TOOLS FOR MS4s: MICROBIAL SOURCE TRACKING

WHY DOES THE SOURCE OF *E. COLI* MATTER?

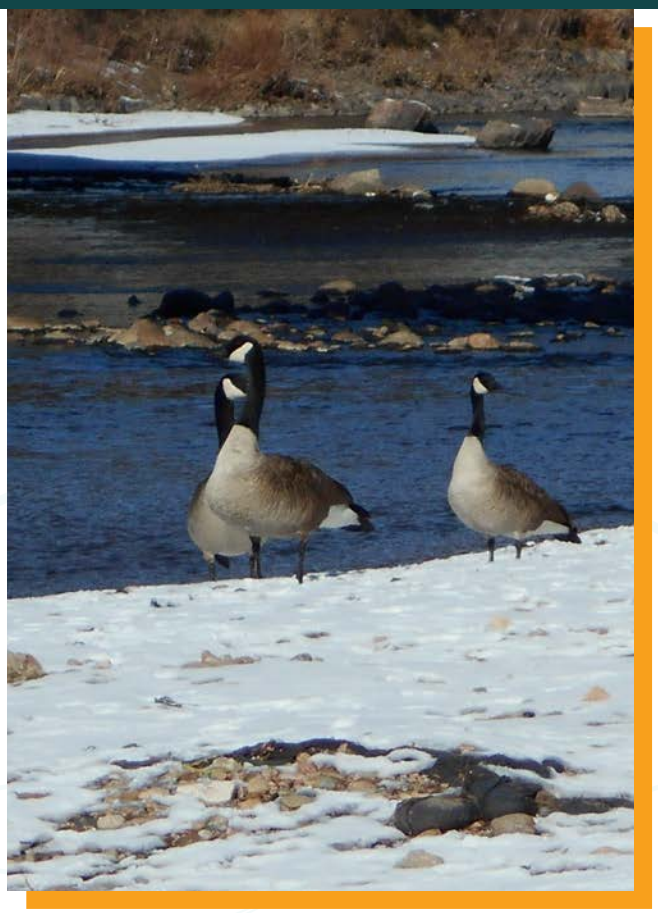
Elevated levels of fecal indicator bacteria (FIB), including *E. coli*, are one of the most common causes of water quality impairment in surface waters across the United States. While some sources of FIB can be identified through a sanitary survey, sources of FIB in many watersheds remain unknown and thus cannot be effectively controlled through targeted management actions. FIB results alone give no indication of the fecal source (human vs non- human). In addition, the health risk associated with exposure to water containing human waste is much greater than that of most non-human sources. Therefore, identifying the source(s) of fecal contamination in waters with chronically high FIB levels is of critical importance to meet recreational water quality criteria and reduce human health risk.

WHAT IS MICROBIAL SOURCE TRACKING?

Microbial source tracking (MST) uses a set of tools that allow for sources of fecal waste to be distinguished. These tools include conventional methods (e.g., ammonia, CCTV, dye testing) that have been used to identify illicit discharges for the past 20+ years, as well as more recently developed advanced laboratory methods that measure DNA specific to humans and other animals known as "markers" to identify sources. However, MST is more than just a set of tools or methods. MST is a process by which potential waste sources are systematically tested and investigated to identify and locate the origin of fecal bacteria in a contaminated water.

WHEN IS MICROBIAL SOURCE TRACKING MOST EFFECTIVE/ADVANTAGEOUS?

The greatest advantage of MST is in the use of DNA markers to identify and locate fecal waste sources in a watershed or storm sewer system (MS4). This information is useful when there are multiple potential sources, and the primary source(s) cannot be determined using conventional tools. MST is most effective when a tiered, hypothesis-driven approach is used creating multiple lines of evidence. Specific questions regarding potentially contributing sources are defined (i.e., hypotheses), and the MST study is then designed to answer those questions. This allows MS4 managers to make informed conclusions about contributing sources and locations, and thus informed management decisions to control or eliminate those sources, leading to water quality improvement and health risk reduction. In most cases, locating and eliminating FIB sources using MST comes at a dramatically lower cost than the use of structural measures to treat or divert contaminated stormwater during wet weather.



WHAT ARE THE LIMITATIONS OF MICROBIAL SOURCE TRACKING?

Due to their advanced levels of sensitivity and specificity (i.e., ability to detect highly dilute waste from specific sources), DNA markers represent a dramatic improvement in the identification and location of fecal sources. However, there are limitations that should be considered before an MST study is performed.

- DNA markers give quantitative, source-specific results, but these results cannot be used to accurately quantify the proportion of FIB that came from each detected source. This is because the bacteria targeted by DNA markers are different than those targeted in FIB analysis. However, quantitative marker results can indicate the relative magnitudes of contributing sources.

- Different human waste sources (sewage, septage, open defecation) cannot be distinguished from each other with human markers alone. A study design targeting specific study hypotheses is used to distinguish between human sources.

- Treated human waste sources (WWTP effluent, reclaimed/recycled water, treated septic effluent) may also be detected using human markers. Therefore, human markers may have limited utility in waters that receive treated wastewater.

- Non-human markers are not capable of distinguishing all animal sources. For example, the dog marker detects domestic dogs and wild canines. Also, DNA markers are not available for all animals.

- Eliminating human waste sources alone may not reduce FIB levels in many areas where multiple sources of FIB are present. However, this may be one of the most effective means of reducing human pathogens and recreational illness risks.

- Low level human markers may persist, even after infrastructure based human waste sources have been eliminated. The methods used to detect DNA markers are extremely sensitive and can detect a sewage signal diluted up to one million times. Therefore, diffuse sources (e.g., open defecation, bather shedding) could contribute to detectable levels in recreational waters.

- Human markers are not conservative once they enter the environment. Markers can decay rapidly under some conditions and are therefore best used as an indication of recent waste inputs. Samples should be collected as near to potential sources as possible (e.g., MS4 discharges). Human markers do not cause illness themselves and therefore there may not be an elevated health risk associated with human marker detections.

WHAT ARE COMMON SOURCES OF *E. COLI* IN URBAN WATERSHEDS?

There are many sources of *E. coli* and other FIB from human and non-human sources. These sources can be categorized into three general groups (Table 1).

HUMAN WASTE SOURCES	NON-HUMAN SOURCES RELATED TO HUMAN ACTIVITIES	NON-HUMAN SOURCES INDEPENDENT OF HUMAN ACTIVITY
Sanitary Sewer Overflows (SSOs)	*Pets (Dogs, Cats, etc.)*	*Wildlife (Raccoons, Birds, Deer, Coyote, etc.)*
Leaky Sewer Pipes (Exfiltration)	*Livestock (Horses, Cows, etc.)*	*Decaying Plants*
Illicit Connections to MS4	*Rodents (Mice, Rats, etc.)*	*Algae and Biofilms*
Leaky or Failing Septic Systems	*Birds (Gulls, Pigeons, etc.)*	*Soil/Sediment*
Porta-Potties	*Dumpsters and Trash Cans*	
Bathers and Open Defecation	*Garbage Trucks*	
Boats and RVs	*Animal Manure/Compost*	
Dumpsters and Trash Cans	*Washwater*	
Garbage Trucks	*Green Waste*	
Illegal Dumping	*Litter*	
Illegal Discharges	*Grease Bins/Traps*	
Gray Water Discharges	*Irrigation Runoff*	
Pools and Hot Tubs		

TABLE 1. COMMON SOURCES OF *E. COLI* IN URBAN WATERSHEDS

The human health risk associated with exposure to water containing *E. coli* varies dramatically based on the source. Human waste sources generally have the highest pathogen levels and therefore highest illness risk, followed by non-human sources from human activities, and finally non-human sources that are not associated with human activities (natural sources). When conducting an MST study, it is often most effective to prioritize sources for investigation by first testing for human sources representing the highest health risk before moving on to non-human sources that may be contributing to FIB exceedances. This allows for risk to be most effectively reduced and for a pathway toward regulatory compliance where human sources must first be demonstrated to be controlled prior to any request for permit relief or water quality standard modification/variance.

WHAT TOOLS ARE USED IN MICROBIAL SOURCE TRACKING?

There are a variety of tools available that range in cost and complexity of analysis. Low-cost tools should be used first to characterize where FIB are elevated and to determine potential waste sources. Where illicit connections or leaks are suspected, tools such as dye testing and closed-circuit television (CCTV) may be used to locate sources. However, conventional tools (e.g., FIB, basic chemical indicators) are limited due to their relatively low sensitivity and inability to discriminate sources. Where sources are dilute or cannot be distinguished, DNA marker analysis is the most effective way to identify and locate sources.

TOOL	DESCRIPTION	COST
Visual Surveys and Outfall Screening	*Field observations to identify flowing outfalls and potential fecal waste sources.*	*$*
GIS	*Essential for planning and analyzing data in relation to infrastructure. Useful prior to field investigations to target areas for more detailed investigation.*	*$*
FIB (E. coli)	*Basic indicator of potential fecal contamination tied to regulatory receiving water limits.*	*$*
Basic Chemical Indicators	*Includes detergents/surfactants, fluoride, ammonia, and potassium. Low-cost field kits may be useful in MS4 networks (e.g., ammonia).*	*$*
Canine Scent Tracking	*Trained dogs used to identify sewage leaks. Useful when real time results and broad spatial coverage are needed, such as in large storm drain networks.*	*$*
Dye Testing	*Visual or fluorometer based detection of dye. Useful for identifying illicit connections and infiltration from sewers to storm drains.*	*$/$$*
CCTV	*Cameras used in the MS4. Useful for locating illicit connections, infiltration from sewers to storm drains, and tracking flow sources.*	*$$*
Advanced Chemical Indicators	*Includes sucralose, caffeine, and cotinine, as well as many contaminants of emerging concern. Useful as a second line of evidence for sewage sources.*	*$$$*
Human Markers	*Most sensitive and specific tool for identifying human waste. Useful for sampling in receiving waters, outfalls, within the MS4, and groundwater.*	*$$$*
Non-Human Markers	*Able to identify non-human sources of waste including cows, dogs, birds, deer, and others. Useful after human sources have been ruled out.*	*$$$*

TABLE 2. TOOLS AVAILABLE TO IDENTIFY E. COLI SOURCES

DNA markers have been thoroughly validated at multiple laboratories and USEPA guidance on their use is under development. However, a toolkit approach utilizing multiple tools is often the most effective way to identify FIB sources. By combining advanced source-specific tools with conventional analytes, GIS, and observational data, waste sources can be identified and eliminated through targeted management actions.

HOW DO I DESIGN A SUCCESSFUL SOURCE TRACKING STUDY?

MST is best used as part of a management strategy to locate and eliminate waste sources. By controlling sources, cost savings can be achieved compared to wet weather treatment of contaminated stormwater using structural BMPs. Listed here are important design considerations that will reduce costs and increase the chances of a successful study that leads to conclusive results and implementable management actions.

- Use local resources to identify potential waste sources. This includes consultation with stakeholders, analysis of historical sampling data, desktop GIS and mapping analysis (including stormdrain and sewer/septic infrastructure), and first hand observational/reconnaissance visits.
- Define specific questions (hypotheses) that will be tested through sampling and analysis. The number of samples, locations, frequency and timing of collection, and analysis methods are determined based on these questions (e.g., more samples are needed to demonstrate a source is absent than to confirm its presence).
- Use a tiered investigation approach to most efficiently identify sources (Figure 1).
- Consult with MST practitioners experienced in study design considerations, selection of methods, analysis of DNA-based data, and interpretation of results in terms of determining the contributing source(s), the extent of contamination, and successful management strategies and regulatory pathways.
- Consult the sources linked at the end of this fact sheet for additional guidance.

FIGURE 1. TIERED APPROACH TO SOURCE TRACKING (ADAPTED FROM GRIFFITH, 2013)

WHAT ARE THE OUTCOMES OF A SUCCESSFUL SOURCE TRACKING STUDY?

The outcome of a successful MST study will depend on the study questions being investigated. It is important to identify specific study questions that can be adequately tested to provide conclusive results that lead to implementable actions. Listed here are several potential outcomes of a MST study that could lead to successful management strategies.

- Human waste sources are identified/located and eliminated through targeted management actions, leading to improved water quality and reduced health risk.
- Non-human waste sources from human activities are identified/located and reduced through targeted management actions leading to improved water quality.
- Human waste sources and non-human sources from human activities are shown to be absent or to have been successfully eliminated, but FIB remain high due to natural sources, leading to a pathway toward regulatory modification (e.g., site specific standard).

In addition to these successful outcomes and cost savings, there are other potential benefits of MST such as the reduction of other pollutants (nutrients and contaminants of emerging concern associated with sewage), IDDE program action in MS4 annual report, public relations and regulator perception that agencies are using the best tools available to improve water quality.

WHERE CAN I FIND MORE INFORMATION ON SOURCE TRACKING?

The information contained in this fact sheet was summarized from multiple sources and is not intended to provide the level of detail necessary to design a MST study. Additional reading and/or consultation with an experienced MST practitioner should be done prior to beginning a study. Below are links to guidance documents useful to MS4 managers containing more detail on how to perform a successful MST study.

For more information on the use of conventional sanitary survey and IDDE tools:

- *Stormwater Effects Handbook: A Tool Box for Watershed Managers, Scientists, and Engineers*
- *Illicit Discharge Detection and Elimination: A Guidance Manual for Program Development and Technical Assessments*

For more information on the use of DNA markers for MST:

- *The California Microbial Source Identification Manual: A Tiered Approach to Identifying Fecal Pollution Sources to Beaches*

For more information on designing a successful MST study that utilizes the toolbox approach:

- *Colorado E. coli Toolbox: A Practical Guide for Colorado*
- *Pathogens in Urban Stormwater Systems*

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