



DETECTION OF WASTEWATER CONTAMINATION

KNOWLEDGE DEVELOPMENT FORUM



IMPORTANT NOTICE

The material presented in this publication has been prepared in accordance with generally recognized engineering principles and practices and is for general information only. This information should not be used without first securing competent advice with respect to its suitability for any general or specific application.

The contents of this publication are not intended to be a standard of the Water Environment Federation® (WEF) and are not intended for use as a reference in purchase specifications, contracts, regulations, statutes, or any other legal document.

No reference made in this publication to any specific method, product, process, or service constitutes or implies an endorsement, recommendation, or warranty thereof by WEF.

WEF makes no representation or warranty of any kind, whether expressed or implied, concerning the accuracy, product, or process discussed in this publication and assumes no liability.

Anyone using this information assumes all liability arising from such use, including but not limited to infringement of any patent or patents.

The Publisher works hard to ensure that the information in this publication is accurate and complete. However, it is possible that the information may change after publication, and/or that errors or omissions may occur. We welcome your suggestions on how to improve this publication and correct errors. The Publisher disclaims all liability for, damages of any kind arising out of use, reference to, or reliance on information included in this publication to the full extent provided by state and Federal law.

DETECTION OF WASTEWATER CONTAMINATION

CONTENTS

AUTHORS AND CONTRIBUTORS	iii
PREFACE	1
OVERVIEW	1
WASTEWATER CONTAMINATION DETECTION	1
Human bacterial markers.....	1
Chemistry techniques: strengths, weaknesses, and where chemistry fits best	3
Canine scent tracking	7
Optical properties of water for prediction of wastewater contamination in surface water	8
Background.....	8
Objectives	8
Study Approach.....	9
Sampling Design	9
Status of Research.....	10
Additional Bacteria.....	11
ROUNDTABLE DISCUSSION SYNTHESIS RE: WASTEWATER DETECTION METHODS	11
What techniques are used within each group and how? List advantages and limitations and cost for each method.	
Are there methods not represented here that should be, and what research is needed?.....	12
What information is needed for investigation and what information is needed for action to be taken?.....	13
How do we interpret results?	14
PRACTICAL APPLICATIONS AND FUTURE TECHNOLOGIES	15
CASE STUDY 1 – Incorporating Molecular Testing as an Evidentiary Tool in Municipal Water Quality Monitoring Programs	15
CASE STUDY 2 –Collection System Infrastructure Microbial Source Tracking.....	18
CASE STUDY 3 –Illicit Discharge Investigation Examples: Unraveling the Spaghetti	21
FUTURE TECHNOLOGIES: MOBILE qPCR, SEQUENCING, AND OTHERS	22
ROUNDTABLE DISCUSSION SYNTHESIS RE: Challenges for implementing wastewater detection programs.....	25
Mapping the collection system.....	25

What are the major hurdles for backtracking? Financial, expertise, administrative, research needs, political will. What needs to happen logistically? Administratively?	26
Can production laboratories help? What turn-around time is needed?.....	26
What financial barriers are there?	26
How can effectiveness in results interpretation be implemented through information sharing among agencies?.....	27
WHAT RESEARCH NEEDS TO BE DONE?	28
Contamination detection and source identification	28
Guidance and tools	28
Remedies.....	29
Management.....	29
REFERENCES	30
10-AUG-2018 KNOWLEDGE DEVELOPMENT FORUM PARTICIPANTS	32

Table 1: Quantitative PCR (qPCR)	2
Table 2: Characterizing Illicit Discharges.....	4
Table 3: Typical Chemical Field Parameters for Illicit Discharge Detection	5
Table 4: Chemical Monitoring, Strengths and Weaknesses	6
Table 5: Techniques for Detection of Wastewater Contamination, Pros and Cons of each Method, and Relative Cost	12
Table 6: The Applications of Lachno3 and Lachno12 Assays on Environmental Samples that were Inconsistent in HB and Lachno2 Assays Results	22
Table 7: Mapping the Collection System.....	25

Special thanks go out to the

Great Lakes Protection Fund
for financial support, and to the

**School of Freshwater Sciences,
University of Wisconsin-Milwaukee**
for hosting the Knowledge Development Forum

National Institutes for Water Resources (NIWR)
Agency project number: 2016WI354G
Project title: Detection of sewage contamination in urban
areas of the Great Lakes
Principal Investigator: Sandra McLellan
for additional funding

AUTHORS AND CONTRIBUTORS

Danny Barker, Environmental Scientist,
Hampton Roads Sanitation District

Annette DeMaria, P.E., Principal Engineer,
Environmental Consulting & Technology, Inc.

Deb Caraco, P.E., Senior Watershed Engineer,
Center for Watershed Protection

Steven R. Corsi, Research Hydrologist,
U.S. Geological Survey

Julie Kinzelman, Ph.D., M.S., M.T., Laboratory Director / Research Scientist,
City of Racine, WI

Barry Liner, Ph.D., P.E., Chief Technology Officer and Sr. Director, Water Science & Engineering Center,
Water Environment Federation

Sandra L. McLellan, Ph.D., University of Wisconsin-Milwaukee, School of Freshwater Sciences

Lisa McFadden, Director, Integrated Technical Programs and Associate Director, Water Science & Engineering Center,
Water Environment Federation

Cheryl Nenn, M.S., Riverkeeper
Milwaukee Riverkeeper

For further information, please contact:

Steven R. Corsi
Research Hydrologist
U.S. Geological Survey
Upper Midwest Water Science Center
8505 Research Way
Middleton, WI 53562
email: srcorsi@usgs.gov

Sandra McLellan
Associate Editor, NPJ Biofilms and Microbiomes
Professor
School of Freshwater Sciences
University of Wisconsin-Milwaukee
600 E. Greenfield Avenue
Milwaukee, WI 53204
email: mclellan@uwm.edu
<http://home.freshwater.uwm.edu/mclellanlab>

PREFACE

The U.S. Geological Survey, the University of Wisconsin-Milwaukee, and the Water Environment Federation in cooperation with the Great Lakes Protection Fund gathered substantive feedback through engagement during a Knowledge Development Forum (KDF) on detection of wastewater contamination for targeted remediation. The event provided opportunity for subject matter experts and interested parties to openly discuss new technology and management practices. Unlike a traditional workshop, the KDF provided an interactive gathering of stakeholders brought together to develop new knowledge, identify collaborative efforts to bridge gaps, and facilitate adoption of new and better ways of problem solving in the water sector. Through a series of highly interactive discussions, experts in the field provided a platform for evaluation and dissemination of information gleaned from recent studies with a focus on:

1. Wastewater detection methods,
2. Wastewater detection methods round table discussions with the KDF participants,
3. Practical applications and future technologies, and
4. Practical applications round table discussions with KDF participants.

OVERVIEW

Wastewater contamination from illicit discharges and leaking sewer infrastructure in the Great Lakes remains a serious source of pollution in tributaries and nearshore waters. These situations result in substantial surface water contamination, and once located, are considered a high priority by municipalities for rapid repair. One of the major barriers for municipalities responsible for mitigation of these contamination sources is locating them in a time-, labor- and cost-efficient manner. Stormwater sewer systems capture runoff from streets, parking lots and rooftops and discharge water directly to rivers. An illicit connection between the sanitary and the stormwater system is expensive and time intensive to locate with standard methods. The Detection of Wastewater Contamination KDF provided an opportunity for industry leaders to collaborate and discuss current techniques and parameters used to identify wastewater contamination sources, the vision of improvements to technology and practices, and next steps, which are catalogued and highlighted in this document.

WASTEWATER CONTAMINATION DETECTION

KDF introductory presentations provided an overview of current methods for wastewater detection in different spatial contexts for short-term and long-term management goals.

HUMAN BACTERIAL MARKERS –

SANDRA L. MCLELLAN, PH.D., UNIVERSITY OF WISCONSIN, MILWAUKEE,
SCHOOL OF FRESHWATER SCIENCES, GREAT LAKES WATER INSTITUTE

To assist in framing the KDF discussion, Dr. McLellan provided the following summary of human bacterial markers, the how, what, and why of microbial source tracking through fecal pollution.

All animals and humans have *E. coli* in their gut including dogs, birds, people, and agricultural animals. Fecal pollution sources such as leaking wastewater and farm run-off are more likely to contain pathogens. For 100 years, easily cultured organisms have been used to track sanitation concerns, starting with *Bacillus coli*, then later total coliforms and fecal coliforms. *E. coli* and enterococci are currently the most common indicators of fecal pollution and have been recommended for use by the USEPA since 1986. *E. coli* and enterococci reliably indicate fecal pollution since they are present in almost all animals and are

easily detectable. However, there are problems with the use of these organisms, as they do not differentiate the source. Human sources have a high likelihood to contain human pathogens, and certain animal sources can be of concern also for specific pathogens (*E. coli* O157:H7, *Salmonella* spp.). Additionally, *E. coli* and enterococci can survive and grow in the environment.

E. coli can be detected because this bacterium can easily be grown on media, but there are hundreds if not thousands of other organisms present in pollution sources such as wastewater. An estimated 90% of human gut bacteria are fecal anaerobes and difficult to culture but may be uniquely associated with humans. Next generation sequencing can be used to inventory and identify organisms that are specifically associated with a host. This analysis has not been available until relatively recently, as cloning sequences individually limited the depth to an estimated 1000 sequences per sample, which only will characterize the most abundant organisms. Next generation sequencing has brought in a whole new era in microbial ecology. Today, next generation sequencing can sequence 10,000,000 bacteria per run – 100,000 per sample.

In addition to sequencing an entire sample, specific organisms can be detected using Polymerase Chain Reaction (PCR). This method amplifies a specific sequence unique for the organisms which are being targeted. For bacteria, the 16S rRNA gene is highly conserved and used to distinguish the diverse range of organisms present and is often used as a marker gene to track organisms that are specifically found in a host source of fecal pollution (humans, cows, dogs, etc.). Quantitative PCR (qPCR) is performed by amplifying DNA using fluorescent tags, allowing for more efficiency and improved real-time results.

Table 1: Quantitative PCR (qPCR)

Assay, target, and reference	Primers	Sequence
Enterococci 23S rRNA gene USEPA2012	EnterolF-G	5'GAG AAA TTC CAA ACG AAC TTG3'
	Entero2R	5'CAG TGC TCT ACC TCC ATC ATT3'
	Enterop	5'[6FAM]-TGGTTCTCTCCGAAATAGCTTTAGGGCTA[MGB-NFQ] 3'
Human <i>Bacteroides</i> (HB) 16S rRNA gene of <i>Bacteroides</i> , Templar 2016, modified from Bernhard and Field 2000 and Kildare et al 2007	HF183F	5'ATC ATG AGT TCA CAT GTC CG3'
	HF241R	5'CGT TAC CCC GCC TAC TAT CTA ATG3'
	HF193p	5'[6FAM]-TCC GGT AGA CGA TGG GGA TGC GTT [MGB-NFQ] 3'
Human <i>Lachnospiraceae</i> , genus <i>Blautia</i> (Lachno2) 16S rRNA gene of <i>Lachnospiraceae</i> , Newton et al. 2011	Lachno2-F	5'TTCGCAAGAATGAAACTCAAAG3'
	Lachno2-R	5'AAGGAAAGATCCGGTTAAGGATC3'
	Lachno2p	5'[6FAM]-ACCAAGTCTTGACATCCG [MGB-NFQ] 3'
Ruminant-Specific <i>Bacteroidetes</i> (RumBacR) 16S rRNA gene of <i>Bacteroidetes</i> , Reischer et al. 2006	RumBacR_f	5'GCG TAT CCA ACC TTC CCG3'
	RumBacR_r	5'CAT CCC CAT CCG TTA CCG3'
	RumBacR_P	5'[6FAM]-CTT CCG AAA GGG AGA TT [MGB-NFQ]3'

In some urban areas, runoff models of land use only account for an estimated 10% of the fecal indicator bacteria, which has large implications for estimating Total Maximum Daily Loads (TMDLs). In these instances, it is important to account for these unknown sources of fecal pollution. Urban stormwater has been shown to be a major delivery route of fecal indicator bacteria and is at the forefront for pollution inputs in cities.

Stormwater should be comprised of rain runoff; however, studies have shown there are areas of wastewater intrusion within the stormwater system, thus high levels of fecal indicator bacteria and likely, pathogens. Urban stormwater is collected from streets and roof tops and is released directly into rivers. A recent survey completed by the McLellan lab group, in collaboration with Milwaukee Riverkeeper and the Milwaukee Metropolitan Sewerage District (315 outfalls n=1500 samples), estimates 30% of stormwater outfalls show high and consistent level of untreated wastewater and 8% had very high levels of wastewater. When considering low to moderate levels, greater than 70% of stormwater outfalls had some evidence of wastewater contamination. Evidence of sewage contamination did not coincide with fecal coliform levels and 50% of the outfalls with high fecal coliforms do not have wastewater.

To track these problem areas within a stormwater system, qPCR may be effective; however, it can be very costly, as well as technically challenging. Dr. McLellan noted that the microbiome of humans and animals has host specific organisms and while qPCR is sensitive and specific, there is a need for better approaches for surveys along pipe network since this type of effort requires a large amount of samples and rapid results.

CHEMISTRY TECHNIQUES: STRENGTHS, WEAKNESSES, AND WHERE CHEMISTRY FITS BEST – DEB CARACO, P.E., CENTER FOR WATERSHED PROTECTION, ELLICOTT CITY, MD

Deb Caraco provided an overview of how chemical monitoring techniques can be best used to identify wastewater discharges, along with other illicit discharges to the storm sewer system or stream network, and the conditions where chemical monitoring is effective at identifying these discharges. The presentation drew largely from the 2004 Center for Watershed Protection guidance, “Illicit Discharge Detection and Elimination: A Guidance Manual for Program Development and Technical Assessments.” (Brown et al., 2004), and was also informed by field experiences and studies since that manual was originally developed.

Characterizing Illicit Discharges

Illicit discharges include both wastewater discharges, and other discharges, such as industrial wastes, that are illegally discharged to the sewer system, or directly to waterways. The best approach to identify and eliminate these discharges depends on characteristics of the discharge, including the discharge frequency and mode of entry (Table 2) and the chemical content of the discharge (Figure 1). The Discharge Frequency is critical to determining how difficult it is to identify a discharge, and is also an indicator of the potential for a discharge to contribute pollutants to water resources:

- Continuous discharges are constantly flowing, such as a broken sewer main that discharges to the storm drain system. These discharges are the most easily captured through regular outfall or instream monitoring, and typically have the potential to contribute the greatest amount of pollution because of their constant nature.
- Intermittent discharges flow certain times of the day, only on certain days, or intermittently throughout the day. Most discharges fall into this class and may include an individual home or building that is cross-connected to the storm drain system, or other discharges that occur only at certain times. There are techniques to capture these discharges through monitoring, but more vigilance is needed. These discharges also have the potential to contribute large pollutant loads, depending on the volume and frequency of the discharge.
- Transitory discharges are very rare or “one time” discharges such as spills or other rare events. While these discharges can have a large short-term impact, they are typically not easily identified through monitoring. A combination of adequate spill-response and educational programs are best suited to addressing these pollution sources.

Discharges can also enter the system either directly (i.e., through a straight pipe entry, or through a cross-connection), or indirectly, such as by wastewater or another contaminant entering the stormwater system or a stream by seepage through soils or groundwater. This distinction is important because discharges that enter the system indirectly are more difficult to detect using traditional monitoring techniques and are better detected using more sophisticated techniques that more suited to identify diluted contamination sources.

Table 2: Characterizing Illicit Discharges

Characterizing Illicit Discharges		
DISCHARGE TYPE	FREQUENCY	MODE OF ENTRY*
Continuous discharges	Occur most or all of the time	Direct and/or Indirect
Intermittent discharges	Occur over shorter/more limited time period	Direct and/or Indirect
Transitory discharges	Occur rarely and without predictable frequency	Direct and/or Indirect

* / Direct includes direct cross connection and/or straight pipe entry
 Indirect includes groundwater seepage and/or septic tank overland flow

Although the most common source of contamination in illicit discharges is wastewater, other sources may contribute as well, such as washwater, either from a single sink or wash machine, or from an industrial source. If flow is detected at a storm drain outfall between storm events, and is a suspected illicit discharge, it is also useful to identify the source as either groundwater (i.e., naturally occurring flow) or tap water. Although tap water released from water main pipes is not typically identified as an illicit discharge, cities try to identify water system leaks to reduce drinking water waste or, for large leaks, to prevent contamination with tap water.

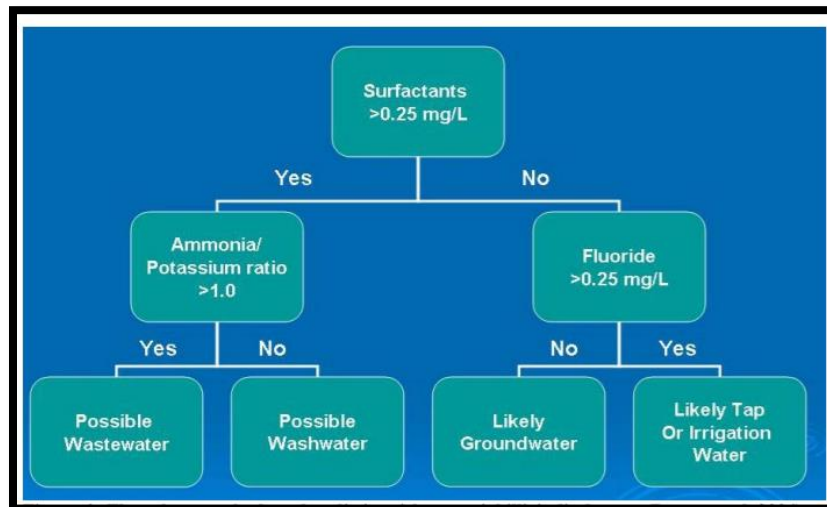


Figure 1: Example of manual decision tree for determination of likely sources (derived from: Brown, et al. 2004).

Appropriate Chemical Parameters

For field work, ideal field parameters are portable, achieve quick results, have safe reagents and are conservative (i.e., do not degrade in the environment). In addition, the ideal parameter has a relatively high concentration in the type of discharge being identified. For example, surfactants (found in detergents) are excellent at distinguishing an illicit discharge (wastewater or washwater) from other natural waters or tap water, because surfactants are not typically found (or are not detectable) in tap water or groundwater. Similarly, fluoride can often be used to distinguish tap water from groundwater, since tap water is fluoridated in most communities. Unfortunately, no single parameter can meet all of these criteria, so a combination of different parameters is often the best approach.

Figure 1 represents a “flow chart” approach (from Brown, et al., 2004) that was proven effective based on a large dataset from Birmingham, Alabama. This approach is fairly robust but can be adjusted based on local data. For example, some communities may not add fluoride to tap water, so a different parameter may be appropriate. Further, alternative parameters may be effective in some settings. For example, boron has shown some promise as a potential washwater indicator (Brown, et al., 2004), but has not been widely used in the field.

In practice, the “flow chart approach” employs simple, immediate field parameters that can identify potential discharges quickly so that field crews can attempt to track down the source of the discharge. Although ammonia is not conservative because it volatilizes quickly, it can be a good, quick indicator of potential wastewater discharges, and typically can be useful for tracking a discharge to its source in the storm drain system. Some typical parameters and their uses are identified below.

Table 3: Typical Chemical Field Parameters for Illicit Discharge Detection

Typical Chemical Field Parameters for Illicit Discharge Detection			
PARAMETER	USES	ADVANTAGES	DISADVANTAGES
Ammonia	<ul style="list-style-type: none"> • Good single indicator of wastewater • Can be used in the “flow chart technique” to distinguish wastewater from other discharges 	<ul style="list-style-type: none"> • Quick results. • Can be analyzed in the field 	<ul style="list-style-type: none"> • Volatilizes easily • May not be sensitive enough to capture dilute or distant discharges • Natural sources can confound results
Boron	<ul style="list-style-type: none"> • Potential indicator of any discharge containing detergents 	<ul style="list-style-type: none"> • Safe reagents, and alternative to surfactants 	<ul style="list-style-type: none"> • Boron is present in natural waters in some areas • May not be detected in dilute discharges
Chlorine	<ul style="list-style-type: none"> • Identify tap water presence 	<ul style="list-style-type: none"> • Simple field test 	<ul style="list-style-type: none"> • Volatilizes extremely quickly
Fluorine	<ul style="list-style-type: none"> • Identify tap water • Used as a flow chart parameter 	<ul style="list-style-type: none"> • Simple test 	<ul style="list-style-type: none"> • May not be effective in all communities, depending on natural waters concentrations and fluoridation policies
Nitrogen/ Phosphorus	<ul style="list-style-type: none"> • An in-stream parameter to identify large-scale wastewater contamination 	<ul style="list-style-type: none"> • Fairly easy to measure. • Often monitored as a part of other efforts 	<ul style="list-style-type: none"> • Not a very useful parameter for outfall monitoring • Other nitrogen and phosphorus sources are common
pH	<ul style="list-style-type: none"> • Identifies some industrial discharges 	<ul style="list-style-type: none"> • Very easy and quick to monitor 	<ul style="list-style-type: none"> • Rarely detects discharges in practice
Potassium	<ul style="list-style-type: none"> • Excellent indicator of industrial discharges • “Flow Chart” parameter 	<ul style="list-style-type: none"> • Easy to use • Robust indicator of industrial discharges 	<ul style="list-style-type: none"> • Not very useful as a “stand alone” parameter in the absence of other indicators • Natural sources can be present

Surfactants (MBAS)	<ul style="list-style-type: none"> • Good indicator of any discharge containing detergents 	<ul style="list-style-type: none"> • Not found in natural waters • Found in almost all detergents 	<ul style="list-style-type: none"> • Hazardous reagents • May not detect very dilute discharges
--------------------	---	---	---

When to Use Chemical Monitoring

Chemical monitoring can be incorporated as a part of an overall effort toward identifying wastewater discharges, but it is not as sensitive as some of the more refined microbial tracking methods. Overall, the relative speed of chemical monitoring becomes more advantageous as the monitoring gets closer to the source of the discharge. While instream chemical monitoring can identify general trends, it can usually only identify persistent or very large discharges. By contrast, sampling at outfalls and moving closer to the discharge itself, chemical monitoring becomes more advantageous.

Table 4: Chemical Monitoring, Strengths and Weaknesses

Chemical Monitoring, Strengths and Weaknesses		
LOCATION	STRENGTHS	WEAKNESSES
In-Stream	Can assist in tracking progress or identifying large discharge	Better detection using bacteria
Outfalls	Beneficial tool for identifying discharges at flowing outfalls	During repairs, less reliable in locating issues at outfalls
Storm Drain Network	Useful in tracking discharges to a source within pipeline(s)	
All locations	Provides for immediate results that allow for rapid community response in locating discharge source(s)	Not very useful for indirect discharges, or for very infrequent discharges, many of these chemicals are not unique to wastewater, and analytical sensitivity may limit the ability to detect wastewater presence

Conclusions

Chemical monitoring can be a useful part of an overall program to identify and eliminate wastewater discharges, as well as other industrial discharges. At the same time, it cannot be relied on alone to detect all discharges, and particularly those that are very small volume, intermittent, or diluted by other sources. Chemical monitoring should be used in combination with other techniques, and is most useful for outfall monitoring, and to track sources through the storm drain network. In these settings, the speed and relatively low cost and flexibility of chemical monitoring are advantageous.

CANINE SCENT TRACKING – CHERYL NENN, MILWAUKEE RIVERKEEPER

Cheryl Nenn, Milwaukee Riverkeeper, provided a synopsis of results pertaining to the use of dogs in wastewater detection. The project results were provided for 2012 and 2014 sampling, using Environmental Canine Services.

In 2012, three stormwater drainage areas were used for the project sampling. In 2014, 10 stormwater drainage areas within the Menomonee watershed, the subwatersheds of Underwood Creek, and the Kinnickinnic watershed were used for the project sampling.

The 2012 results, using two dogs (Figure 2), provided accurate identification of wastewater when compared to 12 lab-tested samples. Samples in the lab were tested for human *Bacteroides*, and *Lachnospiraceae* using qPCR methods. The dogs did not detect wastewater at three locations in which the lab positively identified wastewater (one of these locations had a relatively low detection level, another location had heavy petroleum odor, and the third had evidence of recent dye testing). Additionally, only one dog identified wastewater at a location that the lab positively identified human wastewater. Overall, positive and accurate detection occurred approximately 75% of the time.

The 2014 results, using one dog, provided correct identification of wastewater in six locations that also were positive for wastewater using lab analysis. One sample in which the dog did not correctly detect wastewater was high in human *Bacteroides*, and *Lachnospiraceae*, but low in enterococci and *E. coli*. Similar to 2012, the dog correctly detected wastewater in 75% of the samples taken.

Overall, the dogs correctly detected wastewater in 18 of 24 samples. Canine use in the detection of illicit sources of wastewater is cost effective and the dogs can sample a large area if the humans are well-organized. On the other hand, dogs can get sick and be unable to detect scents as expected and may not be located in close proximity to the project area. Overall, canine scent tracking proved to be a reasonable choice as a screening tool for detecting wastewater contamination in stormwater systems.

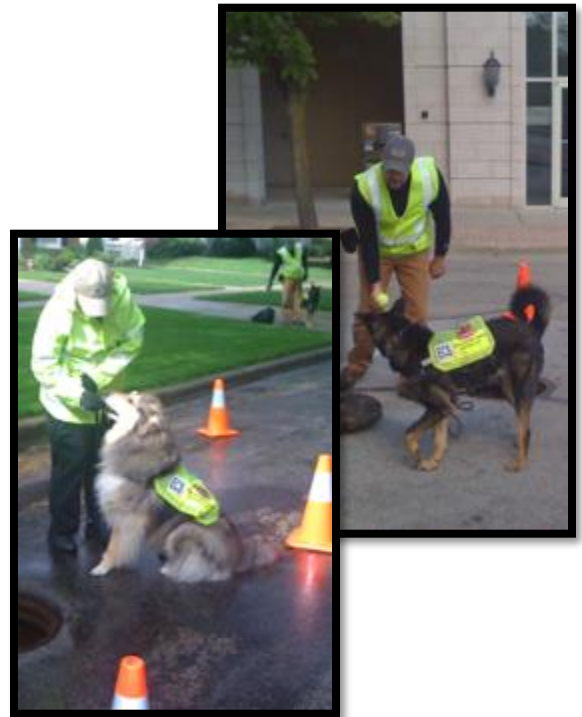


Figure 2: images reproduced with permission, Nenn, C. (2018) "Canine Scent Tracking for Human Wastewater," Milwaukee Riverkeeper

BACKGROUND

Wastewater contamination from illicit discharges and leaking sewer infrastructure in the Great Lakes remains a serious source of pollution in tributaries and nearshore waters. Construction of wastewater infrastructure often includes misconnections into the storm sewer system, and many metropolitan areas in the U.S. have an aging sanitary sewer infrastructure with failures in the system that can result in exfiltration of wastewater. One study by the United States Environmental Protection Agency reported between 12% and 49% of wastewater flows are lost due to leaking infrastructure (Amick, et al., 2000). Contaminants found in wastewater including toxic compounds, pathogens, nutrients, pharmaceuticals, and hormones and other endocrine disruptors can have a substantial effect on the aquatic ecosystem. These situations result in substantial receiving-water contamination, but once located, are considered a high priority by municipalities for rapid repair.

A major barrier for municipalities responsible for administering state and Federal Illicit Discharge Detection and Elimination (IDDE) programs for reducing these contamination sources is locating them in a time-, labor- and cost-efficient manner. Wastewater leaking outward from cracked pipes (exfiltration) or entering storm sewers from illicit connections can migrate into the stormwater system, which acts as a very effective conduit to deliver leaking wastewater into streams. However, the point of entry of wastewater into the stormwater system is very difficult to locate with standard methods. Each outfall represents an area of several acres to hundreds of acres that is drained by a complicated network of storm sewers and/or ditches. In addition, the complex mixture of factors that influence sanitary wastewater systems and the dynamics of urban hydrology and urban stormwater conveyance systems result in highly variable concentrations of wastewater in receiving streams. Defining the quantity, timing, and location of wastewater contamination is a very challenging task that would benefit from new tools that offer improved efficiency in measurement of these parameters.

Optical sensors: Human wastewater has distinct optical properties that are different than those typically observed in natural waters. Optical property analysis includes measurement of fluorescence and absorbance spectra that serve to characterize the composition of dissolved organic matter (DOM) in water (Fellman, et al., 2010). There are many sources that contribute to DOM in natural waters that influence optical property signals. Research has previously been conducted to identify signals that predict wastewater presence in natural waters, but these studies have typically been limited in scope to single study areas and have not been examined thoroughly for transferability to other geographic regions. Identifying reliable optical signals that can predict the presence and relative magnitude of wastewater in real time would provide an additional tool to help improve efficiency for practitioners aiming to define contamination and locate sources.

OBJECTIVES

The overall objective of this research is to identify optical signals that could be designed into a real-time sensor system for detection of wastewater in surface waters. Specific objectives include: (1) to characterize the optical properties in stormwater conveyance systems and surface-water samples in a diverse set of geographic and watershed settings, (2) to define wastewater presence and magnitude by concurrent analysis of human-specific bacteria, (3) to identify optical signals that best serve as surrogates to predict wastewater contamination for development of field sensors, (4) to conduct this research using samples that represent multiple settings including: variable land use, variable hydrologic conditions, through different seasons, and at multiple watershed scales, to define limitations of such signals, and (5) to provide results to stakeholders for development of sensor systems that can detect wastewater presence in real time.

STUDY APPROACH

Samples were collected from areas around the Great Lakes at three different drainage basins scales (figure 3): (1). Watershed scale: Eight tributaries of the Great Lakes were sampled in large watersheds with a gradient of urban to agricultural land use, (2). Subwatershed scale: Three locations representing subwatersheds were sampled on the Menomonee River in the Milwaukee Metropolitan area, and (3). Very small scale: Multiple storm sewers and ditches (n = 163) were sampled in each of three small subwatersheds in different geographic locations in the Great Lakes region: The Kinnikinnic River in Milwaukee, the Middle Branch of the Clinton River in Macomb County, Michigan, and Red Creek in Monroe County, New York.

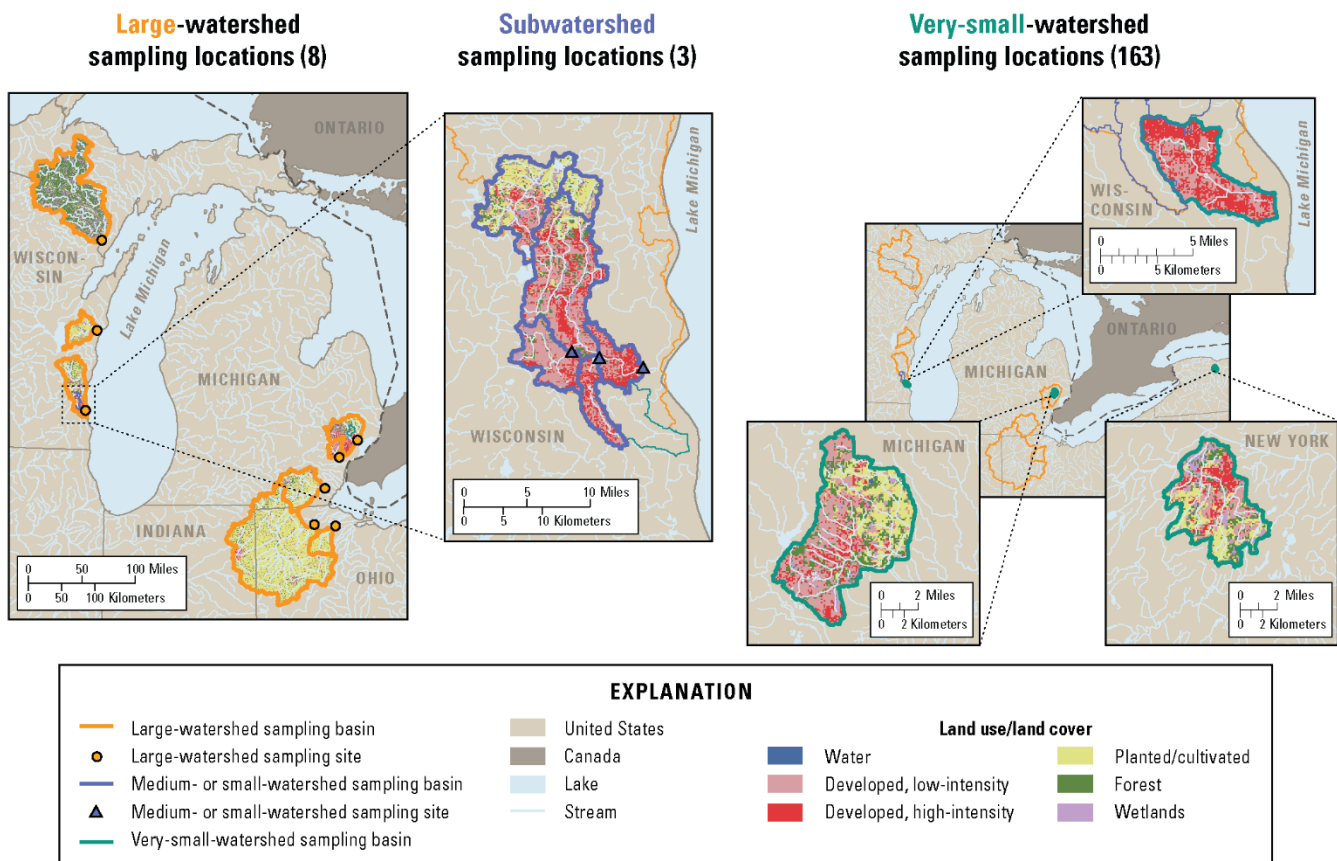


Figure 3: Study locations for three spatial scales in the Great Lakes region

Figure 3. Map of study locations for three different spatial scales in the Great Lakes region. Map composed of various spatial datasets: state and political boundaries (Instituto Nacional de Estadística Geografía e Informática, The Atlas of Canada, and U.S. Geological Survey 2006b), hydrography (Instituto Nacional de Estadística Geografía e Informática, The Atlas of Canada, and U.S. Geological Survey 2006a; National Atlas of the United States 2005), land cover, and watershed boundaries (U.S. Department of Agriculture-Natural Resources Conservation Service, U. S. Geological Survey, and U.S Environmental Protection Agency 2009).

SAMPLING DESIGN

Samples at all three watershed scales were collected during low-flow periods and runoff-event periods (rainfall and snowmelt) to capture variable hydrologic conditions and throughout each season of the year to capture seasonal variations. Samples were analyzed for optical properties of water (fluorescence and absorbance spectra measured in a laboratory setting) and two human-associated bacteria markers (human *Bacteroides* and human *Lachnospiraceae*).

At the large watersheds and subwatersheds, samples were collected to represent conditions throughout the full sampling period which included 24-hr flow-weighted composite samples for low-flow periods, and flow-weighted composite samples collected throughout the period of increased streamflow for runoff-event periods. The large watersheds were sampled over a two-year period for a total of 236 samples and 127 samples were collected in subwatersheds over a four-year period. At the very small-scale watershed sampling locations, discrete grab samples were collected by peristaltic pump or direct bottle submersion. A total of 593 samples were collected during low-flow and runoff-event periods at the very small-scale sites over a two-year period. All data are available in the U.S. Geological Survey National Water Information System at <https://waterdata.usgs.gov/nwis>.

STATUS OF RESEARCH

Human-associated bacteria results indicated a wide variation in the contamination level among the large watershed- and subwatershed-scale samples, with human-associated bacteria concentrations between <225 to 2.6×10^6 count number/100ml (Figure 4). This wide variation in wastewater content collected over multiple geographic regions, all seasons, and variable hydrologic conditions provides the needed information to assess the quality of relations between bacteria and optical signals and to better understand potential for applicability in different settings and environmental conditions.

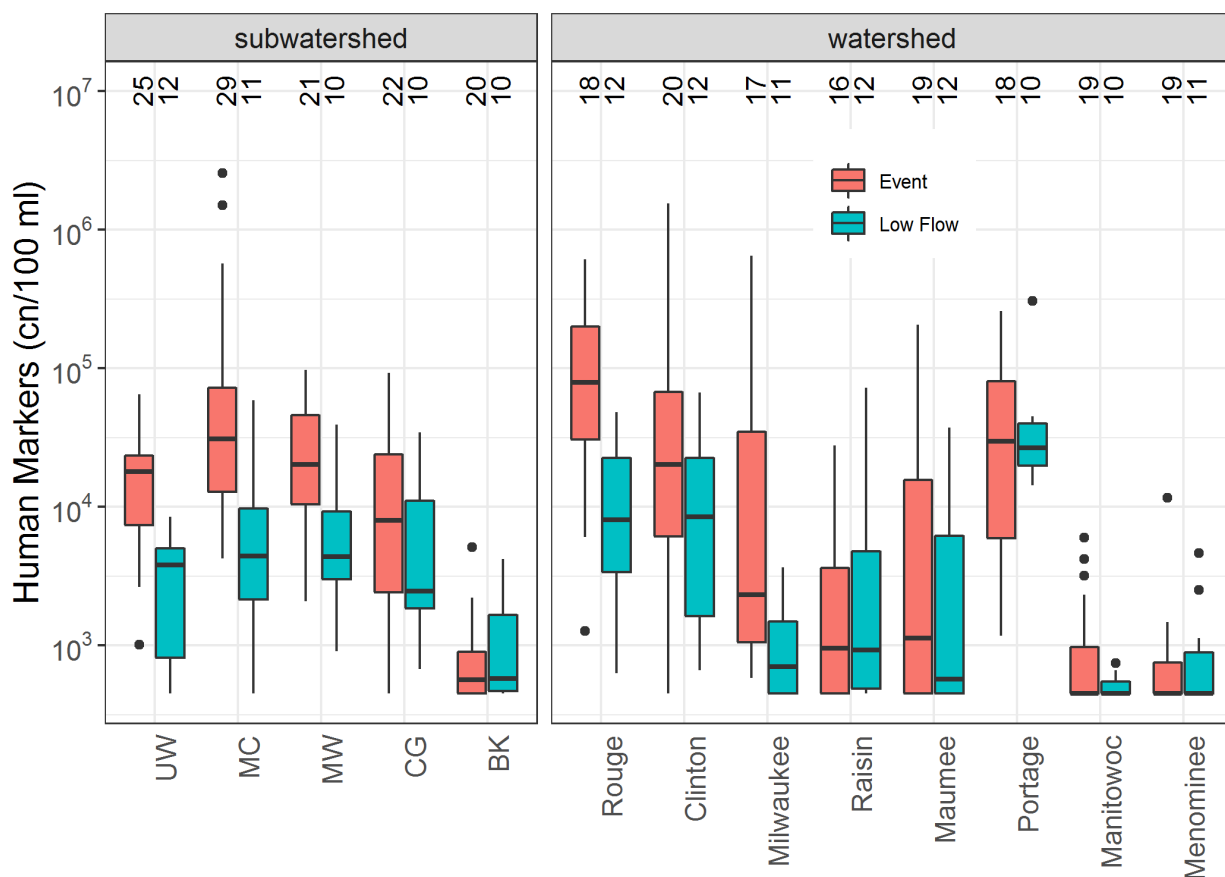


Figure 4: Sum of human-associated bacteria markers *Bacteroides* and human *Lachnospiraceae* at watershed- and subwatershed-scale sampling locations. UW, Underwood Creek; MC, Menomonie River at 16th Street; MW, Menomonie River at Wauwatosa; CG, Milwaukee River at Cedarburg; BK, Bark River; cn, count number. Data availability at <https://waterdata.usgs.gov/nwis>.

The next step planned for this research is to determine which optical signals (fluorescence, absorbance, or a combination of the two) look to be promising predictors of human-associated bacteria. Various regression techniques are planned to be used

to explore these relationships. Given that there are more than 6000 optical signals that were generated with each sample analysis, several steps will be taken to reduce the number of potential signals to a number that would be practical for development of a field sensor system. First, common optical signals that currently exist as field sensors will be examined. This includes sensors that target fluorescence signals such as “tryptophan-like” fluorescence, “colored dissolved organic matter (CDOM)”, and “optical brighteners” because sensors for these signals already exist from several different manufacturers. If regressions of sufficient quality cannot be developed with existing sensor signals, the next step would be to explore additional signals with alternative regression techniques along with more advanced “variable selection” procedures.

Data are planned to be analyzed by various factors to determine potential transferability of results among different watersheds, regions, and drainage-area scales. This will include analysis for different geographic regions and specific areas within a geographic region, different hydrologic conditions, different seasons, and by grouping study sites into those with similar dissolved organic matter composition based on fluorescence spectra. Ultimately, the goal is to reduce the number of optical signals down to a number that would be practical to implement in a field sensor system, and to use optical signals that are effective at multiple or all studied settings. Such a sensor system could be deployed in a fixed location for defining wastewater contamination concentration and loadings in streams, or as a mobile unit to track contamination and help identify source locations.

ADDITIONAL BACTERIA

Although the objective of this project was to identify optical signals to detect wastewater contamination, many stakeholders are interested in the presence and magnitude of fecal indicator bacteria (FIB) as well. The presence of FIB does not necessarily indicate wastewater contamination but is thought to provide information on more general fecal contamination that includes human and non-human sources. In addition to the human-associated bacteria, two genetic markers that represent commonly measured FIB were quantified (*E. coli* and enterococci) concurrently with the samples for optical properties. This optical properties data set will be used to assess the potential for the use of selected optical signals as surrogates for FIB estimation with the same techniques as those used with the human-associated bacteria. This information would expand the utility of this data set beyond the initial intent for limiting analysis to human-specific contamination.

ROUNDTABLE DISCUSSION SYNTHESIS RE: WASTEWATER DETECTION METHODS

The following questions and responses were asked of the participants during the KDF session. Please note that the responses do not necessarily represent the full spectrum of possible responses to these questions, but rather represent only those highlighted during the KDF discussion period. For instance, costs attributed to techniques and methods are estimates based on participant responses and may not accurately reflect actual costs.

WHAT TECHNIQUES ARE USED WITHIN EACH GROUP AND HOW? LIST ADVANTAGES AND LIMITATIONS AND COST FOR EACH METHOD. ARE THERE METHODS NOT REPRESENTED HERE THAT SHOULD BE, AND WHAT RESEARCH IS NEEDED?

The following techniques identified provided associated benefits and limitations, as follows:

Table 5: Techniques for detection of wastewater contamination, pros and cons of each method, and relative cost

TECHNIQUES		PRO	CON	COST
FIELD-BASED PHYSICAL				
1.	Dye testing of homes/facilities	Identifies the specific source	Home/facility access coordination is time consuming and sometimes difficult to secure with private property owners	\$\$\$
2.	Smoke testing/ Televising	Accepted, accurate, can cover a large area fairly quickly	Coordination, time, staff, competing priorities, generally still need to do a dye test.	\$\$\$\$
3.	Acoustic	Cheap, fast, low-tech	Not widely applicable; major issues are favored	\$(labor \$)
4.	Canine Scent Tracking	Rapid results, good geographic range, facilitates visual inspection, cheap screening tool	Capacity, credibility, service provider, mobility, accuracy, non-specific	\$ to \$\$\$/ (economies of scale)
FIELD-BASED CHEMICAL				
5.	Mobile chemical	Easy collection, quick results	Qualitative, data quality, reportability, cross reactive with non-wastewater sources, false negatives	\$
6.	Optical Brighteners	Simple, quick	Non-specific (not targeted, interference), confidence	\$
7.	Surrogate	Simple real-time measurements, prioritize regions of watersheds	Time for calibration, non-biological par., scalability, distribution, limited application	\$\$\$
8.	Optical Sensors	Fluoresce very high measurement resolution; real-time collection	Non-standard yet non-specific (needs more work) – site specific utility	\$ - \$\$\$
LAB-BASED CHEMICAL				
9.	Lab-based chemical	Sensitivity, specificity, and reportability	Time, static (snapshot) rear view, cross reactive with non-wastewater sources, false negatives	\$ - \$\$
10.	Pharmaceuticals and personal care products (PPCPs) /Artificial Sweeteners	Very specific, source specific, credibility, reportability/accepted widely	Time, staffing, expertise, lab equipment, interpretation, detection levels	\$\$\$

MICROBIOLOGICAL				
11.	Rapid Coliform Test - 3M (single type)	Can be done in the field; no lab needed	Non-specific source, background sources exist, less effective with intermittent flows, limited dynamic range	\$
12.	Lab-based microbiological (fecal indicators)	Widely used procedure, established, existing data, EPA risk factors established, established standards	Non-specific source, background sources exist, less effective with intermittent flows, limited sample hold time	\$
13.	Biosensor	Very specific, rapid, configurable	Sensitivity/ chemical oxygen demand (COD), needs vetting, credibility	\$\$
14.	Human bacteria (Genetics Testing) <ul style="list-style-type: none"> • Lab-based PCR method 	Very specific, source specific, credibility, reportability/accepted widely	Time, staffing, expertise, lab equipment, interpretation	\$\$\$
15.	Sequence-based genetic testing (Illumina MiSeq)	In-house, rapid, project level results (prioritize)	Initial Capex; not quantitative, high level of expertise needed for interpretation	\$\$\$ (excluding Capex at onset)

WHAT INFORMATION IS NEEDED FOR INVESTIGATION AND WHAT INFORMATION IS NEEDED FOR ACTION TO BE TAKEN?

The following information was provided by KDF participants as needed for investigation and action to be taken:

- Information on the following items are beneficial to have in advance of investigating sanitary contamination of stormwater:
 - Groundwater levels and flow direction
 - Reporting of sanitary sewer overflows (SSOs) or combined sewer overflows (CSOs)
 - Age of the sewer system
 - Age of pipes
 - Bypass points in the system
 - Current sewer and stormwater infrastructure maps
 - Most recent inspection information (condition assessments)
 - Monitoring flow/level in collection systems in real-time
 - Basement backups
 - Surcharge conditions
 - Catchment characteristics
- For adaptive management action to be taken (further testing with more expensive methods, smoke testing, etc.) the following were identified in which responses might prompt action:
 - A high confidence in the location of wastewater leakage is needed for a remedy to be implemented
 - Single sample exceedance of a criterion calls for more focused examination to confirm risk and identify source(s).
 - On the laboratory side - confidence should be high (80% or greater) before further action is taken to ensure data are sound.
 - Management action depends on the scale of the problem. Situations with high-risk to human health regardless of confidence, and situations with high confidence should be prioritized.
 - Wastewater contamination levels exceed water quality standard that reflect a risk to human health.

- Sufficient redundancy in monitoring must be done to avoid decisions based on false positives.
- Clear directions are needed regarding what the concentrations mean. Is there an action level? What do non-detects really mean? Was the study design and technology choice appropriate for the question being asked?
- Prioritization of affected water bodies based on potential risk to exposure can help direct resources.
- Improved indicators for human sources are still needed. E.coli still has significant nonhuman sources. More research on levels of nonhuman sources that may be a health risk is needed. The frequency and type of human contact with potentially contaminated water should also be considered.
- In coordination with human health risk information, cost-benefit analyses are valuable to leverage available funds when working in systems with multiple potential deficiencies.

The most effective wastewater contamination detection technique or combination of techniques identified by the participants. The best contamination technique or combined technique depends on the context, including risk to human health, and the goal of the investigation. With this in mind, some effective methods are listed below.

- Host-specific markers (qPCR, dPCR) combined with mass balance approaches;
- Dye testing from potential sources for detecting illicit connections;
- Bacteria testing (E. coli, other emerging rapid tests) for surface waters;
- Enzymatic testing (these tests can detect viable organisms that are not actively dividing);
- The use of microbial source tracking can assist in looking for a source, but cost of testing and expertise may limit its applicability at this time

Education is needed so decision makers are well informed about the state of the sciences. These techniques are most useful when they can be applied in the right context and the results interpreted properly. Educational information and other considerations that are needed to empower municipalities and managers to act includes:

- A 'how to document' with successful case studies.
- An understanding of uncertainty in monitoring data.
- Connecting results to human health outcomes and the benefits to the community and watershed.
- Incorporating ratepayers/community members as part of the strategy.
- Realistic preventive maintenance schedules for infrastructure as it ages.
- A need for resilience with respect to climate change including system response to increasing extreme events, clear understanding where citizens interact with water resources, and concentrating evaluation in areas of greatest risk.
- Engaging the community to foster a more proactive management of their systems.
- Communication products that focus on leveraging data to make positive contributions.

HOW DO WE INTERPRET RESULTS?

Participant discussion regarding interpretation of the results led to identification of some important considerations.

Limitations for interpreting results include:

- Canine scent tracking provides only positive/negative results
- Using resulting data to prioritize areas of greatest risk is challenging. Mixed positive and negative results are difficult to interpret.
 - Multiple lines of evidence are needed
 - Methods to treat uncertainty and variability are necessary (parse out seasonality, etc.)
 - Development of a model that considers all factors is needed
- Resulting data from environmental conditions can be highly variable
- Balancing cost, sensitivity, and confidence of methods.

- A tiered approach is beneficial to find hotspots with appropriate methods to screen for contamination with inexpensive methods, and follow up using methods with increased sensitivity and confidence in areas where there is a high likelihood of wastewater contamination
- Competing interests could interpret results differently, for example, different stakeholder may interpret what is “bad” differently.
- There is a gap between research, citizen science, and approved methods (EPA, etc.)
- Improve communication with municipalities on the interpretation of results.

How to Interpret Results:

- Put results into context with the goals of a monitoring program
 - Resources available
 - Consider techniques used and timing of samples
 - Develop manual decision tree for prioritizing areas
 - Use low cost method and follow up with higher cost method where needed
 - Use multiple methods for weight of evidence to develop confidence
- Identify responsible party with great caution.
- Final results should be framed in a way that can be understood by general public/multiple audiences

PRACTICAL APPLICATIONS AND FUTURE TECHNOLOGIES

CASE STUDY 1 – INCORPORATING MOLECULAR TESTING AS AN EVIDENTIARY TOOL IN MUNICIPAL WATER QUALITY MONITORING PROGRAMS – JULIE KINZELMAN, CITY OF RACINE, WI

Dr. Julie Kinzelman, Laboratory Director and Research Scientist for the City of Racine (WI) Public Health Department, provided insights on the way in which the City of Racine incorporates molecular testing in the identification of pollution sources as part of their comprehensive watershed assessment program. An emphasis is placed on visual observation; site surveys (indicators + conditions); and microbial source tracking. Chemical and microbial indicators include: *E. coli*, pH, temperature, turbidity, conductivity, detergents, chlorine, copper, phenols, and nutrients. Fecal source tracking markers include human-specific *Bacteroides* and *Lachnospiraceae*. Utilizing the accumulated data, the City of Racine employs a decision tree approach derived from the correlation between water quality parameters, environmental parameters and physical assessments to guide future actions (Figure 5).

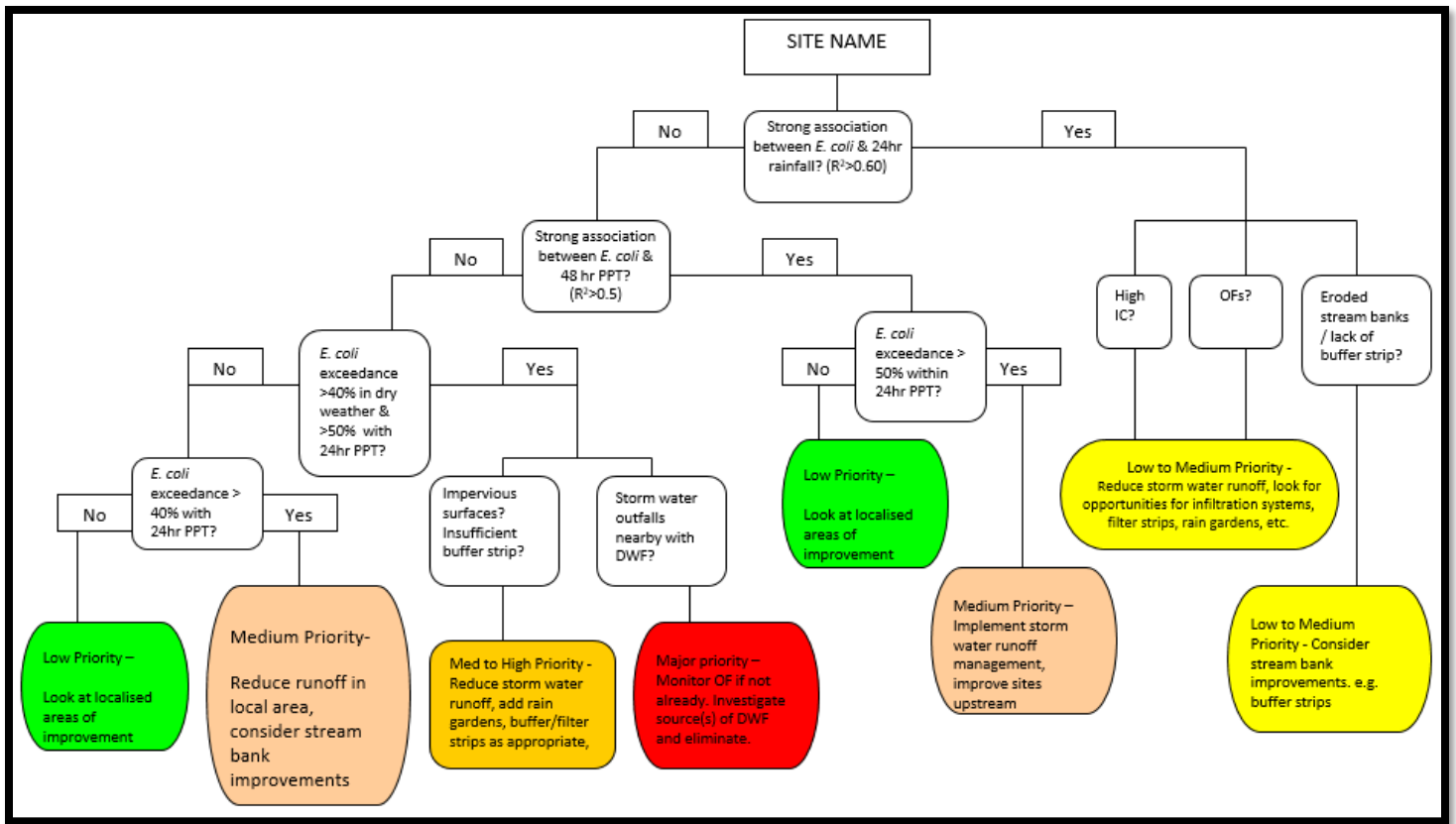


Figure 5: Decision tree template for use of monitoring and observational data to identify contamination sources.

The City of Racine also uses molecular testing to decrease public notification time for recreational waters; provide supportive information for illicit discharge investigations; and develop community profiles (in conjunction with partners at USGS and UWM School of Freshwater Sciences). Utilizing molecular methods for regulatory recreational water quality monitoring provides better public health protection and the ability to respond to changing conditions in near real-time. Microbial source tracking lends to the assignment of fecal indicator bacteria to likely hosts. Profiling assists in capturing relational similarities in environmental samples and may provide common source indications.

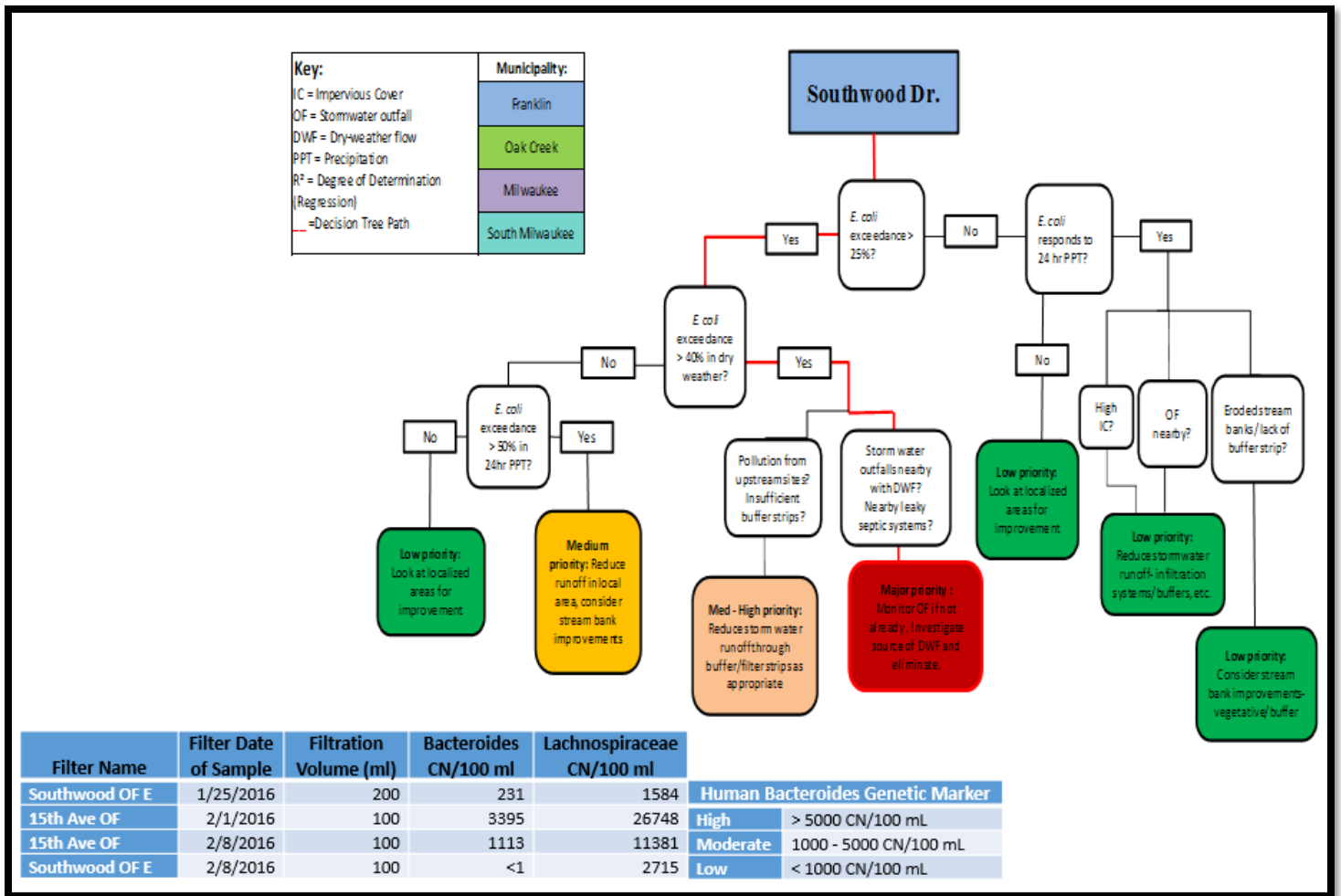


Figure 6: Example of decision tree use to determine likely contamination sources at Southwood Drive in Racine, Wisconsin.

Figure 6 above provides an example of employing the template in the development of a baseline of water quality dataset informative to the Oak Creek Watershed restoration planning process. In this example, *E. coli* concentrations at Southwood Dr. were found to be significantly higher than surrounding locations, and site surveys in conjunction with microbial source tracking pointed to an upstream stormwater outfall as a likely culprit.

In another example from the same study, a tributary located just outside the Oak Creek Watershed boundary had been previously identified as a potential source of impairment to near shore Lake Michigan recreational water quality (Figure 7). Utilizing observational data and site surveys, it appeared that the potential still existed some five years later and that implementing stormwater runoff mitigation would be beneficial. Further information, generated through a community profiling effort in conjunction with UWM School of Freshwater Sciences, indicated that investigation of an upstream lift station might also be warranted.

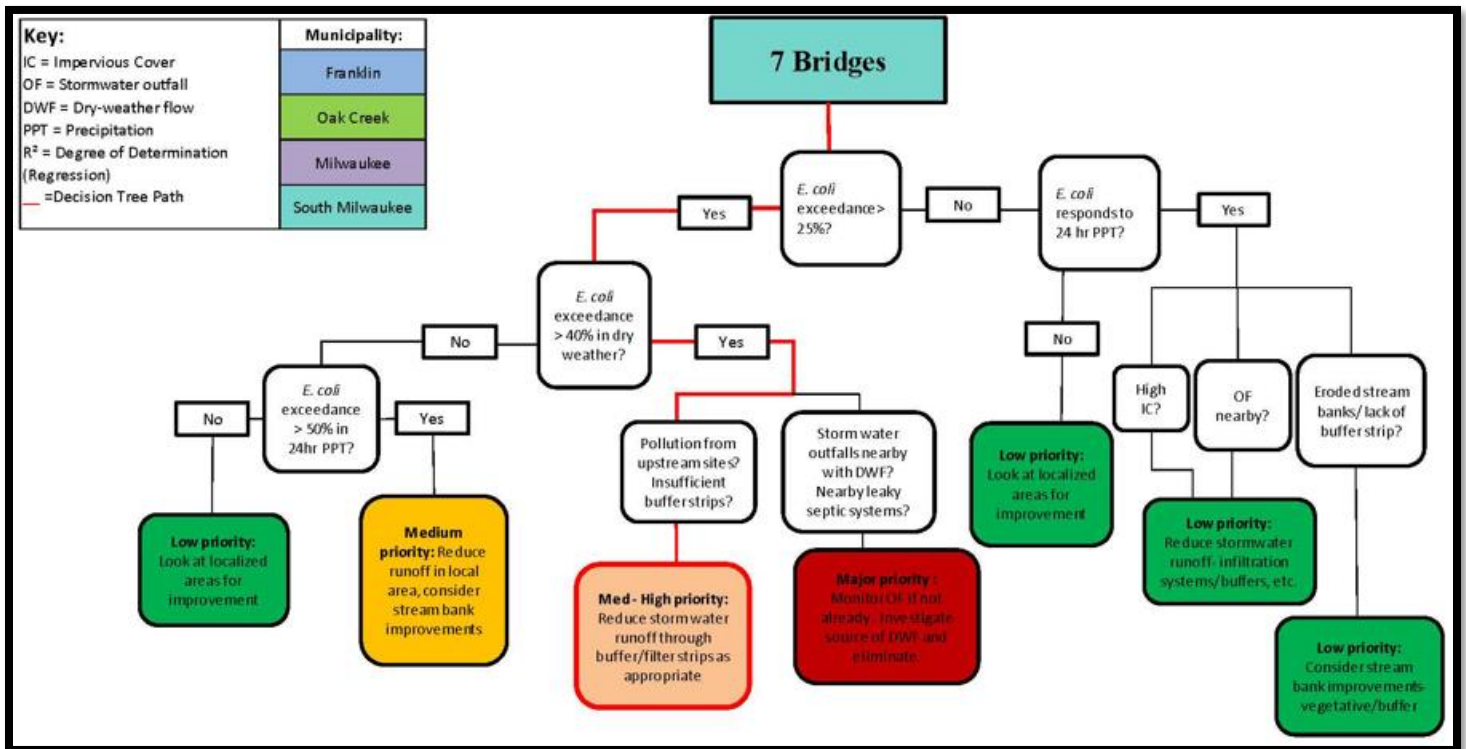


Figure 7: Example of decision tree use to determine likely contamination sources at a tributary to Lake Michigan near Racine, Wisconsin.

Dr. Kinzelman recommends using a stepwise, weight of evidence approach for detection of pollution sources. She routinely employs a toolbox of available methods, including physical assessments, microbial indicators, chemical indicators, alternative and secondary indicators (chemical/optical tracers), Microbial Source Tracking (MST), community profiling, and modeling. While no definitive association (i.e. a “smoking gun”) may result from a single study of limited duration, a snapshot of potential sources is likely to occur, where one can begin to assign relative contribution to various sources, thus focusing future time and money towards that which will result in the greatest improvement.

CASE STUDY 2 – COLLECTION SYSTEM INFRASTRUCTURE MICROBIAL SOURCE TRACKING – DANNY BARKER, ENVIRONMENTAL SCIENTIST, HAMPTON ROADS SANITATION DISTRICT (HRSD)

Hampton Roads Sanitation District (HRSD), a political subdivision of the commonwealth of Virginia, treats southeast Virginia’s wastewater with the mission to protect public health and the waters of Hampton Roads. HRSD serves an area that is over 3,100 square miles and includes 18 cities and counties of southeast Virginia. To serve the 1.7 million people in the service area HRSD works closely with localities to meet their wastewater needs.

As part of the HRSD Vision— “future generations will inherit clean waterways and be able to keep them clean”— a Molecular Pathogen Program was created in the Water Quality Department. A fundamental objective of this program is to perform Microbial Source Tracking (MST) to proactively identify wastewater infrastructure problems (or lack thereof) in our region. Field sample collection, analysis, and reports/recommendations completed by HRSD’s Molecular Pathogen Program are a no cost source identification resource for local municipalities. Program funding is part of HRSD’s annual operating budget.

HRSD’s Central Environmental Laboratory is a NELAP accredited laboratory through the State of Virginia (VELAP) that has been performing MST projects for years and has a state-of-the-art molecular laboratory. The molecular laboratory has several quantitative polymerase chain reaction (PCR) platforms and a digital PCR platform. Internally, HRSD has completed multiple MST projects that highlight capability/proficiency at applying and interpreting molecular host-specific assays.

HRSD began an effort to reduce bacteria contamination in local waterways by proactively identifying and repairing sources of human fecal contamination. To accomplish this objective HRSD has been applying molecular MST tools to stormwater and sewer infrastructure samples with the goal of either identifying compromised infrastructure or defining the fecal contamination origin to a smaller, more manageable area. The backbone of HRSD's source tracking studies has been the implementation of rapid in-house human-associated molecular assays that have high sensitivity and specificity (e.g. Human-associated Bacteroides, detected by the HF183 marker assay). Informed, adaptive results-based decisions used in tandem with local knowledge of sanitary sewer and stormwater maps allow HRSD to trace a human fecal signal through infrastructure to a point of origin.

Due to the large number of potential sites within a collection system's infrastructure, HF183 is used to narrow down and locate compromised infrastructure issues. EPA's HumM2 and/or crAssphage molecular assays are used in tandem with HF183 results to confirm remediation (repair) of infrastructure problems using a weight-of-evidence approach.

The successes of the program include:

- In-pipe infrastructure investigations
 - Elimination of dilution and marker degradation
- Matrix inhibition alleviation
 - Elimination of false negative results
- In-house data turnaround
 - Adaptive sampling design based on prior sample data
 - Knowledge of local marker concentrations
- Engineering tools (e.g. DNA mass balances, infrastructure monitoring)

The program included the investigation of Wayne Creek. Successful downstream to upstream stormwater pipe network investigation and municipality efforts assisted in narrowing contamination findings to their origin, which led to the finding of a cracked pipe (Figure 8).

Wayne Creek Sampling Design

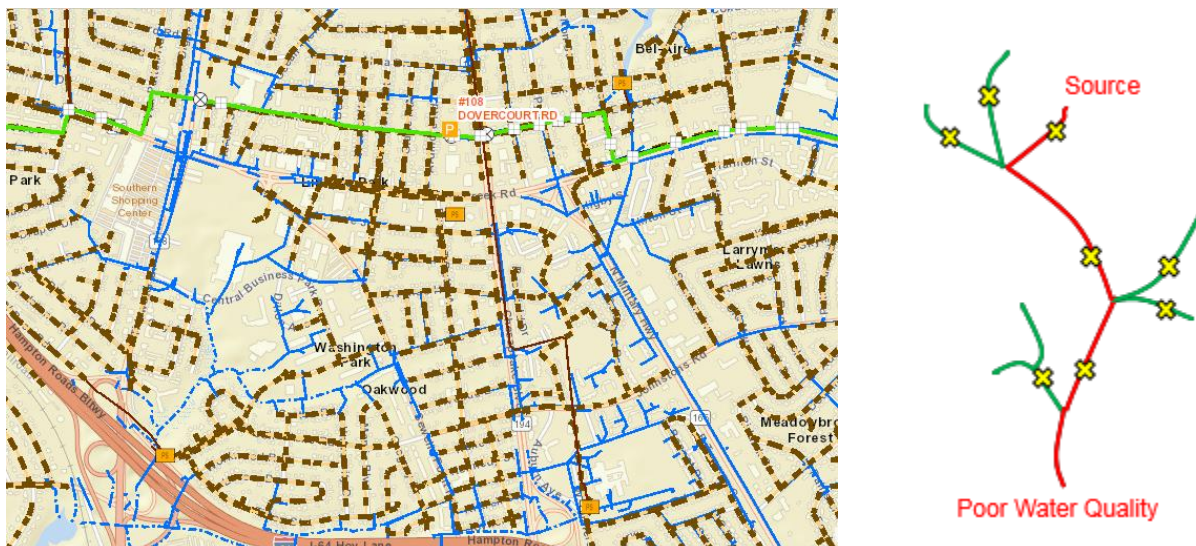


Figure 8: Wayne Creek Sampling Design



Figure 9: Wayne Creek watershed site map used for assisting in location of contamination sources – photographs of the resulting identified cracked pipe as the source.

Additional proactive monitoring efforts are aimed to spatially cover Hampton Roads waterways. An example of these efforts includes the Nansemond River. The Nansemond Watershed drains 161,000 acres of land in Suffolk and Isle of Wight County (Figure 10). Salinity at the mouth of the Nansemond is 15 parts per thousand (brackish) but is only 3 parts per thousand near downtown Suffolk. Human-associated marker surveying provides a snapshot of human contamination hotspots that are used to prioritize remediation.

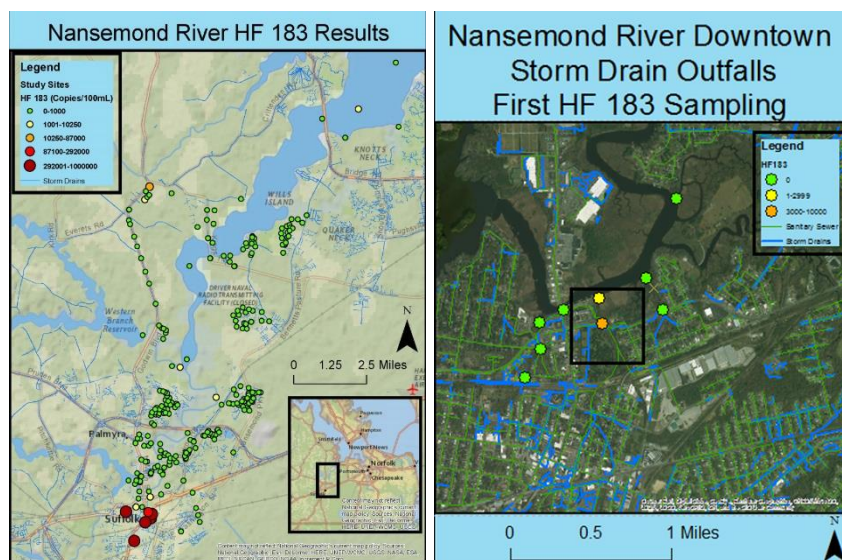


Figure 10: Site map examples for assisting in location of contamination sources in the Nansemond River watershed.

CASE STUDY 3 –ILLICIT DISCHARGE INVESTIGATION EXAMPLES: UNRAVELING THE SPAGHETTI - ANNETTE DEMARIA, P.E., PRINCIPAL ENGINEER, ENVIRONMENTAL CONSULTING & TECHNOLOGY, INC

Traditional investigation techniques, such as visual observations, sampling for FIB, field chemistry kits, sewer inspection video, smoke testing and tracer dye testing assist utility departments in unraveling the underground infrastructure to identify illicit discharges. For example, most of these techniques were employed during the discovery of a 15” sanitary sewer that was illicitly connected to a storm sewer. Complicating this investigation was the existence of multiple utility lines and that the illicit connection occurred as a blind tap – meaning that there was not a manhole structure where the two utility lines were connected (Figure 11). The correction of this illicit connection eliminated 637,300 gallons of wastewater/year from entering the storm sewer system which discharged ultimately into the Clinton River and Lake St. Clair.

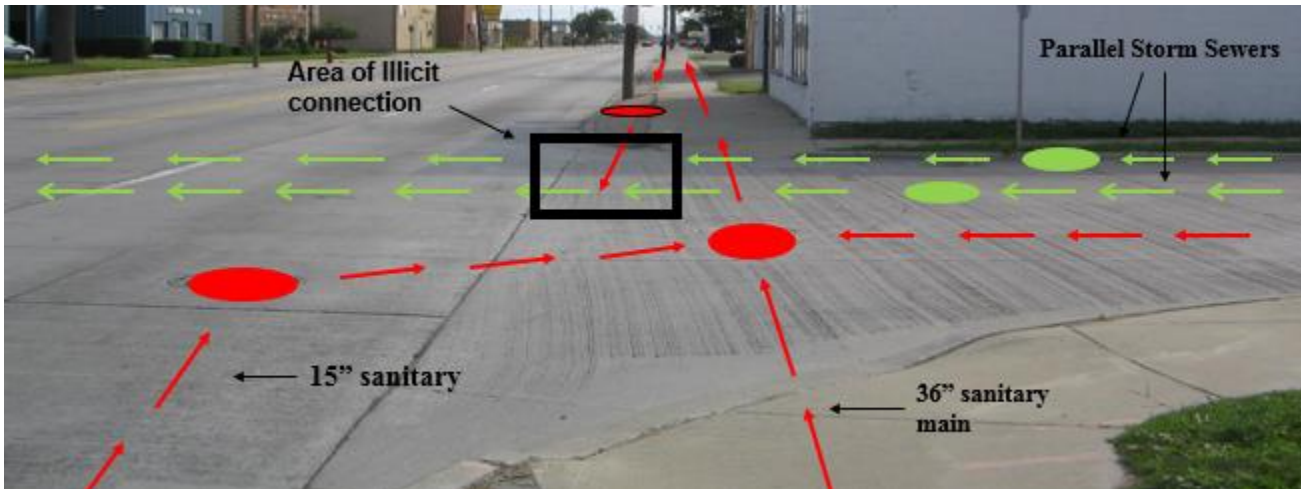


Figure 11: Connection of a Sanitary Sewer to a Storm Sewer via a Blind Tap (Macomb County Health Department) DeMaria, A. (2018) "Illicit Discharge Investigation Examples: Unraveling the Spaghetti," ECT Environmental Consulting & Technology, Inc., Detection of Wastewater Contamination Knowledge Development Forum, presentation slide 5.

Many investigations are time-consuming, because storm sewer maps are often incomplete, there are multiple jurisdictions that own the utilities and there is a lack of sharing of data between jurisdictions. But with perseverance, investigations can lead to corrections that provide positive impact on the communities served. For instance, in a former combined sewer area, a sanitary lead from a restaurant was improperly connected to a storm drain owned by the state department of transportation (DOT) when it should have been connected to the local jurisdiction’s combined sewer. Although not ideal, this was not a problem until the combined sewer was separated. At that point the discharge from the DOT drain (and the wastewater from the restaurant) was directed to a county-owned storm drain which discharged to the Rouge River. This situation went unnoticed for 25 years until the county screened their outfalls for illicit discharges and noted high *E. coli* concentrations (>10,000 MPN/100 mL). After FIB sampling was used to narrow down the origin of the discharge, the drain was televised and the lead from the DOT drain was discovered. However, it was not recognized that the DOT drain conveyed the illicit discharge. Only after dye testing all businesses in the target area was the illicit connection to the DOT drain discovered. To correct the illicit connection, the contractor had to bore under the DOT and county drains to reach the sanitary sewer. The investigation took about 8 months to complete from initial discovery of the high *E. coli* to correction (Figure 12). It was suggested by local staff that the complexity of connecting to the sanitary sewer played a role in why it wasn’t initially completed properly, but this is only speculation.



Figure 12: Map of illicit Discharge Investigation – Multiple Jurisdictions. DeMaria, A. (2018) "Illicit Discharge Investigation Examples: Unraveling the Spaghetti," ECT Environmental Consulting & Technology, Inc., Detection of Wastewater

FUTURE TECHNOLOGIES: MOBILE QPCR, SEQUENCING, AND OTHERS

SANDRA L. MCLELLAN, PH.D., UNIVERSITY OF WISCONSIN, MILWAUKEE,
SCHOOL OF FRESHWATER SCIENCES

Next generation sequencing technologies offer added benefits to finding and remediating issues in collections and conveyance. In screening collection systems, for instance, qPCR using multiple indicators may not be as effective as sequencing approaches that are faster, cheaper, and provide more detailed information. No qPCR marker has 100% specificity. For example, Lachno2 cross reacts with dogs, and human *Bacteroides* (detected with the HB assay) has also had reported cross reactivity with dogs, albeit to a lesser degree. Examples of discrepancies among four human-associated markers is illustrated in the table below.

Table 6: The applications of Lachno3 and Lachno12 assays on environmental samples that were inconsistent in HB and Lachno2 assays results

Sample name	Type	Site	Sample date	HB	Lachno2	Lachno3	Lachno1 2	DogBact	Interpretation of presumptive sources*
				CN /100ml					
FT21217	Rivers	Kinnickinnic River grab	5/3/16	801	27,300	6,510	4,450	0	Human
FT21380	Stormwater	Kinnickinnic River grab	6/7/16	7,500	548,000	173,000	40,500	0	Human
FT20574	Rivers	Kinnickinnic River autosampler	9/8/15	39,700	188,000	75,400	37,300	15,800	Human/Dog
FT21332	Stormwater	Kinnickinnic River Manhole	5/10/16	0	1,350	0	170	19,200	Dog

FT12198	Stormwater	Wilson Park Creek Outfall 25	6/21/12	566	0	0	132	0	Raccoon
FT12431	Stormwater	Honey Creek 05	7/24/12	672	318	151	162	0	Low human
FT14569	Beaches	South shore old beach 001	7/9/13	BLD	1,760	394	391	276	Low human/ Low dog
FT14570	Beaches	South shore old beach 002	7/9/13	166	3,460	1,000	1,430	1,060	Human/Dog
FT14571	Beaches	South shore old beach 003	7/9/13	0	18,100	985	765	27,900	Low human/Dog
FT15268	Stormwater	Kinnickinnic River Outfall 47	10/31/13	3,540	0	0	0	0	Raccoon
FT15280	Stormwater	Kinnickinnic River Outfall New	11/6/13	225	33,700	249	196	8,710	Human/Dog
FT17167	Rivers	Kinnickinnic River	7/22/14	1,381	34,000	6,730	8,900	944	Human/Dog
FT17171	Rivers	Kinnickinnic River	7/22/14	375	6,450	821	1,610	0	Human
FT17708	Stormwater	Wilson Park Creek Outfall 07	8/25/14	BLD**	9,020	1,630	466	839	Human/Dog
FT17713	Stormwater	Wilson Park Creek Outfall 15	8/25/14	0	6,150	107	193	408	Low human/Low dog
FT18040	Stormwater	Wilson Park Creek Outfall 18	10/14/14	BLD**	9,620	1,890	185	0	Human
FT19920	Rivers	Menomonee River	7/9/15	0	675	265	161	0	Human
FT20193	Beaches	South Shore Old Beach 001	8/10/15	0	1,320	132	0	320	Low human/ Low dog
FT20724	Stormwater	Russell Avenue Manhole	10/28/15	8,560	0	45	256	0	Raccoon

Next generation sequencing can be used to inventory host-specific bacteria and has ushered in a whole new era in microbial ecology. Next generation sequencing can sequence 10,000,000 bacteria per run – nearly 100,000 per sample, which gives a very in-depth profile of the organisms that are present. To analyze these complex datasets, there are a variety of bioinformatic tools available. Random forest is one machine learning algorithm that is used to create a classifier by selecting signature of sequences (Figure 13). This type of very high-level resolution data would be useful in sewersheds that have a mixture of fecal pollution sources. Further, the falling cost of sequencing (as low as \$50/sample in some cases) may make the approach feasible in the future.

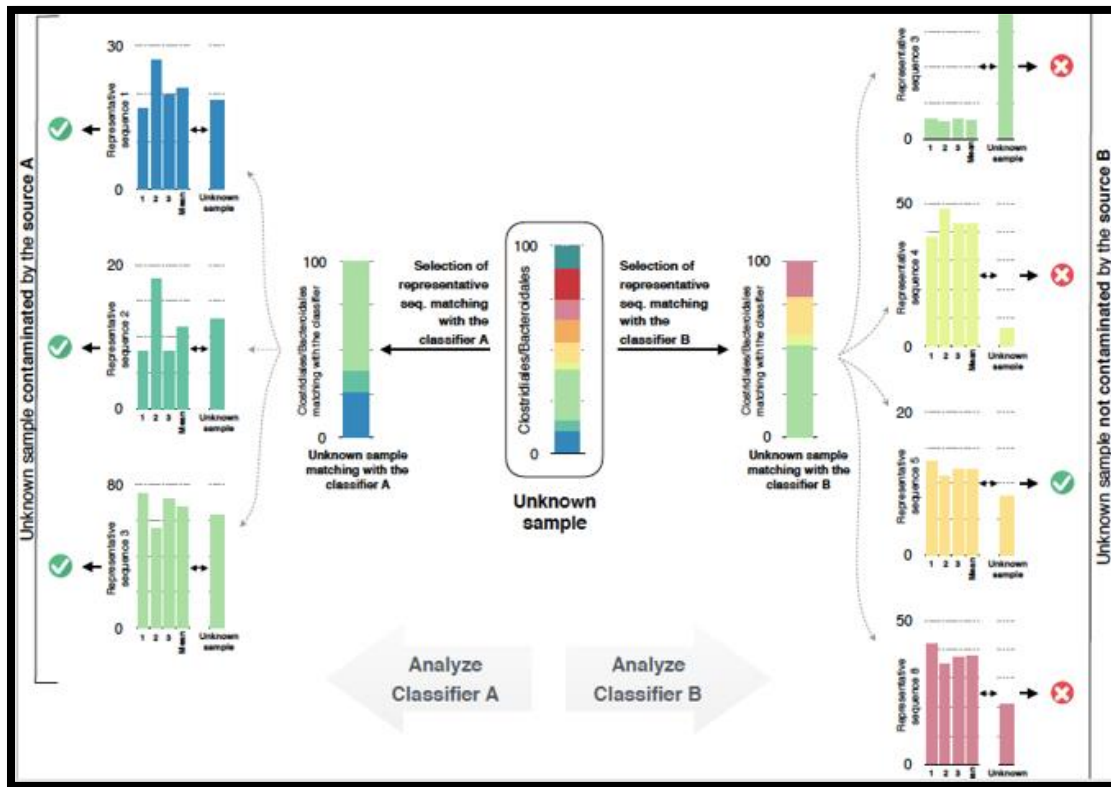


Figure 13: Random forest applications for identifying sources of fecal pollution using microbial community data. Signatures of sequences from different sources are identified by random forest and used to create a classifier. Unknown samples are then compared to the classifier.

Another emerging technology is rapid methods for qPCR. With thousands of miles of pipes, it is very difficult to perform extensive sample collection and analysis in the laboratory, without having results available to guide the sample collection. Further, while qPCR is very sensitive, it is also somewhat expensive. DNA extractions are expensive and labor intensive – 16 samples can take up to 4 hours. Instrumentation, reagents, and other cost estimates include:

- Instrumentation \$40,000
- Technician 1 week for 96 samples, 5 markers
- Reagents \$8-16 per plate
- Disposables \$3 per plate
- DNA extraction \$500 per plate,
- Storage \$15,000 per freezer: life span 7 years

Development of rapid qPCR methods that are field deployable would be useful for stormwater system investigations. Since very high concentrations of wastewater are generally found in up the pipe testing, this approach doesn't require exact quantification. If testing could be completed in a relatively short time, such as <1-hour, sample collection could be dynamic and guided by results. Simplified qPCR methods also would require less technical expertise and could be accessible to personal within municipalities or wastewater agencies.

ROUNDTABLE DISCUSSION SYNTHESIS RE: CHALLENGES FOR IMPLEMENTING WASTEWATER DETECTION PROGRAMS

KDF participants spent time discussing best practices and considerations for mapping the collection system as highlighted in the table below. The following section provides insights into the challenges that occur in wastewater detection program implementation. In order to map and mitigate source contamination, a variety of steps are considered in the process. Identification of areas in which time and efficiencies might be gleaned were discussed, as follows:

MAPPING THE COLLECTION SYSTEM

Table 7: Mapping the Collection System

<p>1. Identify tasks and deliverables</p>	<ul style="list-style-type: none"> • Continuous updating/cataloguing of current and future tasks and deliverables • Convert historical data to electronic data (i.e., GIS) • Ensure performance of “truth check(s)” (to include field observations)
<p>2. Identify resources</p>	<ul style="list-style-type: none"> • Multi-agency cooperation (wastewater district, municipalities – drinking water and wastewater)
<p>3. Identify staff / expertise level</p>	<ul style="list-style-type: none"> • Effort/time and expertise needed • Volunteer involvement • User-friendly apps • Open source
<p>4. Identify methods</p>	<ul style="list-style-type: none"> • “Truth check” with field observations • Quality control • Training • Resolve outdated information, and multiple sources of information • Central repository • Knowledge of data sources • Privacy of data

WHAT ARE THE MAJOR HURDLES FOR BACKTRACKING? FINANCIAL, EXPERTISE, ADMINISTRATIVE, RESEARCH NEEDS, POLITICAL WILL. WHAT NEEDS TO HAPPEN LOGISTICALLY? ADMINISTRATIVELY?

KDF participants discussed the most challenging issues in the process of administering a program for remediating wastewater contamination sources. Some of these challenges are listed below:

- Financial
 - There can be challenges in demonstrating that there will be a beneficial return on investment including an impact on the desired public good.
 - Treatment of environmental justice issues need to be defined
 - Competing priorities can limit resources
 - Long-term payback needs evaluation and justification
- Expertise
 - To keep up with changing technology, expertise needs to evolve
 - The industry can struggle to maintain sufficient expertise to conduct field, laboratory, and administrative tasks
- Research
 - Procurement of financial support to advance technology and methods is an ongoing and challenging process that requires substantial funds
 - Justification is needed to prove worth for research and development investments
- Political
 - There can be a distrust in public-private partnerships in some instances
 - Justification of the benefits can be questioned

CAN PRODUCTION LABORATORIES HELP? WHAT TURN-AROUND TIME IS NEEDED?

KDF participants exchanged views on the option of using production laboratories rather than field techniques to help with monitoring needs and listed the following items to consider:

- Private and municipal laboratories have potentially important roles that they could play.
- Parameters could include fundamental water chemistry and microbiology through advanced analysis such as pharmaceuticals and host-specific microorganisms.
- Assistance with interpretation of results would be helpful, especially for more modern and advanced parameters.
- Cost could be a limitation as well as turn-around times.
- Proximity of laboratories could influence the ability to meet holding time requirements.
- Turn-around time requirements would vary depending on the situation. In general, 24-hour to 2-week time periods would be beneficial for these types of investigations.

WHAT FINANCIAL BARRIERS ARE THERE?

Like many areas of research, financial barriers can prove to be the limiting factor in the scope of projects moving forward, or in halting projects in mid-stream. KDF participants noted financial barriers as one of, if not the, most significant barrier to innovation in research development. The following responses were additionally provided as financial barriers:

- There is often the sentiment that “It is someone else’s problem”
- Garnering the political will to make investments
- Data and evidence-based reasoning sometimes does not convince those who are opposed to support for these efforts
- Regulatory priorities may or may not support the need to remediate wastewater contamination issues depending on the circumstances
- Financial priorities (competing interests) may result in a lack of incentive to provide resources
- Outside contracts may be needed to fill the needed expertise or capacity.

- Stakeholders may not be informed properly to develop the perception that this is important work.
- Not a clear return on investment
- Scalability
- Reduction of federal and state funds (lack of O&M support; federal funding exclusion for infrastructure)

HOW CAN EFFECTIVENESS IN RESULTS INTERPRETATION BE IMPLEMENTED THROUGH INFORMATION SHARING AMONG AGENCIES?

In assessing coordination and collaboration opportunities to improve effectiveness in results interpretation, KDF participants discussed opportunities for furthering information sharing potential, while at the same time noting the need to coordinate with one another to alleviate duplicative activities and ensure that fragmented results are shared for incorporation into higher-level extrapolations.

How can effectiveness in results be improved through information sharing among agencies?

- Learning nuances of strategy and approaches through experience of colleagues
- Sharing of project planning techniques to achieve desired results
- Availability of open source tools
- Share of information is most effective when done in a timely fashion
- Eliminating “silos” would help inform all stakeholders
- Sharing of information would help inform all stakeholders
- More opportunities to be involved in forums to share ideas would be beneficial to bring relevant entities together
- Joint databases would allow seamless data sharing
- Sharing mapping applications among different levels of organizations would increase efficiency and leverage resources
- Collaborative grants would help to provide resources in areas where needed
- Collaborative field work may reduce travel needs

WHAT RESEARCH NEEDS TO BE DONE?

Investment in research and development will assist in fueling advancements in detection of wastewater contamination. The KDF participants provided feedback on research topics that could use some emphasis as follows:

CONTAMINATION DETECTION AND SOURCE IDENTIFICATION

- Development of improved methods for reliable, accessible, and time-relevant wastewater-specific indicators is needed.
- Identification and implementation of multiple reliable microbial source tracking markers are needed to improve confidence in results.
- Innovative development of real-time PCR for rapid estimation (mobile field unit) of microbial contaminants.
- Development of real-time sensors as surrogates for microbial contaminants to better define magnitude and variability throughout contamination events via grab samples or unattended deployments.
- Identification of additional parameters that are specific to human wastewater, easy and inexpensive to measure in a rapid timeframe.
- Field-level methods/sensors are needed to reliably identify and quantify existing human-specific microbial markers or chemical wastewater-specific parameters by direct measurement or surrogate techniques.
- Better definition of safety thresholds for monitoring parameters to understand the risk from human and non-human sources.
- Development of volunteer monitoring/surveillance program to help define potential contamination areas.
- Study the use of remote sensing and heat mapping for locating areas of contamination.
- Development of more effective and efficient methods of identifying, ranking, and prioritizing individual private property source contributors within a specific project area. This could include microbial indicators and water quality parameters, and flow-based assessment to identify suspect properties.
- Consider all physical, thermal, and chemical properties of sanitary water to develop more novel methods of source identification on the micro level for the residential private sector.
- Development of more effective and efficient methods of distinguishing the characteristics of the contribution once a property has been identified by considering flow contribution from a source with a diverse set of metrics (flow, temp, chemical composition) to assess the likely composition of the contribution (% sanitary lateral, % foundation drain, etc).
- Conduct research to determine which combinations of methods in variable conditions provides the most robust, cost effective, and widely applicable means for identifying wastewater components in ambient water.
- Conduct research to define how current approved methods correlate to next generation methods.
- Conduct a formal evaluation of the effectiveness of mobile chemistry test kits to define effectiveness and uncertainties involved in identifying contamination sources with these methods.
- Formulation of methods to limit false positive and false negative errors in detection of wastewater contamination.
- Evaluate the use of rapid *E. coli* and fecal coliform testing methods.
- Consider options for implementing proactive vs. reactive wastewater detection monitoring.

GUIDANCE AND TOOLS

- Development of a toolbox of means and methods for detecting wastewater contamination utilizing traditional microbial, molecular, chemical and emerging methods.
- Development of additional guidance on how to address contamination from private laterals.
- Development of a decision tree framework for locating and prioritizing likely portals of entry/sources of wastewater contamination

- Development of an update of the Center for Watershed Protection document (Illicit Discharge Detection and Elimination. A Guidance Manual for Program Development and Technical Assessments) that incorporates the latest techniques.
- Development of a best practices/industry standard for pollution source investigation.
- Development of guidance for public education (i.e. understanding who is responsible for the pipes, risk to public, working with insurance companies to replace laterals, etc.)
- Development of training protocols for end users to effectively make use of new technologies (markers, instruments, etc.).

REMEDIES

- Conduct research to determine the effectiveness of green infrastructure for wastewater contaminants.
- Evaluation of the feasibility of end-of-pipe treatment before discharging into surface water systems.
- Defining options for products that can be used to minimize risk of infrastructure failures (ex., types of pipes).
- Development of methods to efficiently conduct cost/benefit analysis for repairing leaks and prioritizing remediation efforts.
- Conduct research and development on materials and methods for reducing rehabilitation costs on private property including use of current innovation in the public and commercial private sector and applying it to the residential private sector of sanitary and water infrastructure.

MANAGEMENT

- Identify partners for provision or development of best practice case studies that demonstrate an effective team approach to IDDE; i.e. it may not be collaboration of departments, agencies, and consultants.
- Identify partners for provision or development of examples for practitioners to reference where entities have worked through remedies and red tape to successfully remove sources of wastewater contamination.
- Develop innovative policy and funding approaches to help expand opportunities.
- Investigate opportunities for public/private partnerships in support of this work.
- Work with regulators at local, state, and national levels to approve next generation methods.
- Develop centralized location to store data, accessible to anyone (including metadata of pipe system as well as results).

REFERENCES

- Amick, R. S., and Burgess, E. H. (2000) "Exfiltration in Sewer Systems." EPA/600/R-01/034. Cincinnati, OH: U.S. Environmental Protection Agency.
- Barker, D. (2018) "Collection System Infrastructure Microbial Source Tracking," HRSD, Detection of Wastewater Contamination Knowledge Development Forum presentation.
- Bernhard A.E. and Field K.G. (2000) A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 66. 4571-4574.
- Brown, E., Caraco, D., Pitt, R. (2004) Illicit Discharge Detection and Elimination: A Guidance Manual for Program Development and Technical Assessments," Center for Watershed Protection and University of Alabama, Web., <https://owl.cwp.org/mdocs-posts/idde-guidance-manual/>
- Deb Caraco, D. (2018) "Chemistry Techniques: Strengths, Weaknesses, and Where Chemistry Fits Best," Center for Watershed Protection, Detection of Wastewater Contamination Knowledge Development Forum presentation.
- Corsi, S. R. and McLellan, S.L. (2018) Optical Properties of Water for Prediction of Wastewater Contamination in Surface Water," U.S. Geological Survey and University of Wisconsin-Milwaukee, Detection of Wastewater Contamination Knowledge Development Forum presentation.
- DeMaria, A. (2018) "Illicit Discharge Investigation Examples: Unraveling the Spaghetti," ECT Environmental Consulting & Technology, Inc., Detection of Wastewater Contamination Knowledge Development Forum presentation.
- Fellman, Jason B., Eran Hood, and Robert G. M. Spencer (2010) "Fluorescence Spectroscopy Opens New Windows into Dissolved Organic Matter Dynamics in Freshwater Ecosystems: A Review." *Limnology and Oceanography* 55 (6): 2452–62. <https://doi.org/10.4319/lo.2010.55.6.2452>.
- Instituto Nacional de Estadística Geografía e Informática, The Atlas of Canada, and U.S. Geological Survey. 2006a. "North American Atlas - Hydrography: Government of Canada, Ottawa, Ontario, Canada." Instituto Nacional de Estadística, Geografía e Informática, Aguascalientes, Aguascalientes, Mexico; U.S. Geological Survey, Reston, Virginia, USA. http://nationalmap.gov/small_scale/atlasftp.html?openChapters=%2Cchpwater#chpwater.
- Instituto Nacional de Estadística Geografía e Informática, The Atlas of Canada, and U.S. Geological Survey. 2006b. "North American Atlas - Political Boundaries: Government of Canada, Ottawa, Ontario, Canada." Instituto Nacional de Estadística, Geografía e Informática, Aguascalientes, Aguascalientes, Mexico; U.S. Geological Survey, Reston, Virginia, USA. <http://nationalatlas.gov/atlasftp-na.html>.
- Kildare B.J., Leutenegger C.M., McSwain B.S., Bambic D.G., Rajal V.B., Wuertz S. (2007) 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: A Bayesian approach. *Water Res.* 41:3701–3715.
- Kinzelman, J. (2018) "Incorporating Molecular Testing as an Evidentiary Tool in Municipal Water Quality Monitoring Programs," City of Racine, WI, Detection of Wastewater Contamination Knowledge Development Forum presentation.
- McLellan, S. L. (2018) "Microbial source tracking using new indicators of fecal pollution," University of Wisconsin-Milwaukee, School of Freshwater Sciences, Detection of Wastewater Contamination Knowledge Development Forum presentation.
- National Atlas of the United States (2005) "National Atlas of the United States, County Boundaries of the United States, 2001." National Atlas of the United States. Reston, VA: National Atlas of the United States. <http://nationalatlas.gov/atlasftp.html>.

Nenn, C. (2018) "Canine Scent Tracking for Human Wastewater," Milwaukee Riverkeeper, Detection of Wastewater Contamination Knowledge Development Forum presentation.

Newton R.J., Vandewalle J.L., Borchardt M.A., Gorelick M.H., McLellan S.L. (2011) *Lachnospiraceae* and *Bacteroidales* alternative fecal indicators reveal chronic human sewage contamination in an urban harbor. *Appl. Environ. Microbiol.* 77: 6972–6981.

Reischer G.H., Kasper D.C., Steinborn R., Mach R.L., Farnleitner A.H. (2006) Quantitative PCR method for sensitive detection of ruminant fecal pollution in freshwater and evaluation of this method in alpine karstic regions. *Appl. Environ. Microbiol.* 72: 5610–4.

Rutsch, M., Franz, T., & Krebs, P. (2007) Transferability of exfiltration rates from sewer systems. *Journal of Soils and Sediments*, 7(2), 69–74.

Templar H.A., Dila D.K., Bootsma M.J., Corsi S.R., McLellan S.L. (2016) Quantification of human-associated fecal indicators reveal sewage from urban watersheds as a source of pollution to Lake Michigan. *Water Res.* 100:556–567.

U.S. Department of Agriculture-Natural Resources Conservation Service, U. S. Geological Survey, and U.S Environmental Protection Agency (2009) "The Watershed Boundary Dataset (WBD)." Vector digital data. Fort Worth, Texas. <http://datagateway.nrcs.usda.gov>.

USEPA (2012) Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. EPA-821-R-12-008.

10-AUG-2018 KNOWLEDGE DEVELOPMENT FORUM PARTICIPANTS

1	John	Attridge
2	Danny	Barker
3	Benjamin	Benninghoff
4	Christopher	Bocciardi
5	Joseph	Boxhorn
6	Marty	Collins
7	Annette	DeMaria
8	Shannon	Donley
9	Zac	Driscoll
10	Raul	Gonzalez
11	Justin	Hart
12	Graham	Hoppe
13	Shannon	Johnson-Windsor
14	Julie	Kinzelman
15	Brandon	Koltz
16	Adrian	Koski

17	Peter	Lenaker
18	Barry	Liner
19	Matthew	Magruder
20	Lisa	McFadden
21	Sandra	McLellan
22	Julie	McMullin
23	Dan	Murray
24	Cheryl	Nenn
25	Nicklaus	Neureuther
26	Amy	Nitka
27	Hayley	Olds
28	Christopher	Palassis
29	Katie	Rademacher
30	Beth	Sauer
31	Nancy	Schultz
32	Corinne	Wiesner
33	Tracy	Wilkins

