- Coordination with volunteer samplers for receipt of field samples.
- Processing and analysis of samples collected according to established SOPs and QA/QC procedures.
- Creation and maintenance of final spreadsheet of primary and secondary data.

David Price of the Town Branch WWTP of the LFUCG will be responsible for the following activities:

- Collaboration in pilot sampling design as required.
- Technical assistance with secondary data as required.
- Quality Assurance officer.

Figure 1: Project Organizational Chart



A5. Problem Definition/Background

Rationale for initiating the project

The surface water systems in Lexington are contaminated with pathogen indicators from fecal sources of multiple origins; the most problematic in terms of human health risk from recreational contact with originate from sanitary sewage intrusion. The USEPA has entered into an agreement with LFUCG to improve the overall quality of surface waters with respects to pathogen indicators. However, the major sources of fecal contamination from sanitary sewage and other sources of human fecal materials need to be identified for remediation and their potential impact on the overall fecal load apportioned in support of watershed remediation schemes. As well, it is expected that the difference in the overall fecal indicator burden as represented by concentrations of *E. coli* may not provide the precision to denote improvements in watershed quality that happen as a result of remediation in these sites, due to the multiple sources of fecal wastes, many of which are not under the control of LFUCG Division of Water Quality (urban wildlife). Therefore, it has been agreed upon that a multi-indicator pilot study will be initiated along an urban watershed to: 1. Pinpoint and document areas within the selected watershed receiving proportionately large loadings of human fecal material for remediation, and 2. Create a baseline against which to assess water quality improvements with respects to the fecal age and proportion of human sourced fecal materials in the pilot areas.

Objectives of the project

- Define the unique pattern of indicators that define regions of local urban streams contaminated with proportionally great amounts of human/domestic sewage (hotspots) from those contaminated by other, less hazardous fecal sources.
- Establish baseline values for the indicators in these urban streams, and relative risk categorizations, to be used to evaluate future data against to illuminate water quality improvements or changes.

Anticipated Outcomes of the project

- Increased awareness of the impacts of sanitary sewage leaks, overflows, and spills on the environment.
- Improved understanding of opportunities to reduce environmental impacts in urban streams.
- Improvement of environmental quality in a target region or watershed.
- Increased recognition of environmental leaders of all involved parties among key stakeholders.
- Greater remediation efficiency and more effective allocation of LFUCG resources.
- Cost savings for the LFUCG.
- Development of a policy approach that could be used in other urban areas, agricultural areas, states, and regions.
- Improved communication and understanding between regulators and the regulated community.
- Greater collaboration among involved parties and state agencies.
- Enhanced networking and peer mentoring within the community.

Anticipated Decisions arising from the project

- Based on the findings of this project, LFUCG may modify its approach to monitoring its urban watershed and engage in follow-up projects to demonstrate to the State and Federal agencies continuous water quality improvements as remediation within its watershed are completed.

Regulatory information, applicable criteria and action limits

Section 303 (33 U.S.C. 1313) of the Clean Water Act (CWA) requires States and authorized Tribes to adopt water quality standards for waters of the United States within their applicable jurisdictions. Such water quality standards must include, at a minimum: (1) Designated uses for all water bodies within their jurisdictions, (2) water quality criteria necessary to protect the most sensitive of the uses, and (3) antidegradation provisions consistent with the regulations at 40 CFR 131.12. To meet ambient water quality standards, the city's new stormwater permit and the U.S. Environmental Protection Agency's Consent Decree require LFUCG to prevent water pollution to the maximum extent possible. The consent decree outlined specific tasks for the LFUCG to complete relative to stormwater and sanitary sewers. The decree states that:

> "LFUCG shall carry out assessments and engineering analyses necessary to identify all measures needed to ensure that LFUCG's Sanitary Sewer System complies with the requirements of the Clean Water Act, the regulations promulgated thereunder, the Kentucky pollution control laws, the regulations promulgated under such laws, and National Pollutant Discharge Elimination System Permits Nos. KY0021504 and KY0021491 and then shall implement all such measures in a timely manner, with the objective of eliminating all cross-connections and Recurring SSOs from the Sanitary Sewer System and Unpermitted Bypasses at the LFUCG's WWTPs.

While the activities outlined in this pilot project are not specifically specified in the consent decree, they are related to improving the water quality in Lexington's urban creek systems. The pilot project will provide information essential for creating a watershed management decision system that can detect and prioritize stream regions impacted by sanitary sewage overflows, leakages, and cross connections into stormwater systems. Further, the activity specified will create baseline data against which future studies can assess the effectiveness of remediation activities undertaken in the areas studied in support of the City's attempts to improve the overall water quality with respects to pathogen indicators. The approach proposed has only one indicator that will be measured that has ambient criteria established; *E. coli*. The current KY 401 KAR 10:031 surface water standards for *E. coli* in recreational waters are as follows:

> "Section 7. Recreational Waters. (1) Primary contact recreation water. The following criteria shall apply to waters designated as primary contact recreation use during the primary contact recreation season of May 1 through October 31:
>
> (a) Fecal coliform content or Escherichia coli content shall not exceed 200 colonies per 100 ml or 130 colonies per 100 ml respectively as a geometric mean based on not less than five (5) samples taken during a thirty (30) day period. Content also shall not exceed 400 colonies per 100 ml in twenty (20) percent or more of all samples taken during a thirty (30) day period for fecal coliform or 240 colonies per 100 ml for Escherichia coli. Fecal coliform criteria listed in subsection (2)(a) of this section shall apply during the remainder of the year; and (2) Secondary contact recreation water. The following criteria shall apply to waters designated for secondary contact recreation use during the entire year:
>
> (a) Fecal coliform content shall not exceed 1,000 colonies per 100 ml as a thirty (30) day geometric mean based on not less than five (5) samples; nor exceed 2,000 colonies per 100 ml in twenty (20) percent or more of all samples taken during a thirty (30) day period; "

A6. Project/Task Description

Project overview

This project will allow LFUCG to explore whether an approach modeled upon the novel, multi-indicator approach to stream classification created by Dr. Gail Brion can assist them in improving the water quality of urban streams in Lexington, KY.

Project summary and work schedule

This project's major tasks and timeline are outlined in the table below.

Table 2: Schedule of Major Project Tasks

Task Name	Task Description	Start Date	End Date
Outreach	Outreach to internal and external stakeholders about the project.	May/2010	August/2010
Goals identification	Finalize the goals of this project, upon which metrics will be based	May/2010	June/2010
Measures identification	Finalization of metrics to be tracked by this project.	May/2010	May/2010
Stream identification	Determine the exact characteristics of streams to be included in this project, and compile a list of streams from reliable sources. Select target sample sites in collaboration with LFUCG and Friends of Wolf Run personnel. 20 sites expected to be sampled.	May/2010	May/2010
Data input & management	Development and implementation of an approach to inputting and managing all primary and secondary data.	May/2010	August/2010
QAPP finalization & approval	Finalize QAPP based upon results of the measures identification, statistical methodology, and data management tasks. Primary data collection will not occur before relevant parts of the QAPP are finalized and approved by LFUCG.	May/2010	August/2010
Baseline Sample Site inspections	Inspections at selected stream sites to assure accessibility and discover potential problems	April/2010	May/2010
Baseline analyses of indicators	Analysis of indicators in grab samples of water from each sample site to establish expected ranges under wet and dry conditions.	April/2010	April/2011
Selective follow-up analyses	Resampling and analyses of indicators at sites under dry and wet conditions until results for 5 samples per site, per condition are achieved.	April/2010	April/2011
Post-sampling inspections/investigations	Inspections at sample sites to establish whether conditions have changed since the baseline. Inspection data also used to cross-check conditions reported to LFUCG	May/2010	April/2011
Data analysis	Analysis of data to create categories of relative impact with respects to domestic sewage intrusion based on fecal load, source, and age.	Dec/2010	March/2011
Reporting to LFUCG	Reporting shall include initial and final reports.	Dec/2010	June/2011

Table 2: Schedule of Major Project Tasks

Task Name	Task Description	Start Date	End Date
Tech Transfer	Report findings to interested local, national, and international groups	Dec/2010	Dec/2011

Geographic focus

The area for study is within the LFUCG limits. While initial consultations have selected a region of the city, and suggested sample sites within that area and at a clean-comparison site located outside of urban influences, the actual region and sites will be established in collaboration with all parties during the initial stages of the project and detailed in the amended QAPP.

Resource and time constraints

Getting repeat measures for this number of sample sites and replicate events under two different weather conditions is dependent upon the normal weather patterns holding true to past behavior. It may be difficult to obtain 5 samples under both wet and dry conditions depending upon the weather. Historically, the best sampling times for wet weather sampling is generally during the months of March through June. Based on past sampling efforts within the LFUCG area, there should be at least 5 separate rain events that could be sampled during this period by the volunteers. Dry weather sampling is best accomplished from July through September when the weather pattern has fewer rain events and stream flow is stabilized. More wet weather samples can be taken in October through December when significant storms influence water quality. The volunteers and the lab will have to be flexible and coordinate closely to assure that the sampling events occur as planned.

A7. Quality Objectives and Criteria

Quality objectives

Gail Brion and all involved parties recognize the importance of ensuring that data are of sufficient quality to meet the needs of the project. Friends of Wolf Run, LFUCG, and the University of Kentucky are committed to collecting primary data and obtaining secondary data of the highest quality possible within the constraints of project resources. Data quality can be characterized in terms of precision, bias, representativeness, completeness, comparability, and sensitivity.

Precision

For environmental measurements, Gail Brion will encourage all involved parties to meet the precision standards achievable by the use of EPA-approved analytical methods with proper sample collection and handling protocol.

For example:

- The ERTL analytical laboratories will use, where possible, EPA approved methods and common laboratory QA/QC methods. Where EPA methods have not been developed and approved (AC/TC and PCR analysis) the best methods documented by other researchers will be used.
- Volunteers with The Friends of Wolf Run collecting samples will be required to document their anticipated, and actual, data collection methods
- Volunteers will receive guidance in the form of voluntary or mandatory training sessions.

Bias

The following kinds of bias may impact the ability to draw conclusions from the data: incompleteness or lack of representativeness is a reasonably anticipated source of bias. To reduce concerns about bias in the reporting of project results, progress reports and the final project report will report potential biases in the data and justify all conclusions reached on the basis of project data, and project data will be open to inspection for [5] years.

Representativeness

To ensure representativeness of physical water samples, all parties will review the sampling plan to ensure that environmental sampling will be collected in accordance with guidelines and "best practices" established by the state or EPA. While prior knowledge of the urban watershed to be sampled may bias sample site selections, this knowledge is not common to all parties involved in the design of the sampling plan. The final sampling plan will represent a blend of perspectives and should reduce bias towards overly contaminated sites.

Completeness

Completeness goals for this pilot study are that usable data for all analytes from at least 10 selected sample sites that were sampled at least 3 times under replicate conditions of dry or wet weather be completed. It is expected that some sample sites may be dropped and others added, but the goal is to have 10 sample sites resampled for the length of the project with laboratory analysis providing quality results for all 3 classification of fecal indicators selected.

Comparability

The most important comparisons to be made in this project are between data obtained from sites known to be greatly impacted by sanitary sewers and those not directly and or greatly impacted. In general, all quantitative comparisons (e.g., among sites or between sites before and after a

remediation will be normalized whenever appropriate normalization data can be obtained (i.e. total PCR signal for *Bacteroides*). If normalization is not possible, the final report will make note of any considerations that would affect confidence in the comparison. Data from different sources will never be combined unless they were collected in a comparable manner.

Sensitivity

For environmental measurements, Gail Brion will encourage facilities to meet the sensitivity standards achievable by the use of EPA-approved analytical methods with proper sample collection and handling protocol.

A8. Special Training/Certification

To the extent possible, Tricia Coakley of ERTL and Ken Cooke of The Friends of Wolf run will assure that training sessions to key parties to ensure quality data collection, are completed to the extent practicable. Training sessions will be delivered to the following individuals to ensure quality data collection:

- All Volunteers collecting, handling, and delivering samples to lab.
- QA/QC personnel (if any additional training is needed to familiarize them with the project)

- Training will be augmented by debriefing personnel shortly after their tasks have begun, to correct and clarify appropriate practices. Volunteers who grab samples or supervise the sampling streamside will be required to complete a Standard Sampling Training Module developed by the Training Committee and approved by the Science Advisors Committee of the ICC that addresses:
  - Sample container handling
  - Sample collection
  - Sample preservation
  - Sample transport and storage
  - Documentation and chain of custody record completion
  - QA/QC procedures including duplicate samples and field blanks
  - Communication with Event Coordinators and lab staff

- The module includes a demonstration, ideally streamside, of sample container handling, collection, and preservation, and requires the volunteer to demonstrate competency. A PowerPoint of the Standard Sampling Module is posted on the Watershed Watch website:
- http://kywater.org/watch/workshops/. Ken Cooke of the Friends of Wolf Run is responsible for ensuring that all personnel involved with sample collection have the necessary training to successfully complete sampling tasks and functions and have on file the form, "Volunteer Monitor Participation Agreement," to document that a training participant has

satisfactorily completed the Standard Sampling Module under the supervision of a certified trainer.

A9. Documents and Records

Report format/information

The format for all data reporting packages will be consistent with the requirements and procedures used for data validation and data assessment described in this QAPP.

Document/record control

The recording media for the project will be both paper and electronic. The project will implement proper document control procedures for both, consistent with best practices. For instance, hand-recorded data records will be taken with indelible ink, and changes to such data records will be made by drawing a single line through the error with an initial by the responsible person. The Project Manager will have ultimate responsibility for any and all changes to records and documents. Similar controls will be put in place for electronic records.

The LFUCG Quality Assurance Officer shall retain all updated versions of the QAPP and be responsible for distribution of the current version of the QAPP. The LFUCG Quality Assurance Officer and the Project Manager will approve all updates. The Project Manager shall retain copies of all management reports, memoranda, and all correspondence between the LFUCG and all project personnel identified in A4.

Dr. Brion will be in control of all data until the generation of the final report and the verified electronic copy of the database of analytes. At that time, LFUCG and Friends of Wolf Run will be provided copies of the spreadsheet database with all analysis data recorded. The electronic data generated by this project is to be considered public and will be made available to interested parties upon written request to Dr. Brion, Ken Cooke, or Charlie Martin.

Other records/documents

Other records and documents that will be produced in conjunction with this project include:

- Chain of custody forms.
- Sampling and observation logs.
- Outreach materials, including workbook, fact sheets, brochures, etc.
- Amended QAPP.
- Readiness reviews (see below).
- Data handling reports.
- Progress reports.
- Project final report (to include discussion of QA issues encountered, and how they were resolved).

Storage of project information

While the project is underway, project information will be stored in a central file within the ERTL laboratory facility. Upon completion of the project, paper records, photographs, and audio-visual material will be retained for [5] years at ERTL in a central file. Electronic records will be stored for [5] years on the Project Managers computer with a copy kept on Tricia Coakley's computer in ERTL.

Backup of electronic files

A backup copy of electronic files will be made to removable hard disk that will be stored in the file cabinet with the paper documents.

B DATA GENERATION AND ACQUISITION

B1. Sampling Process Design (Experimental Design)

A key task in this project will be to develop a sound sampling plan of selected urban watersheds for analysis of the selected indicators of fecal age, load, and source in the water in order to draw inferences related to the selected objectives. The major quality objective will be to collect representative data that truly reflect the conditions of the urban watershed that this project focuses upon in two distinct weather conditions, wet and dry. Data generated by this project is of two types: (1) analytical data generated from analysis of the grab samples of water obtained from the urban watershed, which will be collected by trained volunteers from the Friends of Wolf Run and analyzed by Tricia Coakley of the UK-ERTL labs or a student specifically trained for the task by Tricia Coakley, and (2) observations and information available from secondary sources such as maps, stage level recorders, other written and oral reports.

B2. Sampling Methods

As described above, the primary data collected and used by this project will come from a series of sampling events along urban streams within selected watersheds. Samples will be collected by trained volunteers in vessels appropriate for the analyte according to EPA methodology and best standard practices. Volunteers will collect samples according to the existing QA/QC procedures found at the website http://kywater.org/watch/qa.htm. The Inter-Basin Coordinating Committee for Watershed Watch has developed this QA/QC material in consultation with the Kentucky Division of Water for submitting data from synoptic sampling events to the Division for consideration for use in regulatory processes, such as development of the Division's 303(d) and 305(b) reports. These procedures will be followed by the volunteers trained for this project.

The precise sampling plan will be designed to characterize sources of POTW effluent and untreated human sewage influencing each watershed and create baseline data on resultant microbial water quality in the watersheds during periods of baseflow (dry) and wet weather. The sites will be chosen based on their ability to be easily located, safely accessed and their potential for recreational contact. Sample locations are to be selected through a mixture of input from local authorities, sanitary survey reconnaissance, and segmentation of the watershed into incremental units using a "point of interest" methodology and topographical and field surveys. One sample site per sampling event will be representative of domestic sewage and will be taken at either the entrance to the sewage treatment plant or overflowing manholes. The sampling sites are to be correlated with map coordinates obtained from GPS and identified by these coordinates and other identifying features that will be logged into the record for reporting purposes so that others may identify these locations. Sample sites will be assigned unique ID numbers. Samples will be collected at each of the proposed sites during the months of March-September. Specific dates for sampling will be set to ensure that a diversity of flow conditions (high and baseflow) are included in the sampling plan. The final sampling plan is expected to be reflective of the advice given in EPA's *Generic Guide to Statistical Aspects of Developing and Environmental Results Program* (2003) and input from other water professionals, especially local water authorities familiar with the watershed. The sampling design assumes that during the collection of dry-weather samples the freshwater system is at or near baseflow conditions. Baseflow conditions will be characterized based upon review of real-time river/stream gauges and antecedent rainfall events. Also, during water sampling events it is assumed that, ambient water is laterally and vertically well mixed throughout creek/stream/river cross-sections, and water samples collected are representative of water at that location.

B3. Sample Handling and Custody

A standard chain of custody form will be developed and used for all samples collected by this project. Samples collected will be stored on ice in coolers and holding times will be met to insure the accuracy of the results. Sampling events will be arranged so that samples are delivered to the lab within 6 hours with analyses for *E. coli* to be initiated within 8 hours of sampling times (if this time not achieved, sample results will be flagged in reporting documents). All analyses for culturable *E. coli* bacteria analyzed by IDEXX quantitray and the filtration of samples for qPCR will be done on the day of sampling. Filters for qPCR will be stored at -20◦C until extraction. Sample aliquots used for the analysis of the AC/TC ratio will be processed within 24 hours of sampling times and will be stored under refrigeration until processing. Samples and sample containers will be maintained in a secure environment at all times when they are not in the laboratory. Once samples are received in the laboratory, the SOP for normal custody will be followed. Transfer of samples to the laboratory will be accomplished using a signature on the field log sheet that denotes transfer time, date, and responsible lab personnel. If custody is not maintained, then a note must be made on the accompanying sample forms. All frozen and/or archived samples are to be stored in a locked freezer (-20oC) accessible only to authorized laboratory personnel. The laboratory analyst is responsible for the samples from arrival to analysis and final disposal.

Data entry QA procedures

Personnel participating in the study will catalog all methods, results, dates, conditions, and data in lab books with permanent ink. Copies of the data from lab books, field sheets, lab analysis sheets, and chain of custody sheets will be kept in a centralized file until entry into electronic spreadsheet. Procedures for entering hand-written data into the database will follow standard quality assurance procedures (e.g., verification using independent double key entry). Files created from the centralized spreadsheet for modeling or analysis will have 10% of the data entries random record checked to assure that manipulation of the file did not corrupt the data. Errors caught during cross-checking will be flagged and corrected, to the extent possible, in consultation with data collection staff and appropriate parties.

B4. Analytical Methods

This project will follow well-recognized analytical methods for surface and drinking water samples. The membrane filter and broth culture methods to be used are standardized (SM9222b for the AC/TC ratio obtained from the m-endo broth based, membrane filter analysis for total coliforms, IDEXX Quanti-Tray 2000 for *E. coli*). The IDEXX analysis will be done per published procedural manuals from IDEXX. Basically, 100 mL samples of water are mixed with pre-packaged amounts of media, and then distributed into a sterile multiple well Quanti-Tray and incubated at 35 degrees C for 24 hours ± 2 hours before counting the number of wells with blue florescence. The numbers of large and small positive wells are used to provide a statistical estimate of the most probable number of bacteria per 100 mL of sample to be read from a chart provided by IDEXX. The AC/TC ratio analysis will require colony counts for two types of bacterial colonies grown on m-endo fed membrane filters, those presenting as total coliforms (dark red with sheen) and those presenting as atypical colonies (pink to red, no sheen). The AC/TC ratio reported is produced by dividing the number of atypical colonies per 100 mL by the number of typical coliform colonies per 100 mL. The AC/TC ratio reported is unitless.

Extraction methods for qPCR extracts will be standardized by using commercially available, pre-packaged kits or EPA methods. Sewage or cloned DNA product will be used for the positive controls and matrix spikes. Records will be kept of PCR efficiency and qPCR results will be reported as DNA copies per unit volume. Dr. Brion will review all microbial data for consistency and quality. Data that shows substantial discrepancies from known precisions or variances will be discarded and the events surrounding the value investigated. Dr. Brion will determine and record the appropriate corrective action as required on a case-by-case basis.

Bacteroides qPCR analyses will use the AllBac and HuBac markers and protocol designed by Alice Layton at the University of Tennessee in Knoxville, TN and possibly the quantitative HF183 human specific marker designed by Sylvia Seurink at Ghent University in Belgium or the HumM2 or HumM3 markers by Orin Shanks at the USEPA. These markers have not yet been proven to be effective in our local watersheds, so the emphasis will be on the AllBac and HuBac markers that have been used prior and have been proven with our local fecal sources. The PCR

protocol for the analysis of AllBac and HuBac markers is as follows. The PCR reaction mixture consists of 12.5uL BioRadTM IQ supermix, 1uL each of 10uM forward and reverse primer (Layton, 2006), 0.5uL of 10uM fluorescently labeled probe (Layton et al., 2006), 2uL of template DNA, and enough dilution water to produce a final reaction volume of 25uL. Calibration curves were made using serial dilutions of plasmid DNA containing the cloned 16s rRNA from *Bacteroides*. (Layton, 2006) Calibration covers a range of 101 – 107 target copies/uL. All qPCR reactions are run in triplicate using a BioRadTM iCycler IQ real-time PCR thermocycler. The thermocycler program consists of 1 cycle at 50◦C for 2 minutes and 95◦C for 10 minutes followed by 50 cycles of 95◦C for 30 seconds and 60◦C (AllBac and HuBac) or 57◦C (Bobac) for 45 seconds. (Layton, 2006) The qPCR protocol for the analysis of the qHF183 marker includes 25 uL reactions containing BioRadTM Sybr Green supermix. The thermocycler profile will include a 10 minute denaturation at 95◦C followed by 40 cycles of 30 seconds at 95◦C, 60 seconds at 53◦C and 60 seconds at 60◦C. (Seurinck et al., 2005) If HumM2 or HumM3 markers are analyzed, published methods by Orin Shanks of the USEPA will be followed for qPCR. Threshold cycles from samples are compared with the calibration curve to determine concentration of target in copies per uL and then the final report value in copies/mL is calculated based on the volume of original water sample filtered.

B5. Quality Control

Standard laboratory QA/QC for membrane filtration and IDEXX Quantitray methods will include, but not be limited to the following practices: a positive control will be done for each new batch of media (calibration); a negative control in the form of a field blank will be run each sampling event (reagent blank, sampler competence); a negative control for media quality will be done at the beginning and end of each sampling event (reagent blank, calibration); each sample for membrane filtration will have a minimum of 3 dilutions/aliquots assayed with 2 replicate plates per dilution analyzed (data quality); only counts from plates with >20 or <80 colonies will be used to calculate sample concentrations (data quality); only counts from plates with clearly separable colonies will be used, and colonies that touch each other will be counted as a single colony (data quality); anomalous counts will be excluded from data reporting {ie. Counts that vary inexplicably, such as those obtained when students forget to filter sample, or have added sample twice} (data quality); a 15 sample, duplicate precision test run by the student/person in charge of that analysis will be done to establish acceptable precision (operator competence, duplicate analysis); duplicate samples run on 10% of samples and compared against the precision test and corrective measures taken as appropriate. (operator competence, data quality). When possible, calculations of the final concentration of microorganisms will be made from the maximum volume of sample, even if it includes counts from different dilutions/aliquots. The total number of colonies observed will be divided by the total amount of sample filtered, adjusted to CFU/100 mL, and reported. Initial precision and recovery (IPR) for PCR extract recovery will be done on a series of samples that have been spiked with a source of *Bacteroides* into laboratory water. EPA QA/QC guidelines for PCR methods will be followed and include, but not be limited to: a PCR positive control per each PCR run; a PCR negative control (from the sample blanks); a PCR method blank with each batch of samples processed; a method positive control with every sample batch; an initial matrix spike/inhibition check repeated if water conditions change radically.

Crosschecking data

Dr. Brion will review all microbial data generated by Tricia Coakley for consistency and quality. Data that shows substantial discrepancies from known precisions or variances will be discarded and the events surrounding the value investigated. Dr. Brion will determine and record the appropriate corrective action as required on a case-by-case basis. Data will be provided to compare with duplicate samples taken by the Friends of Wolf Run and analyzed by other laboratories on an as-requested basis.

Data anomalies

Procedures for handling data anomalies (such as outliers and missing data) will be handled based on standard statistical procedures.

B6. Instrument/Equipment Testing, Inspection and Maintenance

The PCR equipment is on a maintenance contract that includes a yearly preventative maintenance visit by a BioRad specialist.

B7. Instrument/Equipment Calibration and Frequency

Calibration is part of the yearly PM visit..

B8. Inspection/Acceptance for Supplies and Consumables

Supplies and consumables are certified sterile or PCR grade. All media expiration dates are reviewed to assure fresh media was supplied.

B9. Non-Direct Measurements (I.e., Secondary Data)

This project will rely upon secondary data to identify conditions that might impact the water quality, such as rainfall, stage level, temperature, overflow events, etc.)

Table 3: Non-Direct Measurements (i.e., Secondary Data)

Data Sources	Intended Use	Rationale for Use	Acceptance Criteria
USGS	Weather and stream flow data	Commonly accepted source	All records will be accepted unless USGS denoted quality issues
LFUCG	Verification of overflow events or sanitary sewer issues	Is agency responsible for sanitary sewers.	All records will be accepted unless Charles Martin or David Price indicate quality issues

Key resources/support facilities needed

The Project Manager will require access to the data sources mentioned above, and this information will be managed within the database created/utilized for the overall project. No obstacles are anticipated to this approach.

Determining limits to validity and operating conditions

Database containing secondary data will be designed such that the original source for all data is marked, and procedures will be in place such that only the Project Manager can officially remove an entry from the final database.

B10. Data Management

As part of this project, Gail Brion, Charlie Martin, and Ken Cooke will continue to develop a data management strategy, and amend the QAPP based upon the strategy as needed. The Project Manager is responsible for ensuring that the strategy is developed and that the QAPP is amended to reflect that strategy. As mentioned prior, hard copies of sample custody sheets, raw data, and laboratory records will be kept in a central file within the ERTL labs. These hard copies will be kept for a period of 5 years after the study is completed. The final data from the project will be compiled into an Excel spreadsheet form along with information on rainfall amounts, intensity, level of detection limits, and comments as an appendix to the final report. A hard copy of the final data spreadsheet and final report will be printed and kept on file with the in-house data. Copies of the final report with appended data file will be sent both hardcopy and electronically to Ken Cooke and David Price at the end of the study. Gail Brion, Ken Cooke, and David Price (of LFUCG Quality Officer designate) are responsible for controlling the dissemination and use of the report and data after the final report has been approved and submitted. Up till the time of the approved final report, data access is to be controlled by Project Manager Gail Brion and all requests for dissemination prior to the production of the final report must be approved by Gail Brion with input from David Price and Ken Cooke. After the production and approval of the final report and the final spreadsheet of results, any and all parties may use and distribute the

results. Requests for the results of this study after the project has closed can be made to Gail Brion, Charlie Martin, and Ken Cooke (or their designees).

C ASSESSMENT/OVERSIGHT

C1. Assessment and Response Actions

The Quality Assurance Officer will conduct a Readiness Review immediately prior to the major data collection tasks. The QA Officer will report findings to the Project Manager, who will take corrective action (if any is necessary) before the data collection task begins. Further, the Project Manager and QA Officer will thoroughly debrief project implementation staff a short time after beginning their respective implementation tasks, to identify emerging/unanticipated problems and take corrective action, if necessary.

C2. Reports to Management

Two kinds of reports will be prepared: readiness reviews (described above) and the project final report. Reports will note the status of project activities and identify whether any QA problems were encountered (and, if so, how they were handled). Project final report will analyze and interpret data, present observations, draw conclusions, identify data gaps, and describe any limitations in the way the data should be used.

Table 4: Project QA Status Reports

Type of Report	Frequency	Preparer	Recipients
Amended QAPP	Once, before primary data collection begins	Gail Brion Project Manager	All recipients of original QAPP
Readiness Review	Before beginning field sampling	David Price QA Officer	Gail Brion, Charlie Martin
Final Project Report	Once	Gail Brion	Charlie Martin

D DATA REVIEW AND EVALUATION

D1. Data Review, Verification and Validation

This QAPP shall govern the operation of the project at all times. Each responsible party listed in Section A4 shall adhere to the procedural requirements of the QAPP and ensure that subordinate personnel do likewise.

This QAPP shall be reviewed at least once to ensure that the project will achieve all intended purposes. All the responsible persons listed in Section A4 shall participate in the review of the QAPP. The Project Manager and the Quality Assurance Officer are responsible for determining that data are of adequate quality to support this project. The project will be modified as directed by the Project Manager. The Project Manager shall be responsible for the implementation of changes to the project and shall document the effective date of all changes made.

It is expected that from time to time ongoing and perhaps unexpected changes will need to be made to the project. The Project Manager shall authorize all changes or deviations in the operation of the project. Any significant changes will be noted in the final report, and shall be considered an amendment to the QAPP. All verification and validation methods will be noted in the analysis provided in the final project report.

D2. Verification and Validation Methods

To confirm that QA/QC steps have been handled in accordance with the QAPP, a readiness review will be conducted before key data collection/analysis steps, and data handling reports will be prepared after each step. These reviews and reports will be consistent with UK-ERTL's SOP. Standard statistical tests (described below in Section D3) will be used to determine the extent to which inferences can be drawn from the sample data.

D3. Evaluating Data in Terms of User Needs

It is the goal of this project to establish universal correlations between cultured indicator bacteria and qPCR signals for *Bacteroides* for use in categorizing relative risk levels with respects to proportion of fresh human sewage in urban watersheds. However, it may be that the trends and correlations found between culture methods and qPCR results are site specific, not universal. Before completion of this section, input will be required from the final users, therefore this part of the QAPP is unfinished. The data collected from the study will be analyzed using accepted microbiological protocols and statistical analyses. Microbial results for *E. coli* will be reported with detection limits and expected analytical variances according to the IDEXX charts used to interpret the results of the Quanti-tray. The microbial data will be log-transformed prior to any

statistical analysis to prevent errors related to non-normal distribution of the results. Geometric means will be reported for *E. coli* data with standard deviations for each site and for the watershed as a whole under normal and rainy conditions. Where *E. coli* numbers exceeded the lower or upper detection levels of the Quanti-tray analysis, the lowest or highest level will be used for calculations with reference made of the impact of this assumption. Correlation analysis (parametric and non-parametric) will be done to illuminate trends between the indicators selected. The 3 indicators will be used in concert to classify sites into low, mid and high concern categories. This classification is expected to follow the scheme using *E. coli* levels >500 or <500 MPN/100 mL and AC/TC levels >20, <20, and/or <10 as decision points. It is expected that the PCR results will be broken into classifications based on the overall fraction of human specific marker to total marker present in samples. However if the newer, more specific human marker is used, different interpretation of the percentages will need to be established relative to our prior findings with HuBac/AllBac and it is likely that the lower level of detection will be used to calculate the percentage of the total *Bacteroides* signal in samples that show no human marker. PCR results using the HuBac marker in relation to the AllBac marker will be divided into 3 categories: <20, >20, and >50 % (HuBac/AllBac *100) and compared to the other two indicators. A 3-way cluster analysis will be done to see if there are any identifiable groupings of data. The final results of this study will present the areas and conditions in the watershed of most concern to LFUCG for use in establishing a priority list for remediation actions and future study.

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Approach to managing unusable or incomplete data

Upon occasion, methods of analysis do not provide data with known concentrations. Examples of this are when the analyte of interest is present in concentrations lower or higher than detectable with the method selected. Since microbial samples cannot be repeated due to time constraints, results that are either less than or greater than the detection limits of the analysis will be assumed to have the concentration of the relevant detection limit to prevent calculated values that are zero or undefined. If toxicity or matrix interferences prevent an analysis from producing results, then all attempts will be made to provide another estimate of the data (from a duplicate sample). If there is no reasonable way to fill a data point from other information (interpolation or simulation), then that datapoint will not be used in any statistical analysis, but may be presented in the final report and spreadsheet of results with notation as N/A. All statistical analyses and results that rely upon data simulated from other sources will be identified and the potential bias noted. .

A Multi-parameter Approach for the Identification of Leaking and Overflowing Sanitary Sewers in the Wolf Run Watershed

Tricia Coakley[1,2] and Gail Brion[1]

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LEXINGTON FAYETTE URBAN COUNTY GOVERNMENT
KENTUCKY
1775
UK
UNIVERSITY OF KENTUCKY
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Friends of
Wolf Run

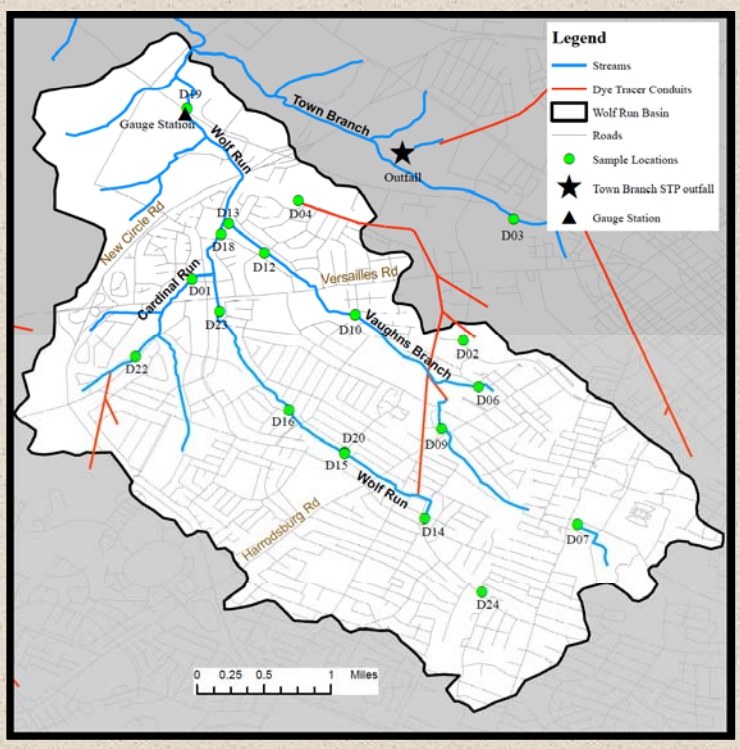
Objectives

- Identify locations of leaking sanitary sewer lines and overflows within the Wolf Run watershed of Lexington

- Establish baseline values of fecal indicators and relative risk categorizations for comparison with future data to show water quality improvements

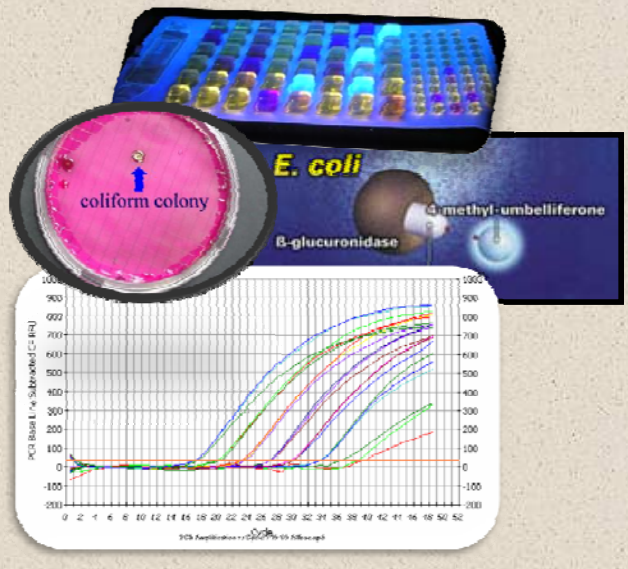
Spatial and Temporal Variability

- 19 stream locations
- WWTP Raw Influent
- 10 sampling events
- April – August 2010
- 4 dry events
- 6 wet events



Analytical Methods

- *E.coli* by Idexx Quantitray 2000
- AC/TC by mEndo broth and membrane filtration
- *Bacteroides* host specific fecal markers by qPCR with primers and probes developed by Alice Layton (University of Tennessee)
  - Hubac = human specific
  - Allbac = general

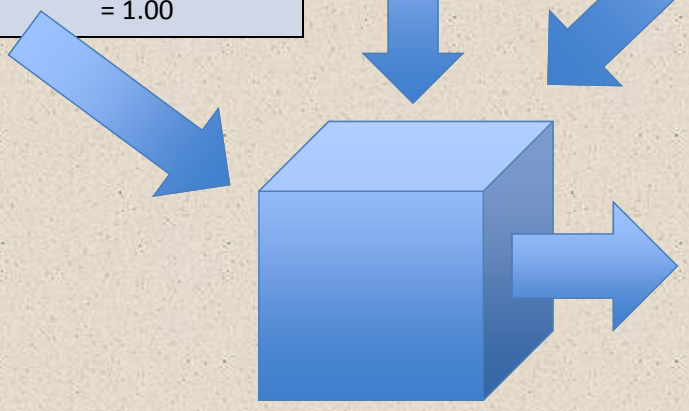


Model to determine Sanitary Category Value (SCV)

Fecal Load (E.coli MPN/100mL)	
< 235	= 0
235 – 575	= 0.17
576 – 999	= 0.33
1,000 – 1,999	= 0.5
2,000 – 9,999	= 0.67
10,000 – 23,999	= 0.83
24,000 +	= 1.00

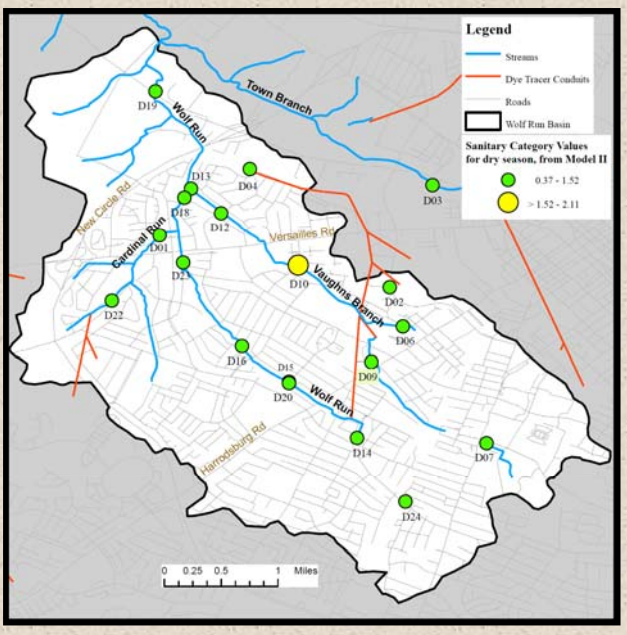
Fecal Age (AC/TC)	
20 +	= 0
19 – 15	= 0.25
14 – 10	= 0.50
< 10	= 1.00

Fecal Source (Hubac)
Log_{10}Hubac/Log_{10}MaxHubacSewage
= 0.1 – 1



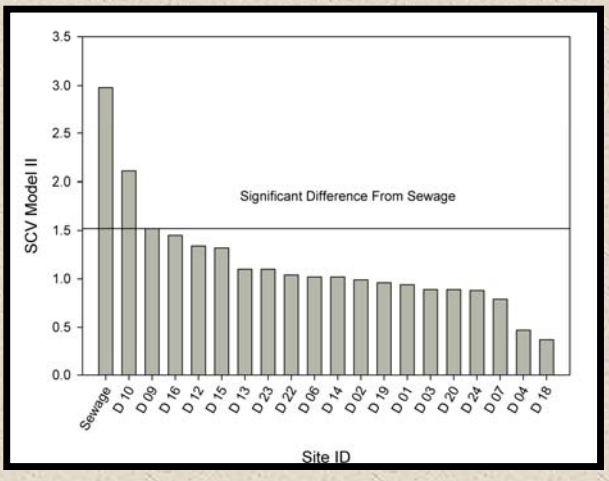
SCV
= 0 – 3.0

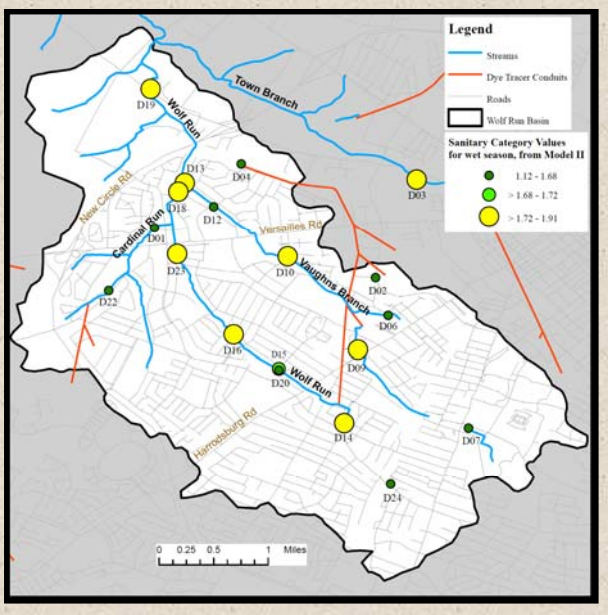
Site ID#	Average SCV All Days (SD)	Average SCV Wet Days (SD)	Average SCV Dry Days (SD)	Difference in SCVs Wet minus Dry
D 01	1.31 (0.62)	1.56 (0.59)	0.94 (0.50)	0.62
D 02	1.28 (0.60)	1.48 (0.62)	0.99 (0.50)	0.49
D 03	1.44 (0.58)	1.80 (0.37)	0.89 (0.36)	0.91
D 04	0.94 (0.54)	1.25 (0.35)	0.47 (0.44)	0.78
D 06	1.30 (0.57)	1.48 (0.55)	1.02 (0.53)	0.46
D 07	1.05 (0.50)	1.23 (0.44)	0.79 (0.53)	0.44
D 09	1.66 (0.57)	1.76 (0.52)	1.52 (0.69)	0.24
D 10	1.99 (0.55)	1.89 (0.41)	2.11 (0.73)	-0.22
D 12	1.52 (.050)	1.65 (0.58)	1.34 (0.34)	0.31
D 13	1.54 (0.82)	1.83 (0.67)	1.10 (0.92)	0.73
D 14	1.55 (0.67)	1.90 (0.62)	1.02 (0.31)	0.88
D 15	1.56 (0.58)	1.72 (0.58)	1.32 (0.58)	0.40
D 16	1.69 (0.66)	1.85 (0.66)	1.45 (0.67)	0.40
D 18	1.21 (0.86)	1.78 (0.62)	0.37 (0.08)	1.41
D 19	1.48 (0.61)	1.82 (0.45)	0.96 (0.42)	0.86
D 20	1.02 (0.36)	1.12 (0.36)	0.89 (0.37)	0.23
D 22	1.37 (0.69)	1.58 (0.65)	1.04 (0.69)	0.54
D 23	1.59 (0.71)	1.91 (0.62)	1.10 (0.57)	0.81
D 24	1.36 (0.76)	1.68 (0.54)	0.88 (0.84)	0.80
Sewage	2.98 (0.02)			
Overflow		2.88		



Dry weather sampling results

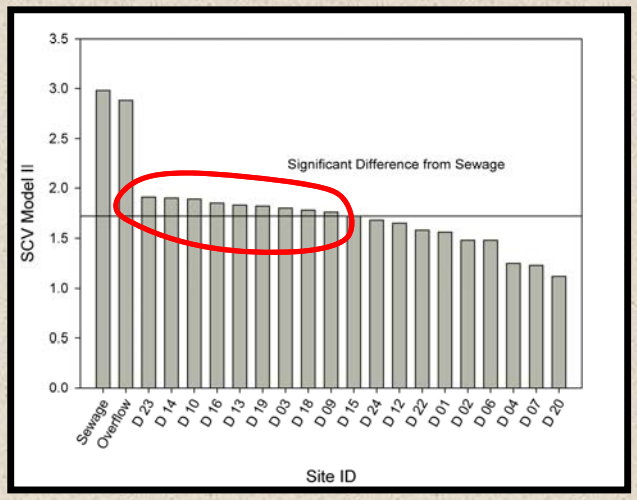
- Most sites significantly different than sewage
- D10 not significantly different than sewage (P<0.01)
- D04 & D18 significantly different than D10 (P<0.472)





Wet weather sampling results

- Many sites <u>not</u> significantly different than sewage
- D10 <u>not</u> significantly different than any other site



SCV change (wet vs. dry)

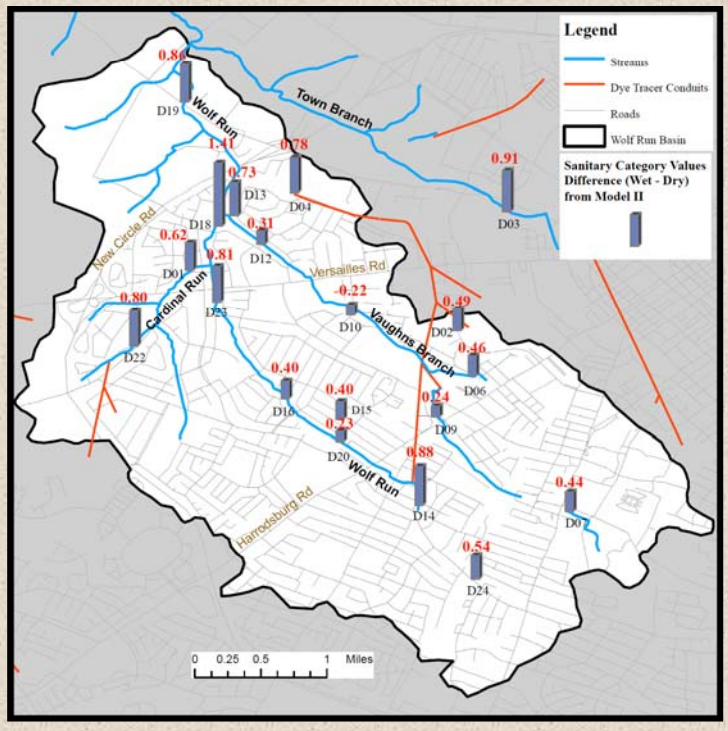
Differentiation between overflows and leaks

Vaughn's Branch

Consistent leaks indicated by little change from wet to dry (average SCV of D10 decreases following rain events)

Wolf Run

Overflows indicated by large changes from wet to dry



Conclusions

- Teamwork
- Spatial and temporal variability required
- Multiple indicators needed to categorize sites
- Fecal pollution from consistent leak may be differentiated from overflows
- Baseline established for future comparison

Cheryl Taylor, Charlie Martin, David Price of the Lexington Fayette Urban County Government

Ken Cooke and the Friends of Wolf Run

Jean Watts, Tracey Knowles and the students of Bluegrass Community Technical College

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