Tributary Pollution Assessment Manual

Final **July 2006**

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Executive Summary

This manual outlines methods for assessing, tracking, and mitigating fecal microbial contaminants in surface waters. Assessment and direct identification of sources of fecal contaminants is confounded by many variables inherent in the use of indicator organisms for monitoring and by variables in the dynamics of microbial populations in various substrates and environmental conditions. This manual examines some of these questions and presents the results of recent research in microbial source tracking (MST) in the context of guidelines for assessment and development of corrective actions for listed impaired water bodies.

<u>TMDL Background</u> The Clean Water Act Water (CWA) of 1972 established water quality standards and criteria for designated functions, or uses, of the nation's waterbodies. More than 30 years later, many rivers, lakes, and estuaries are classified as impaired and fail to meet the standards for their designated use. In Florida, and across the nation, many impaired waters fail to meet their designated use because levels of fecal microbial indicators exceed the established water quality criteria. Section 303c of the CWA requires states to establish Total Maximum Daily Loads (TMDLs) for impaired waters.

<u>Assessment and Source Identification</u> TMDLs are developed by calculating or modeling a theoretical amount of pollutant that a water body can assimilate without resulting in an exceedance of water quality standards. Two important parts of this process are:

- the assessment of the contributing area, and
- the allocation of pollutant loadings among contributing sources.

This manual addresses methods of pollution assessment and microbial source tracking (MST) for fecal microbial contaminants in tributaries in Duval County, Florida.

Microbial impairment is evaluated by measuring concentrations of indicator bacteria including fecal coliform bacteria, *Escherichia coli* (*E. coli*) and *Enterococcus* spp. It would be dauntingly time and cost prohibitive to attempt to directly monitor every pathogenic microbial organism that can affect water quality. Therefore, indicator organisms have been used for decades to monitor and indicate the presence of potentially harmful microbial contamination (Bitton, 2005).

The tributary pollution assessment methodology described in this Manual has been developed to address pollution sources in tributaries impaired by indicator organisms (frequently termed "pathogens"). The

Manual incorporates a decision tree that considers appropriate methods to define sources to a level at which remedial actions can be defined and successfully implemented, while also considering cost. A weight-of-evidence approach is described in which the results of the decision tree are used in conjunction with background knowledge of the watershed and area land use patterns to assess the contribution of various potential sources to water bodies impaired by high indicator organism levels.

The determination of contributing sources is particularly important with respect to fecal microbial exceedances because the measured indicators often do not readily discriminate between human, livestock, and wildlife sources. The ultimate goal of the TMDL process is to implement actions necessary to improve downstream water quality. The development of best management practices (BMPs) and corrective actions require the specific knowledge gained from the assessment of the contributing area and an understanding of the relative contribution of identifiable point sources. Corrective actions will vary considerably for identified sources such as faulty sewage systems or intense agricultural operations versus unknown or dispersed sources transported in stormwater runoff. In addition, because pathogens from human sources present the highest potential for infection, identification of the type of source (human, livestock, or wildlife) affects the evaluation of risk. Therefore, attempts at source identification are critical to achieving the end goal of implementing action to improve water quality and protect human health.

Corrective Action   Once TMDL plans are accepted, state and county agencies work together to establish a combination of regulatory and non-regulatory practices that are intended to reduce pollutant loading. Non-regulatory actions can include the voluntary implementation of BMPs, pollution prevention activities, and habitat preservation or restoration. Regulatory actions can include issuing or revising permits to impose effluent limitations or required use of a combination of structural and non-structural BMPs.

Corrective actions described in this manual focus on the three principle sources of fecal coliform contamination identified in Duval County (not listed in any particular order): 1) sewer infrastructure; 2) septic tanks; and 3) stormwater (as a conveyance system). Effective preventative and predictive maintenance and the identification of problem areas in the existing infrastructure can assist in the reduction of sanitary sewer overflows (SSOs) and decreased need for large-scale replacements of the sewer system. Many of the identified corrective actions pertaining to septic tanks involve the implementation of programs to address effective onsite system operation and maintenance, or the phase-out of septic systems and subsequent transition to sanitary sewer. The use of constructed wetland and bioretention systems are highly effective BMPs in treating stormwater and wastewater effluents, in some cases resulting in coliform removal rates above 90%. Other potential corrective actions include alum injection and ultra-violet light or ozone disinfection. Treatment systems can be incorporated into new development designs or can be retrofitted to existing landscapes. BMPs such as constructed wetlands and bioretention systems may be particularly effective for bacterial contamination when placed adjacent to or downstream from septic systems and septic drain fields and/or used as vegetative filters intercepting runoff to recreational waters.

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List of Acronyms

ANOVA	Analysis of Variance
ARA	Antibiotic Resistance Analysis
BMPs	Best Management Practices
CAFO	Concentrated Animal Feeding Operations
CWA	Clean Water Act
DA	Discriminant Analysis
DCHD	Duval County Health Department
DLS	Data Log Sheets
DNA	Deoxyribonucleic Acid
EPA	United States Environmental Protection Agency
EQD	Environmental Quality Division
FDEP	Florida Department of Environmental Protection
GPS	Global Positioning System
GIS	Geographic Information System
GPR	Ground Penetrating Radar
IFAS	University of Florida Institute of Food and Agricultural Services
FOG	Fats, Oils and Grease
MPN	Most Probably Number
MS4	Municipal Separate Storm Sewer Systems
MST	Microbial Source Tracking
NRCS	Natural Resources Conservation Service
OSTDS	Onsite Sewer Treatment Disposal System
PCR	Polymerase Chain Reaction
POD	Point of Discharge
rep-PCR	Repetitive Extragenic Palindromic Polymerase Chain Reaction
SJRWMD	St. Johns River Water Management District
SSO	Sanitary Sewer Overflows
TAT	Tributary Assessment Team
TMDL	Total Maximum Daily Load
UV	Ultra Violet
WBID	Water Body Identification
WSEA	Water and Sewer Expansion
WWTF	Wastewater Treatment Facility

Section 1. Introduction to the Tributary Pollution Assessment Manual

As introduction to the Tributary Pollution Assessment Manual, this section discusses the purpose of the document, defines the fecal microbial contamination problem, and discusses the potential sources of contamination and the techniques that are available to identify the fecal source.

1.1 Purpose of Document

This Tributary Pollution Assessment Manual (hereinafter referred to as the Manual) was developed to provide a blueprint for conducting site assessments and evaluating sources of fecal contamination in impaired waterbodies within Duval County, Florida.

Duval County has 194 tributary Water Body Identifiers (WBIDs) classified by the Florida Department of Environmental Protection (FDEP). The City of Jacksonville, JEA, the Duval County Health Department, and the FDEP (Northeast District Office) have been working on a cooperative effort to reduce fecal coliform bacteria levels in the tributaries within Duval County. The intention is to reduce coliform levels sufficiently to restore waterbody segments within Duval County that are designated as potentially impaired or verified impaired for fecal coliform bacteria to their designated use for recreation.

This Tributary Pollution Assessment Manual was developed to provide a blueprint for conducting sanitary surveys and assessing sources of fecal contamination in impaired water bodies.

This Manual provides a schematic for several processes pertaining to tributary pollution assessments focusing on fecal microbial contamination. These procedures include:

- obtaining background data for the WBIDs;
- categorizing the WBIDs;
- conducting site assessments;
- gathering field data via sampling;
- analyzing the data in conjunction with the background information;
- determining a potential source of contamination; and, ultimately,
- identifying corrective actions (for types of sources).

The scope of this Manual is a general assessment methodology that can be used for any tributary in Duval County. In conjunction with developing the Manual, the assessment methodologies proposed herein are also being tested for six specific WBIDs in Duval County. Specific information regarding these six WBIDs is provided in Appendix A.

1.2 Problem to be Addressed

The Clean Water Act of 1972 (CWA) employs a variety of regulatory and non-regulatory tools to sharply reduce direct pollutant discharges into waterways, finance municipal wastewater treatment facilities, and manage polluted runoff. A significant portion of waterbodies in the U.S. do not meet water quality standards for their designated uses due to high levels of fecal indicator bacteria.

The problem to be addressed in this Manual is the contamination of waterbodies with microbiological indicators of fecal contamination (frequently termed "pathogens" in TMDL-related documents). Microorganisms from fecal sources that enter waters used by the public can pose a health threat, whether the water is utilized as a drinking water source, for recreation, or for raising food such as shellfish. In addition to public health risks, fecal contamination can result in adverse economic impacts due to the closure of recreational beaches and shellfish harvesting areas. Indicator organisms, such as *Escherichia coli* (*E. coli*) and *Enterococcus* spp. (enterococci), found in the gastrointestinal tract of humans as well as warm-blooded and some cold-blooded animals (Harwood et al., 2000), have been used for over a century to warn of the risk of fecal contamination in water and food. These organisms, along with enteric pathogens, are shed in feces that can contaminate surface and ground waters.

In order to effectively reduce fecal contamination to waterbodies, the sources of such contamination must be identified prior to implementing remediation techniques. Reliable and accurate fecal source identification methods are imperative for developing best management practices (BMPs) to control fecal contamination in order to protect the public from water-borne pathogens and to preserve the integrity of drinking water sources (Environmental Protection Agency (EPA), 2005; included as Appendix B). Techniques used for microbial source tracking are explained in Section 1.5.

1.3 Fecal Contamination Thresholds

Water quality standards define a designated function for a waterbody (e.g., primary contact recreation) and set specific water quality criteria to achieve that function. Under the CWA Section 304(a), the EPA is required to publish water quality criteria that accurately reflect the latest scientific knowledge for the protection of human health and aquatic life. The EPA's Ambient Water Quality Criteria for Bacteria -1986 was developed for the protection of waters designated for recreational uses (EPA, 1986). Epidemiological studies conducted by the EPA demonstrated that for fresh water, *E. coli* and enterococci are best suited for predicting the presence of pathogens that cause illness, and that for marine waters, enterococci are most appropriate (EPA, 2002a; included as Appendix C). The Florida Department of Environmental Protection (FDEP) has adopted Criteria for Surface Water Quality Classifications [Chapter 62-302.530, Florida Administrative Code (FAC)].

Parameter	Units	Class I	Class II	Class III: Fresh	Class III: Marine	Class IV	Class V
(6) Bacteriological Quality (Fecal Coliform Bacteria)	Number per 100 ml (Most Probable Number (MPN) or Membrane Filter (MF))	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 5 samples taken over a 30 day period.	MPN shall not exceed a median value of 14 with not more than 10% of the samples exceeding 43, nor exceed 800 on any one day.	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.		
(7) Bacteriological Quality (Total Coliform Bacteria)	Number per 100 ml (Most Probable Number (MPN) or Membrane Filter (MF))	≤ 1,000 as a monthly avg., nor exceed 1,000 in more than 20% of samples examined during any month, nor exceed 2,400 at any time, using either MPN or MF counts.	Median MPN shall not exceed 70, and not more than 10% of the samples shall exceed an MPN of 230.	≤ 1,000 as a monthly average; nor exceed 1,000 in more than 20% of the samples examined during any month; ≤ 2,400 at any time. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period, using either the MPN or MF counts.	≤ 1,000 as a monthly average; nor exceed 1,000 in more than 20% of the samples examined during any month; ≤ 2,400 at any time. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period, using either the MPN or MF counts.		
(8) Barium	Milligrams/L	≤ 1					
(9) Benzene	Micrograms/L	≤ 1.18	≤ 71.28 annual avg.	≤ 71.28 annual avg.	≤ 71.28 annual avg.		

62-302.530, Criteria for Surface Water Quality Classifications

1.4 Potential Sources of Contamination

Fecal contamination results from the input of untreated fecal material from humans, other warm-blooded animals, and some cold-blooded animals into the waterbody. This Manual addresses two categories of sources: putative (suspected) sources and non-putative (unknown) sources. A putative source is essentially a "smoking-gun," an obvious potential source of contamination.

Putative sources can be recognized through a detailed Site Assessment (explained in more detail in Section 3). The Site Assessment begins with the compilation and review of existing information [preferably Graphic Information System (GIS) data] about the WBID, including but not limited to: land use cover and classification, historical sampling data, infrastructure, and recorded incidents. Once all pertinent information has been overlaid with the WBID, potential sources may be apparent and can be field-verified (depending on source). Putative sources may include:

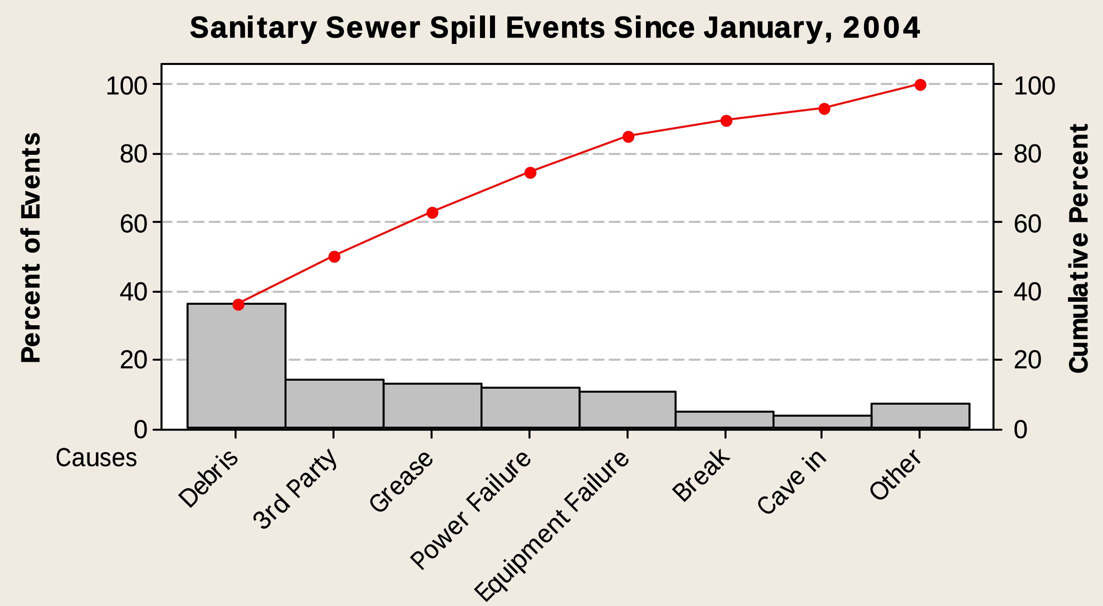
- Sanitary sewer overflows;
- Failing on-site sewage treatment and disposal systems (i.e., septic tanks); and/or
- Specific land uses (e.g., cattle farms, aquaculture, marinas, dog parks).

1.4.1 Sanitary Sewer Overflows

Sanitary sewer overflows (SSOs) are releases of untreated sewage into the environment, and are generally prohibited under the CWA. General information regarding SSOs can be found on EPA's website (http://www.epa.gov/npdes/sso/control/). The following information more specific to the regional sewer system in Duval County based on internal investigations conducted by JEA, the regional service provider.

Spills of untreated sewage from the regional sewer system in Duval County are addressed immediately and investigated thoroughly. When a SSO occurs, the cause is usually listed as a recent, immediately traceable condition, such as a pipe break or pump failure. However, merely repairing the ruptured pipe without understanding the underlying cause of its failure may not protect against future SSOs.

In every case of spillage of any untreated wastewater, JEA, the regional service provider, conducts a two part investigation into the cause of the spill. The first is to address the immediate cause and the second is conducted to address any underlying causes. Based on internal investigations conducted by JEA for events that have happened since January 2004, the causes of events are portrayed below:



	Debris	3rd Party	Grease	Power Failure	Equipment Failure	Break	Cave in	Other
Count	36.02	14.08	12.68	11.87	10.46	4.43	3.42	7.04
Percent	36.0	14.1	12.7	11.9	10.5	4.4	3.4	7.0
Cum %	36.0	50.1	62.8	74.7	85.1	89.5	93.0	100.0

Historically, pipe breaks, cave-ins and grease have been major causes of spill events. Significant investments in pipe replacement focused on areas with the highest occurrence of events have significantly reduced the number of events related to breaks and cave-ins. The introduction of an effective FOG (Fats, Oils and Grease) program has reduced the number of spills associated with grease stoppages. Continuing focus on actions to reduce the causes of the events is the primary assignment of the Waste Water Preventive Maintenance team at JEA.

1.4.2 Failing On-site Sewage Treatment and Disposal Systems

On-site sewage treatment and disposal systems (OSTDS) consist of a septic tank and a subsurface wastewater infiltration system, or drain field, which relies on naturally occurring soils to provide wastewater treatment. The drain field and underlying soils are the most critical components of septic tank systems for treatment of wastewater. A failing septic system is considered to be one that discharges effluent with pollutant concentrations that exceed established water quality standards. Failure of OSTDS can be due to a number of causes, including unsuitable soil conditions, improper design and installation, or inadequate maintenance practices. Improperly functioning septic systems are recognized as a significant contributor of pollutants, including microbiological pathogens. These failing systems can discharge inadequately treated sewage, which may result in obvious sanitary nuisances such as ponding on the ground and run off into surface waters. More subtle failures may also occur in which a direct impact to water supply wells may also be an issue if a sufficient vertical distance from ground water is not maintained (http://cfpub.epa.gov/npdes/stormwater/menuofbmps/illi_1.cfm).

1.4.3 Land Use

Land use cover and classification data, when overlaid with the WBID boundaries, can be used to determine putative sources of fecal contamination. Certain land uses are likely to impact microbial loading to surface waters, including agricultural and aquaculture activities and marinas. Runoff from agricultural areas containing animals (e.g., livestock grazing, dairies, cattle farms, concentrated animal feeding operations (CAFOs)) can contribute a significant amount of fecal contamination to surface waters. Aquaculture facilities have concentrated animal populations and can also be a significant contributor to fecal contamination to the resident waterbody. Marinas that provide on-site waste disposal areas (flush-out pumps) can leak or overflow and can dump raw sewage directly into the waterbody. Although the identification of putative sources may seem like an obvious exercise, the initiation of remedial measures may well not be undertaken without further testing and "proof" of the contamination source.

For some waterbodies, there is no obvious putative source of fecal contamination, in which case a more exhaustive search is required. The background information collected on the WBID should be supplemented with a thorough field examination. In some cases, more advanced techniques such as fluorometry and aerial infrared thermal imagery (discussed in greater detail in Section 1.5) may be deemed appropriate as part of the field examination. These methods could be particularly helpful in the identification of illicit discharges to a stormwater system. Once combined, visual inspections, sampling results, and historical data generally suggest potential source(s) of fecal contamination.

1.5 Available Assessment Techniques

The microbial source tracking (MST) methods summarized below are tools that can be used to help identify sources of indicator bacteria pollution. These methods, including library-based and non-library-based techniques, have not been adopted by regulatory agencies but have been incorporated into a draft guide document recently released by the EPA (2005; included as Appendix B). Consistent with other emerging fields in the environmental sciences, development, assessment, and validation of each of the methods is an adaptive process.

1.5.1 Suite of Indicators

The standard method for characterizing human health risks in drinking water, wastewater discharges, and surface waters is the use of indicator organisms. A recent literature review by Bitton (2005; included as Appendix D) provides a thorough description of the ideal indicator organism criteria. Fecal coliform bacteria, *Escherichia coli* (*E. coli*, a member of the fecal coliforms), *Enterococcus* spp. (enterococci), and coliphage (viruses that infect *E. coli)*, can be used as a suite of indicators to better characterize microbial pollution in sediments and the water column. It is important to note that different indicators may be better suited for use in specific aquatic environments. For example, *E. coli* and enterococci are both appropriate for use in freshwater while enterococci are better suited as indicator organisms in the marine environment (EPA, 2002a). Since indicator organisms are present in the gastrointestinal tract of a variety of warm- and cold-blooded animals and are shed as feces that can contaminate surface and ground waters, it is often impossible to identify a specific source of contamination without discriminatory techniques to identify the genotypic or phenotypic properties of these organisms within a particular sample (Harwood et al., 2000; Harwood, 2004). State of Florida standards for water quality can be found at http://esetappsdoh.doh.state.fl.us/irm00beachwater/default.aspx. Additional water quality criteria information is available from the EPA (1986).

1.5.2 Fluorometry

Fluorescence is a characteristic of certain molecules that absorb specific wavelengths of light and consequently emit longer wavelengths of light. Optical brighteners, a common additive in laundry and dishwashing detergents, absorb light energy below 400 nm and emit light in a general range of 430 nm to 450 nm. Fluorometry can be used to detect and estimate quantities of optical brighteners. Since laundry water is generally discharged to sanitary sewers or OSTDS, optical brighteners are typically present in most human waste treatment and conveyance systems. It should be noted that some older residential structures may have drainage systems for laundry wastewater that are separate from either the septic tank drain field or a sewer line. Releases of residential wastewater into water bodies may contain these compounds.

Fluorometry has been used successfully to detect plumes from OSTDS in surface waters (Grant, 1998; TAMU, 1999) and has been proven to be a cost effective and reliable technique for identifying domestic wastewater contamination when used in conjunction with fecal coliform bacteria (Waye, 1999; Dixon et al., 2005); however, a methodology has not been approved by the EPA (Charlotte Harbor Environmental Center, Inc and Water Resources and Issues, 2003). One of the primary advantages to this technique is that it can provide real-time results when a field instrument is employed. This technique may prove most beneficial when utilized as a flow-through instrument on a boat, which produces continuous sampling output and patterns of fluorescence; however, this strategy would restrict the use of the method to navigable waterways. Despite the establishment of a continuous output using this method, multiple sampling events would be required to represent various patterns of laundry use. Furthermore, in an estuarine environment where naturally-occurring substances, such as humic acids or tannins also fluoresce, the use of optical brighteners as an indicator of human wastewater demands extensive post-processing to effectively separate the fluorescence signal of humics from that of the brighteners (Dixon et al., 2005). Peer-reviewed literature on this topic is limited; however, additional research is anticipated to be conducted by the Mote Marine Laboratory and the FDEP for a Department of Health (DOH)-sponsored study regarding optical brighteners and numerous other water quality indicators (Dixon, personal communication).

1.5.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a process whereby many copies of a specific DNA sequence can be synthesized from a small amount of starting material (template). The process of making many copies of the DNA sequence is called amplification, and generates enough material to be visualized or manipulated for further investigation. PCR applications related to microbial source tracking frequently target host-specific genes or markers present in particular microorganisms. This manual describes two distinct types of PCR techniques: 1) library independent, presence/absence testing, using PCR with human or ruminant specific primers and 2) library-based identification using repetitive extragenic palindromic polymerase chain reaction (rep-PCR).

1.5.3.1 Library independent presence/absence testing using PCR with human or ruminant specific primers

The library independent presence/absence testing using PCR utilizes primers that target specific microbial DNA sequences unique to microbes from a specific source, such as humans or cattle. For example, *Bacteroides,* a fecal anaerobe genus present in both human and animal intestines, is used as a target for this type of source identification technique (Bernhard and Field, 2000a; Bernhard and Field, 2000b). PCR analysis of a fecal sample from a ruminant source using primers designed to target a specific genetic sequence found only in a human source, would show no amplification. In contrast, PCR analysis of samples containing microbial DNA from a human source and a ruminant source would result in amplification of the genetic material (or "presence") with both human specific and ruminant specific primers. Lastly, PCR analysis of samples without microbial DNA from a human or ruminant source would result in a lack of amplification of the genetic material (or "absence") with both human specific and ruminant specific primers. Another target organism for this type of PCR technique is *Enterococcus faecium*, one of the dominant enterococci found in human feces. The virulence factor, entercoccal surface protein (*esp)*, that is found in this species is linked to sewage originating from humans, not animals (Scott et al, 2004). Targeted sampling of another species of enterococci, *Enterococcus faecalis*, which is strongly associated with sewage, can be used to locate probable sewage inputs. For instance, a sampling assay that focuses on several points upstream and downstream of each intensive site should help to locate pollutant inputs from wastewater. Although *Enterococcus faecalis* is usually the dominant member of the enterococci isolated from human sewage, it can also be found in the feces of other animals, thus, its presence should be interpreted in the context of supporting tests. One study (Kuntz et al., 2003) found that between 37% and 58% of enterococci isolated from raw sewage, a sewage lagoon, and a water body impacted by the sewage were *Enterococcus faecalis.* In a more recent study (McDonald et al., 2006), fecal contamination in samples with *Enterococcus faecalis* percentages of around 30% of total enterococci were judged to be from bird sources. This source attribution was supported by the absence of detection of the human-associated *esp* gene.

1.5.3.2 Library-based identification using repetitive extragenic palindromic Polymerase chain reaction (rep-PCR)

Library-based methods require the establishment of large known-source databases (libraries) of patterns ("fingerprints") derived from indicator bacteria. The function of the library is to provide a database to which patterns derived from water samples can be matched in order to predict the most probable source. In order to be useful, a library must be "representative," that is, it must contain patterns from the dominant sources that might impact water quality, and enough host individuals (e.g. cattle, dogs) or sites (e.g. sediments, wastewater treatment plants) to represent the diversity of possible impacts on the water (EPA, 2005). For MST applications, the library consists of indicator bacteria fingerprints from human and animal feces from the sample region. Repetitive extragenic palindromic polymerase chain reaction (rep-PCR) uses primers that amplify the DNA fragments between repetitive sequences of the microbial genome. The DNA fragments are resolved in agarose gels, producing a rep-PCR fingerprint. Analysis of water samples is carried out in a similar manner; enough bacteria are isolated and typed by rep-PCR from each water sample to reflect the diversity of organisms (number of patterns) in the sample, and these patterns are matched against the library. Due to variations in bacterial strains from the same source (cattle, for example), the size of the library is very important to ensure accurate identification of the probable source of contamination. If the profile library is of adequate size, and fingerprints of bacteria from different sources (e.g., humans and cattle) form distinct groups, rep-PCR amplified microbial DNA from an environmental sample can be matched to rep-PCR amplified microbial DNA from a specific source in a given watershed.

Libraries are typically established using one specific indicator organism. *E. coli* or *Enterococcus* spp. are generally used since they are both recognized indicator organisms and are widely distributed in feces (Harwood, 2004). Harwood's group recently demonstrated that phenotypic fingerprinting of *Enterococcus* spp.by antibiotic resistance analysis (ARA) generally had a higher predictive capability for fecal source than similar fingerprinting of *E. coli* (Harwood et al., 2003; Moore et al., 2005). Furthermore, ARA fingerprinting of enterococci more accurately predicted the sources of blind challenge samples than did genotypic fingerprinting of *E. coli* (Moore et al., 2005).

To date, peer-reviewed publications discussing the effectiveness of rep-PCR as a genotyping method for creating MST libraries have most frequently used *E. coli* as the indicator organism; however, source identification using *Enterococcus* spp. is the suggested library-based technique for this manual (Hassan et al., 2005). In this instance, enterococci are isolated from water and sediment at the intensive sites and the genomic profile of randomly selected isolates are determined by rep-PCR. The profiles are then matched to a database, or library, of *Enterococcus* profiles from bacteria isolated from known sources in the study region. One such library, developed by Valerie J. Harwood (University of South Florida) and funded by the FDEP, was developed for the Jacksonville, Florida region in 2004.

1.5.4 Aerial Infrared Thermal Imaging

Aerial Infrared Thermal Imaging can be used as a tool to locate potential sources of pollution, including illicit discharges, in and around water bodies by utilizing an infrared image to detected temperature gradients. This technique is based on the premises that the pollution source is typically warmer than the surface waters it is released into and that warm water emits more infrared radiation than cool water. Black and white infrared images show the contrast in temperatures with shades of gray (lighter colors indicate warmer water). Infrared images can indicate anomalies, or unexpected temperature spikes or dips along the water body. In order to enhance temperature differences, it is necessary to collect images at night and during the colder winter months. Ground verification of the anomalies, aided by the use of aerial photographs and a tool referred to as "Picometry," is used to determine if a particular source of pollution is responsible. Picometry allows the latitude/longitude of a specific thermal signature to be searched in an aerial database. Due to potential obstructions caused by vegetation, especially throughout Florida, it is suggested that the ground-truthing process also occur during the cooler months when vegetation is somewhat diminished (COJ WQB, 2006).

An evaluation of the potential for using infrared technology to identify the location of illicit discharges to the surface waters of three tributaries in Duval County was conducted by City of Jacksonville's Environmental Quality Division (EQD). Photographs for this study were taken in February 2005 and were ground-truthed between May and November 2005. The types of features or sources that were observed to produce thermal signatures included: 1) surficial groundwater seepages; 2) standing or slow-moving water; 3) discharge from stormwater conveyance structures; 4) permitted wastewater discharges; 5) artesian well discharges; 6) dewatering activities; and 7) swimming pool discharges. Despite limitations, primarily including difficulty in determining signatures and the abundance of permitted discharges identified as potential sources, the results of the study indicated that the technique provides useful information for identifying discharges, and depending on cost, could be a useful tool for detecting pollution sources. An important benefit of Aerial Infrared Thermal Imaging is that it can be used to quickly assess a water body, as hundreds of miles can be scanned in one night under optimum conditions.

1.5.5 Monitoring of Groundwater Wells

Favorable environmental conditions of groundwater, including the lack of ultra-violet (UV) light and low nutrient flow, often extend the life expectancy of bacteria and viruses as compared to other water systems. This characteristic allows for using groundwater wells as a method for tracking microbial contamination, especially from septic systems.

Bacterial dispersal in groundwater is dependant on water flow, water volume, and grain size of the substrate. In addition, it is important to note that bacteria do not tend to travel as far or as fast as viruses. Therefore, if the objective is to focus on potential impacts of septic systems, it would be more useful to focus the efforts of the investigation on bacteriophages rather than on coliforms. Groundwater has also been proven as a valuable tool for tracking the spread of antibiotic resistance genes (which serve as markers for resistant bacteria) originating from the gastrointestinal tract of production animals. Koike (2004) reported that samples collected from groundwater wells consistently detected the presence of antibiotic resistance genes originating from animal production systems. Not surprisingly, the study also reported that hydro-geological conditions influenced sample results, with a higher frequency of detection in down gradient wells.

Section 2. Categorization of WBIDs

A categorization scheme was developed to effectively assess the environmental factors potentially influencing WBIDs. These categories are used throughout the remainder of this *Tributary Pollution Assessment Manual*; therefore, categorizing the WBIDs is one of the first steps to be completed in the assessment process. This scheme was developed based on land use classifications and putative / non-putative sources of fecal contamination. Both are explained in this Section of the Manual.

2.1 Land Use Classifications

Land use classifications from the St. Johns River Water Management District (SJRWMD) were used to develop categories of WBIDs. These categories were intended to focus source assessment efforts and essentially constitute a pre-assessment based on the best available existing data. The land use classifications were aggregated into three primary categories: Urban, Suburban, and Rural. Table 2.1 identifies how the land use cover and classification designations were differentiated into the three categories.

Table 2.1 Land Use Classifications

URBAN	**SUBURBAN**	**RURAL**
Auto Parking Facilities	Airports	Abandoned Mining Lands
Bus and Truck terminals	Cemeteries	Agriculture
Commercial and Services	Community Recreational Facilities	Borrow areas
Commercial and Services under construction	Disturbed Land	Extractive
Communications	Food Processing	Holding Ponds
Electrical Power Facilities	Golf Course	Inactive Land**
Electrical Power Transmission Lines	Marinas and Fish Camps	Low Density under construction
Governmental	Medium Density under construction	Open Land
High Density under construction	Military	Oil and Gas Storage*****
Industrial	Other Recreational	Pre-stressed Concrete Plants
Industrial under Construction	Racetracks	Reclaimed Lands
Institutional	Recreational	Residential, Low Density
Oil and Gas Processing	Residential, Medium Density	Residential, Rural
Other Heavy industrial		Rural Land****
Other Light Industry		Sand and Gravel Pits
Parks and Zoos		Sand other than beaches
Port Facilities		Solid Waste Disposal
Railroads		Spoil Areas
Residential, High density		Timber Processing
Roads and highways		Upland Forests
Sewage Treatment Plants		Upland Non-forested
Ship building and repair		Water
Stadiums*		Water supply plants
Transportation under construction		Wetlands

*Stadiums: Those facilities not associated with High Schools, Colleges, or Universities
** Inactive Land with street pattern but without structures
*** Auto Parking Facilities - when not directly related to other land uses
**** Rural Land in transition without positive indicators of intended activity

Land use data (percent per classification) should be obtained from the SJRWMD for each WBID to be evaluated. The classifications should be compiled according to Table 2.1 and totaled for each category (Urban, Suburban, and Rural). The WBID should be categorized as Urban, Suburban, or Rural based on the highest preponderance of aggregated use.

The WBID should be categorized as Urban, Suburban, or Rural based on the highest preponderance of aggregated use.

If there is not a clear distinction between the preponderance of use, knowledge of the surrounding areas should be used to determine the most appropriate category.

2.2 Putative Source Designation

As discussed in Section 1.4, putative sources are suspected sources. Designation of a WBID as belonging to a non-putative source category indicates that there are no suspected sources within the boundaries of the water basin. For the purposes of this Manual, certain parameters are recommended for classifying WBIDs as having a putative source or having no putative source. These parameters consider data from existing databases, including GIS data, from Federal, State, and Local agencies. Sources of data are discussed in more detail in Section 3. If the WBID includes at least one of the following, it could be classified as having a putative source:

1. repetitive, recent and localized sanitary sewer overflows (SSOs) that intersected surface waters;
2. significant number of identified illicit discharges to stormwater system;
3. a septic nuisance and/or failure area adjacent to or intersecting a waterbody; or
4. targeted land use (e.g., cattle farms, aquaculture, marinas).

Other known or suspected sources of fecal contamination should be documented and used to categorize the WBID as having a putative source. These sources might be identified in a pre-assessment evaluation with aerial infrared thermal imaging or fluorometry.

2.3 Categories of WBIDs

Based on the two factors described above (land use classification and potential source of contamination), WBIDs should be categorized into one of the following six categories:

- 1a. Urban – Putative Source
- 1b. Urban – No Putative Source
- 2a. Suburban – Putative Source
- 2b. Suburban – No Putative Source
- 3a. Rural – Putative Source
- 3b. Rural – No Putative Source

These categories are used in the sampling and analysis portion of the assessment (to provide validation that the sampling methods are useful and appropriate in a variety of land use/pollution source scenarios), and in formulating corrective action recommendations.

Section 3. Site Assessment

3.1 Introduction

The objective of the site assessment is to conduct an effective and comprehensive review of the tributaries through preliminary research and fieldwork. The site assessment should apply basic scientific information and a working knowledge of available GIS information. A significant portion of the initial assessment process involves the utilization of highly accurate GPS/GIS technology. For instance, locations of illicit discharges should be mapped and overlaid over features such as roads, aerial photographs, utilities locations, and parcel boundaries to determine if they are a potential source of contamination.

This section of the Manual focuses on site assessments designed to address the pathogen pollutant load element of Section 303 (c) of the CWA of 1972 which, in turn, addresses the total maximum daily loads (TMDL) that must be established for impaired waters. The TMDL is the driving force behind the development of methodology established to distinguish human from animal sources.

3.2 Preliminary Research

The six key elements that should be addressed during the preliminary research aspect of the site assessment process are:

1. land use planning;
2. land conservation;
3. soil characteristics;
4. historical data;
5. stormwater management practices; and
6. non-stormwater discharges.

Table 3.1 describes several items and their respective potential source(s) that could assist in providing a comprehensive understanding of the tributary site and should be collected during this portion of the site assessment. The majority of this information can be obtained through the GIS departments of the state and local governments, as well as, the city and local utilities. Suggested contacts in the local area include:

- Amy Miller (JEA);
- N. George Chakhtoura (City of Jacksonville);

- Grazyna Pawlowicz (Duval County Health Department); and
- Joe North (DEP).

Table 3.1 Examples of Available GIS Data

Data	Description	Source
SSO	Sanitary sewer overflow	Utility
Cave-Ins	Pipe cave-ins	Utility
Drainage Projects	City drainage projects	City
Agricultural Zones	Parcels zoned agricultural	City
Proposed Pipe Repairs	Proposed pipe repairs and line extensions	Utility
Sewer Footprints	Footprints of pipe bursting project areas	Utility
Sewer Infrastructure	Sewer mains, lift stations, and manholes	Utility
Pipe-Bursting Projects	Completed Pipe-Bursting Projects	Utility
Historical Sampling Data	Tributary sampling stations and historical fecal data	City/ Utility/ State Environmental Protection
Stormwater Infrastructure	Stormwater Infrastructure	City Public Works Dept
Septic Tank Repair Permits	Onsite Sewer Treatment and Disposal System (OSTDS) repair permits issued by date	Health Dept
Contamination Sites	Sites with contamination problems	Health Dept / State Environmental Protection
Septic Tank Failure Areas	Location of septic tank failure and nuisance areas	Health Dept
Landfills and Dumpsites	County landfills and dumpsites	Health Dept / City / State Environmental Protection
Illicit Connections	Illicit Connections	Utility / Health Dept
Land Use / Land Cover	Land use and land cover classifications as of 2000	Water Management District
Hydrologic Features	Water features, 1:24,000	Water Management District
WBIDs	WBID boundaries	State Department of Environmental Protection

3.2.1 Land Use Planning

Section 2 of this Manual described how land use classifications were divided into three categories: Urban; Suburban; and Rural. Table 2.1 of this manual provides examples for each category.

3.2.2 Land Conservation

While land conservation is a critical tool used for sensitive watersheds, it is also imperative that it be utilized for other types of resources. Each watershed should have its own land conservation strategy based on its management category, inventory of conservation areas, and land ownership patterns. Identification of these areas would assist in understanding potential animal sources of fecal coliform.

3.2.3 Soil Characteristics

Soil types and classifications for specific areas can be obtained from the Soil Survey of City of Jacksonville, Duval County, Florida (NRCS, 1998) which provides additional information regarding their suitability, limitations, and management for particular uses. For example, the document includes discussion of the degree and type of soil limitations, such as permeability, depth to a high water table, depth to bedrock, and flooding, that influence septic tank absorption fields, sewage lagoons, and sanitary landfills. This information can be used to cross reference areas deemed unsuitable for such sanitary facilities with areas that actually support these facilities to gain better insight into the potential for specific source-types.

3.2.4 Historical Data

Examination of historical data is imperative to gaining a comprehensive understanding of the status of a particular water body over time and could be helpful in determining potential sources of fecal contamination in tributaries. There are two main types of historical data that should be identified and investigated: fecal coliform concentration and rainfall data. Whenever possible, it is important to examine both types of data in relation to one another. For example, sources of fecal contamination may include transient animal populations that change with weather and season (EPA, 2005) or stormwater-related sources that could be connected to rainfall events. In addition rainfall events can greatly affect the bacteriological patterns of a tributary by increasing the probability that fecal contamination will reach surface waters, or eventually diluting a contamination signal.

In Duval County, Florida, the FDEP and the City of Jacksonville have both implemented individual monitoring programs aimed at tracking fecal contamination levels of specific WBIDs. Rainfall data, available from the SJRWMD is available at http://arcimspub.sjrwmd.com. Whenever possible, rain data relevant to the WBID in question should be utilized to perform a linear regression analysis to determine if there is a correlation between fecal concentrations and rainfall events. It is suggested that the antecedent rainfall amount for 1 day, 3 days, and 7 days be examined in this analysis since there is a lag time between when rainfall occurs and when runoff reaches the sampling location. This type of data is beneficial to current and future assessment efforts because it allows any patterns to be discerned, can help in determining critical sampling times, and could provide predictive capabilities for beach or shellfish harvesting restrictions based on rainfall alone. Similarly, the hydrologic conditions of a tributary may contribute to either the dilution of sources or to dry seasonal patterns and should therefore be evaluated. If specific local and relevant rain data to perform this type of analysis is not available through the SJRWMD, various online weather local and regional services, such as intellicast.com, could be utilized to gain basic insight into the approximate rainfall in a particular area during a specific time period. This information could be used in conjunction with sampling results to gain a better understanding of potential source types.

3.2.5 Stormwater Discharges

Stormwater treatment areas employ a variety of applications to minimize the amount of pollutant loading that will eventually enter our tributaries and waterways. Examples of typical stormwater generators include industrial and domestic wastewater treatment facilities Municipal Separate Storm Sewer Systems (MS4) Permits, construction activities (sediment and erosion control), and agricultural and recreational uses. In some cases, the stormwater collected is conveyed to a stormwater treatment pond prior to discharging into the surface water body. In some cases, these conveyance systems and stormwater ponds may themselves play an integral role in perpetuating high bacterial counts since both fecal coliforms and fecal streptococci are able to survive and reproduce in the sediments that are often found in these structures (Burton et al., 1987; Marino and Gannon, 1991). More obvious, is the importance of locating the stormwater pipes and where there is a potential for intersection or contamination from the wastewater treatment facility (WWTF) collection system lines.

3.2.6 Non-Stormwater Discharges

Non-stormwater discharges can contribute to the fecal coliform levels in surrounding tributaries. Possible sources of non-stormwater discharges are septic tanks, wastewater treatment facilities, biosolids land application sites, parks and recreation facilities, Concentrated Animal Feeding Operations (CAFOs), industrial sites, and abnormal events such as sanitary sewer overflows, combined sewer overflows, and transport truck accidents.

On-site sewage treatment and disposal systems (i.e., septic tanks) were initially the only technology available to treat wastewater. Since the CWA was mandated in 1972, more advanced methods have been developed to treat wastewater. It is important to locate septic tanks and evaluate them for the possibility of leaking tanks or faulty drain fields. The local health department may have a septic tank inspection routine, or a septic tank phase-out program that would provide the necessary information to determine if the tributary areas are impacted by septic tanks. For example, in Duval County, the septic tank inspection program administered by the Duval County Health Department, is complemented by a septic tank phase-out program directed by the Water and Sewer Expansion Authority (WSEA). The WSEA was established as an independent authority by the City of Jacksonville in 2003 to provide a voluntary program through which individual property owners are provided with the opportunity to finance water and/or sewer infrastructure in their existing developed neighborhoods. In new neighborhoods, this infrastructure is installed by the developers and the costs are passed to the new buyers (http://www.coj.net/Departments/Independent+Boards+and+Agencies/Water+and+Sewer+Expansion+Authority/default.htm).

In most cities today, the majority of the domestic and industrial wastewater generated is treated through a WWTF. The wastewater generated is collected through lift stations and collection system lines prior to being treated at the WWTF. After being treated at the WWTF, the resulting effluent can be discharged in several different ways. For example, the effluent could be discharged to a surface water body through a point source commonly known as the point of discharge (POD), or it could be released to a land application site. Water reuse is another method of managing wastewater. With the appropriate and effective control of pathogenic organisms through practices such as membrane treatment technologies and ultraviolet disinfection, reuse has the ability to drastically reduce environmental impacts related to the discharge of wastewater effluent to surface waters and provide an alternative water supply for several activities that do not necessitate potable quality water (e.g., irrigation and toilet flushing) (Reuse Coordinating Committee and the Water Conservation Initiative Water Resource Work Group, 2003). It is imperative, for purposes of the site assessment, to locate the individual lift stations, the WWTF collection system lines, and any associated WWTF effluent PODs.

Biosolids are the nutrient-rich organic materials resulting from the treatment of sewage sludge (the name for the solid, semisolid or liquid untreated residue generated during the treatment of domestic sewage in a treatment facility). Once processed, biosolids can be safely recycled and applied as fertilizer to improve and maintain productive soils and stimulate plant growth. Only biosolids that meet the most stringent standards described in the Federal and State rules can be approved for use as a fertilizer.

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Presently, community-friendly practices will also be followed through a Voluntary Environmental Management System currently being developed for biosolids by the National Biosolids Partnership. Although cities decide how best to manage their biosolids, the EPA is obligated and continues to provide the public with educational information regarding the safe recycling and disposal of biosolids.

Natural areas, including wild habitat and parks, CAFOs (ie. dairies, chicken farms, etc.), and recreational areas also need to be located and observed during the preliminary research process. These natural areas may contribute to the high fecal coliform bacteria levels in the water bodies due to direct input or sheet flow rainfall runoff into the water bodies.

Industrial facilities may also contribute to the high fecal coliform bacteria levels by providing source elements or nutrients which the bacteria could obtain as food for survival. Some local counties or cities are under an industrial pre-treatment program regulated by the State. This means that anyone classified as an industrial discharger will be permitted through the City or State, depending on the size of the facility and discharge location. Some examples of industrial facilities are dry-cleaning sites, concrete batch plants, paper mills, containment sites, and car wash facilities.

Abnormal incidents or events should be reviewed and cross-referenced to the potentially impacted water bodies. Abnormal incidents or events, such as SSOs and overturned tanker trucks, cannot be planned for during the environmental impact review. Once these events have been identified, it is important to evaluate and sample the impacted site, at upstream and downstream locations for environmental damage or threat. The local and state environmental agencies may have a compliance and enforcement program instituted to assist in compiling this data.

Other methods for obtaining potential source data or information are available. One example, Aerial Infrared Thermal Imaging Technologies, was identified in more detail in Section 1.5 of this Manual.

GIS mapping of the information identified above should be used to help develop corrective action plans by allowing a spatial analysis of existing utilities infrastructure locations with respect to deficient septic tank areas, failing sewer systems, small wastewater treatment facilities, identified illicit connections, and also natural habitats that may harbor wildlife or agricultural areas used for livestock grazing. A corrective action plan is provided later in this manual.

3.3 Field Site Assessment

It is suggested that the following items be included in the field portion of the site assessment to ensure a safe and productive site visit:

1. maps of the sites in question, cross referencing any of the known sources;
2. a list of the known information for each site;
3. field instruments;
4. labeled sample bottles for the appropriate sample collection techniques;
5. a cooler with enough ice for preservation of the samples;
6. bags, scissors and tape to seal samples for shipping;
7. shipping labels;
8. field data sheets;
9. digital camera;
10. appropriate attire including long sleeve shirts, long pants, and wader boots;
11. bug spray (use caution when sampling as to not contaminate the samples and compromise the results);
12. a device to cut through tall brush; and
13. a list of emergency contacts and property owner contacts.

During the field portion of the site assessment, observations should be recorded regarding anything with the potential to influence bacteriological levels of that tributary (e.g., a potential illicit discharge). For example, if a pipe is observed in the area, pipe location, type of pipe, pipe diameter, and any type, amount, color, or odor of discharge should be noted.

In most circumstances, the term illicit discharge refers to either a sewage cross-connection or straight pipe. A cross-connection can occur when a sewer pipe is improperly connected to a storm drain system and produces a continuous discharge of raw sewage. A straight pipe is a relatively small diameter pipe which is used to intentionally bypass a sanitary sewer connection or septic drain field, producing a direct discharge of improperly treated sewage into open channels or streams (Brown et al., 2004).

If any potential illicit discharges to a water body are noticed, they should be reported to the local Health Department for further investigation. The Duval County Health Department (DCHD) has a dedicated phone number where citizens can easily report illicit discharge and pollution concerns. During normal business hours, any person that would like to submit a complaint or incident report should contact (904) 630-3260. DCHD staff may request contact information from the person submitting a complaint; however, anonymous complaints are accepted. The location of the potential illicit discharge will also be requested; property address and property owner information should be provided when possible. A prompt investigation of each complaint by a trained inspector always follows a reported incident, usually within 24 hours. Jacksonville residents could also call (904) 630-CITY (630-2489) to report potential illicit discharges identified in Duval County.

In situations where the amount of sewage discharge is equal to or greater than 1000 gallons, it is necessary to contact the Florida State Warning Point at 1-800-320-0519. Please remember, in any emergency situation that is immediately life threatening, first call 911.

Field observations including the weather, the general appearance of the water body, and the type and location of any odors noticed should also be recorded. It is also recommended that photographs be taken to document all observations. An example of a field data sheet is located in Appendix E.

Section 4. Developing a Tributary Pollution Sampling and Analysis Plan

4.1 Introduction

This section describes recommendations for developing and implementing a tributary pollution assessment sampling and analysis plan. One of the most important aspects to consider when devising any sampling protocol is that it be science-based and hypothesis-driven so as to ensure adequate spatial and temporal representation and the ability to be statistically analyzed to the maximum extent possible. Therefore, sampling programs should employ experimentally-based designs and incorporate statistical analyses whenever feasible. This would enable the investigator to identify the limits of the conclusions drawn from the resultant data. As a result, prior to the commencement of sampling, the investigator should determine a method for data collection that would produce statistically valid, quantitative information that meets the designated objectives.

A great deal of planning is required to determine an appropriate approach to MST including sampling design, level of data collection, and type of analysis required to successfully identify pollutant sources. Despite drastic differences among potentially impaired tributaries, there are five basic facets that must be considered in order to effectively assess individual systems:

1. when to sample;
2. where to sample;
3. what and how to sample;
4. how to analyze samples; and
5. how to interpret sample results.

The answers to these questions are dependent on the objectives of the investigation (EPA, 2005). For purposes of this Manual, the study objective is to identify sources of fecal contamination to the impaired water bodies. As with any study, staff and funding limitations may necessitate that a lesser number of samples or sampling locations be collected or utilized, respectively.

Other details that should be addressed include:

- sampling station locations and depth;
- sampling dates and times;
- how samples will be collected and packaged for transport; and
- where and how samples will be transported.

The EPA (2002a) provides additional types of information that would be useful to include in the sampling and analysis plan such as how to report data and requirements for repeat sampling. This document is included as Appendix C.

4.2 Basic Factors for Consideration

4.2.1 When to Sample?

Sources of fecal contamination may include transient animal populations that change with weather and season (EPA, 2005) or stormwater sources that are related to rainfall events. As a result, it is imperative that water quality assessments occur over both wet and dry seasons. At a minimum, it is suggested that sampling events be scheduled on a monthly basis for at least four months during each season. If a stormwater source, such as runoff from a dairy farm or a residential development is suspected and time and finances allow, additional events should be added within three to seven days after a measurable rainfall event. All rainfall events within one week of sampling should be noted and considered when attempting to identify a potential source.

Special attention to tides should be considered when sampling tidally-influenced freshwater systems. Whenever possible, samples should be taken during outgoing tide to reduce the potential for outside influences that may affect the sample (e.g., salinity, intrusion by another tidally-influenced water body). In addition, samples should be consistently collected at approximately the same point in the tidal cycle to help account for changes in microbial concentrations that may be associated with tidal flow. For example, considerable dilution of the signal is likely during the lowest point of the tide. In order to allow general field observations to be made so as to identify any unusual events or changes to the system over time, sampling should be conducted during daylight hours. If time-constraints are an issue, it is suggested that the timing of sampling at the different stations within each tributary be coordinated around the tidal schedule (e.g., sample upstream stations first on an outgoing tide).

4.2.2 Where to Sample?

Historical data, putative (suspected) contamination sources, land-use types, hydrologic features, and site accessibility should all be considered when determining sampling locations. If historical data are available at consistent locations for the selected tributary, it would be beneficial to continue using the same sampling stations to help identify long-term trends and potential causes for declines in water quality. If study limitations prevent this practice, historical sampling locations should be bracketed by the new sampling stations to better assess impairments.

Another crucial factor in determining the location of the sampling stations is the presence or absence of a putative contamination source. This information can be used in conjunction with the land-uses characterizing the contributing watershed, the characteristics of the tributary itself, and the accessibility to the desired location. Capturing the variability of water quality within the tributary can be accomplished by increasing the number of sampling stations; however, many studies are limited by time, staff, and financial constraints. A minimum of three permanent sampling stations should be used for any given tributary. These three stations will provide consistency to the sampling program across seasons. If a putative source is present, these stations should be located such that one is as close as possible to the putative source, one is downstream from the putative source, and one is upstream from the putative source. The upstream sampling station would serve as a reference station. If the water body branches at any point, it is also important, whenever possible, to sample each branch, especially if different land-uses are represented.

In addition to the three permanent stations, supplemental stations may need to be added during the duration of the sampling program in order help focus the investigation toward areas containing more likely sources. For instance, supplemental stations may be added to test the water quality within a tributary upstream and downstream of a stormwater pond. Additional stations may also be utilized to further investigate potential illicit discharges or to help locate or confirm areas of failing infrastructure or septic tank seepage once a human source has been identified. If a putative source is not present, a minimum of three stations may be required to characterize the spatial extent of the watershed.

4.2.3 What and How to Sample?

Appropriate preparation and specific sampling procedures are described in this section. Appendix C (EPA, 2002a) contains additional important recommendations regarding sample collection techniques. The number of samples collected per site for each type of analysis is subject to the individual study design and financial constraints. It is suggested that whenever possible, three samples be collected per site.

4.2.3.1 Water Samples for Fluorometry Analysis

The following procedure for collecting water samples for fluorometry analysis was obtained from the Mote Marine Laboratory (July 2005). Water samples should be collected in pre-cleaned 125 mL Amber Glass bottles with Teflon-lined lids. Sample bottles should be filled directly from the water body or from an intermediate container. If an intermediate container (stainless or glass beaker) is used, rinse the container with site water prior to obtaining the sample. Measures should be taken to prevent non-representative organic particulates from contaminating the sample, as these will absorb optical brighteners. It is important to avoid filling the sample bottle more than 3/4 –full to prevent breakage upon freezing the sample. All field and laboratory technicians should wear polyethylene (not latex, nitrile, or other plastic) gloves. Caution should be used to minimize any contact of the sample with plastics to the extent possible. Sample bottles should be labeled, placed in individual Ziploc bags, stored on ice while in the field, and shipped immediately on blue ice to a qualified laboratory for analysis. If immediate shipment is not possible, samples should be frozen in the upright position for a later date. Overnight delivery should be used if the samples are shipped. Prior arrangements for Saturday delivery may be necessary.

4.2.3.2 Water Samples for Bacteriological Assays

Water samples for bacteriological assays should usually be collected using the grab sample method described in the 9060A Standard Methods for the Examination of Water and Wastewater (1992). All field and laboratory technicians should wear sterile latex gloves throughout the collection and analysis process; gloves should be changed between each sample collection. Prior to use, one-liter Nalgene bottles should be autoclaved with the lids loosely in place for sterilization. After cooling, the lids should be tightened until sampling begins. All bottles should be placed into a cooler containing ice for delivery to the sampling locations. Upon arrival at a sampling site, the needed bottles should be taken from the cooler and the lid removed just prior to collection of the sample. The lid should not be placed onto the ground, but should be held by an assistant. The operator should lower the bottle 0.25-0.5 m below the surface of the water to collect a one-liter volume of the sample. The lid should be placed back onto the bottle and tightened. Each bottle should be labeled with the sampling station name, date and time of collection, and initials of the sampling technician. The bottle should be placed back into the cooler where it will remain until processing. A field log sheet, including all pertinent information regarding the samples, should accompany the containers to the laboratory and be initialed and logged in by the receiver upon arrival.

4.2.3.3 Sediment Samples

The collection of sediment samples utilizes a similar process as used in the water column with the following exceptions: 1) a Ponar® sampler is used to retrieve the sample from the surface of the substrate; 2) pre-sterilized 50-mL conical tubes are used to collect 40 g of sediment from the Ponar®; and 3) the Ponar® is rinsed five times with distilled water between sampling stations.

4.2.3.4 Additional Field Data

In addition to taking water samples for laboratory analysis, additional field data such as salinity, conductivity, temperature, pH, turbidity, and dissolved oxygen are helpful in interpreting water quality trends. These measurements should be obtained using a calibrated water quality meter and collected at 0.5-meter or 0.2-meter intervals from bottom to surface in deeper and shallower waters, respectively.

Additional observations regarding water color, clarity, surface oils, and odor or the presence of sediment odors or oils should be noted. Comments regarding weather conditions and general tributary characteristics (i.e. local watershed erosion, evidence of non-point source pollution, percent canopy cover or shading, artificial channelization) would also be useful in determining sources of fecal contamination. These measurements and observations should be taken, whenever possible, during each site assessment.

All samples should be transported on ice to a qualified laboratory for analysis. All bacteriological samples should be analyzed as soon as possible; samples should not be stored for more than 12 hours prior to analysis. Chain of custody forms should be utilized when samples are transferred between parties. These forms should follow state-applicable guidelines and should be filed upon receipt.

4.2.4 Data Analysis and Interpretation

The tributary pollution assessment methodology described in this Manual has been developed to address impaired tributaries and incorporates a decision tree-based approach. The process considers cost and appropriate methods to adequately define sources to a level at which remedial actions can be defined and implemented with a high degree of certainty of success. This approach follows the recommendation of Bitton (2005) which states that a “methods-battery approach” that utilizes a combination of library-dependent, library-independent, and chemical tracer methods is useful in MST. This reduces the need for statistically powerful results from individual methods, which have not yet been developed, and places more weight on the positive or negative result of a series of methods (i.e., weight-of-evidence approach). A brief summary of each technique included in this approach is provided in Section 1.5. A qualified laboratory, with experience in performing the described microbiological techniques, the ability to provide results consistent with the established standards for precision and accuracy, and expertise in interpreting the results should conduct this type of work.

A diagrammatic representation of the decision-tree is provided in Appendix A. The decision-tree begins with an identification of putative sources, if known, followed by intensive site sampling of the indicator suite, over time and space as described above. The use of fluorometry, employing a flow-through approach as discussed in Section 1, to detect optical brighteners (if applicable) can also be an initial step. State of Florida standards applicable to this type of sampling can be found at http://esetappsdoh.doh.state.fl.us/irm00beachwater/default.aspx. Additional water quality criteria information is available from the EPA (1986). As indicated in the diagram, progression through the different tests included in the approach process is based on the results of the previous tests. The final step involves linking the concluded source from the testing process to the potential sources observed in the field in order to confirm the results. It is recommended that enough samples be collected from each sampling station to enable interpretation of temporal and spatial trends and correlations with other factors, such as rainfall. Samples can be prepared ahead of time for evaluation on an as-needed basis throughout the evaluation process. For example, DNA can be extracted and filters can be frozen after each sampling event and saved for future assessment depending on the results of each test in the decision tree.

The entire decision tree should be implemented following the first sampling event. Initially, all of the PCR tests should be conducted simultaneously in order to better identify all possible sources. As the sampling program progresses, it is expected that the use of the tests included in the decision-tree would become continually more site-specific. The use of subsequent tests should be determined using the decision-tree together with information gained from the first round of results; individual test results, site characteristics and remaining funds should be considered when deciding which additional tests are needed and when. All tests should be administered on an as-needed basis in order to provide the necessary weight-of-evidence to support the identification of a specific source. For example, a source or source-category identification should not be made based solely on a single test result; test results should be considered in conjunction with one another or should be confirmed by background knowledge of the area or observations made in the field.

In addition to those tests included in the decision tree in Appendix A, it is suggested that sediments, in addition to the water column, be sampled for the suite of indicator organisms. The results of such examinations could be used to determine whether, 1) fecal coliform contamination exists and possibly regenerates within the sediments and 2) the contributing source of fecal contamination is new or residual in the sediments from an older source. The results of the indicator suite alone will reveal whether microbial contamination is present in the sediments; however, additional testing is needed to determine if the source is relatively old or new. In this case, library-based *Enterococcus* rep-PCR tests could be run to compare the pattern of the genomic profile of the sediments to that of the water column. If the pattern for the sediment exhibits a greater level of similarity than that of the water column, this indicates that there is a reservoir of fecal coliform contamination within the sediments that, during a re-suspension event, may cause elevated levels of contamination in the water column.

A weight-of-evidence approach, as described above, should be used to assess the contribution of various potential sources to indicator organism contaminations observed at a given site. Several MST methods that rely upon different genetic identifiers of particular microorganisms are utilized in a study. Possible sources of error include sampling error (the microorganism was present at the site, but sample volume was too small), temporal variability, and analytical error. Sampling error can be reduced by analyzing multiple samples (replicates) of adequate volume or concentrating technique. Temporal variability can be accounted for by sampling several times over a time period.

The most probable types of analytical error vary depending upon method type. The non-library PCR methods (*Bacteroides*, *Enterococcus*) tend to be highly reliable when they are positive (they produce few false-positive results). In contrast, they may produce a false-negative result when contamination from a specific source is present, but the particular host animal(s) did not carry the distinct bacterial strain required for positive PCR results. Furthermore, inhibitory compounds in the water samples can be a problem, and careful controls must be run to ensure that negative results are not due to PCR inhibition.

The library-based PCR methods are used to identify many bacterial isolates from a water sample. No library is entirely accurate; therefore, there is some expected frequency of mis-identification of the source of isolates, termed the ‘rate of misclassification’ for the library (Whitlock et al., 2002). The rate of misclassification can be used as a “cut-off” for determining whether a particular source, i.e. human, should be considered a “significant” source of contamination in a particular sample (Harwood et al., 2003). A source of ambiguity in library-based analysis is the isolates whose fingerprints do not match those in the library. They are assigned to the “unknown” source category and can confound the analysis when they comprise a high percentage of the isolates.

Temporal variability can be used to help identify potential sources. If, for example, bacteria samples are consistently elevated during both wet and dry season sampling, a leaky sewer line or malfunctioning septic system may be a likely source. Rainfall events can greatly affect the temporal trends in fecal contamination of surface waters. Statistical analysis of microorganism concentrations or presence/absence with antecedent rainfall amounts can also assist with source identification or to eliminate suspected sources. The positive samples for each MST method can be correlated with environmental parameters such as rainfall and temperature to assess their relationships.

Conflicting results between methods may also occur. For example, the non-library PCR methods might be negative for human contamination in a given sample, while the rep-PCR library might indicate significant human contamination. Some of these methods are experimental and studies on the sensitivity and specificity of each technique are underway (Harwood, 2004).

4.2.5 Data Management and Analysis

It is recommended that all data be entered in Data Log Sheets (DLS) and transferred to computer spreadsheets for analysis. Quality control procedures should include confirmation that the data has been correctly transferred, that holding times have been met, and that reported values are reasonable compared to historical data, if available.

A variety of useful statistical analyses can be performed, depending on the experimental design implemented, to help identify trends within or between stations or water bodies. For instance, Analysis of Variance (ANOVA) can be used to compare variation in indicator organism concentrations between sites and linear regressions can be performed to correlate indicator organism concentrations to watershed characteristics, rainfall, or stream flow. Discriminant Analysis (DA) can also be used to assess the relative confidence of accurately identifying a microbial source so that further levels of the decision-tree can be evaluated.

Section 5. Synthesis of Findings

5.1 Introduction

Results from the testing of six priority WBIDs indicate that the dominant source of fecal coliform contamination in Duval County is human-related. More specifically, the three main source-types (not listed in order of importance) are: 1) sewer infrastructure; 2) septic tanks; and 3) stormwater (as a conveyance system) (Appendix F). Although background levels of fecal coliform bacteria originating from non-human sources are present and expected in these tributaries, it appears that concentrated animal populations, such as CAFOs, dog parks, or bird rookeries are necessary to result in the extremely elevated numbers associated with the most impaired water bodies. In these cases, the most reliable specific-source identification relies on the investigator's general knowledge of the site and surrounding area, field observations and site assessments. This section contains information on how a specific source of fecal coliform contamination could be identified and verified once the sampling results have been obtained and analyzed, and it has been concluded that the contamination belongs predominantly to a human-related source. Section 6 provides suggestions for corrective actions that could be implemented once the source type has been identified.

5.2 Specific Human-Source Identification

Insight gained through preliminary research (as described in Section 3) and observations recorded throughout the sampling program should be utilized in conjunction with the sampling results as part of the weight-of-evidence approach used in the process of identifying specific sources prior to investing in the more costly tests described below. For example, if the preliminary GIS data gathered indicates that numerous septic tank repair permits have historically been reported in an area, the soil characteristics are inadequate to properly support these facilities, and the source-tracking tests indicate a human source, then special attention should be directed towards septic tanks and drain fields in that area. If, by contrast, the sewer system is responsible for treatment of sanitary waste in a particular area, the investigator should focus on locations where sewer pipes and lift stations cross or are situated in close proximity to the impaired tributary. During field surveys, investigators may notice suspicious pipes, pools of water or potential illicit discharges that require further inspection and should be reported to the local branch of the Department of Health using the protocol described in Section 3.3. Preliminary analysis using the indicator suite as described in Section 1.5.1 should be considered for selected potential illicit discharges as a confirmation technique. In some cases, further investigation of these occurrences may lead to the identification of a specific source of contamination.

In the majority of cases, where the source of fecal coliform contamination is human-related, practices included as part of the regional utility's current preventative or predictive maintenance procedures could be utilized or expanded upon to help identify more specific sources. These procedures generally include, but are not limited to: 1) using remote camera equipment to inspect the lines, 2) cleaning lines, 3) inspecting manholes, and 4) testing pumps at lift stations to ensure proper function.

JEA, as the regional water and sewer provider for Duval County, has the capacity to perform a variety of tests that could assist in the identification of specific sources within a given portion of the sewer infrastructure. For example, dye testing can be utilized in pipes, particularly where they cross the tributary, as well as in manholes and lift stations. This method is limited in that it can only be used for specific sections of pipe and would not be useful in detecting underground leaks. Smoke-testing of the stormwater system, performed in conjunction with a thorough examination of the stormwater connections, is another useful tool for identifying specific sources. In this case, broken pipes or exit points in the system could be revealed. Smoke-testing can also be used on the gravity portion of the sewer system. In addition, video camera inspections of wastewater collection system lines, stormwater systems, septic tank system lines, and, on rare occasions, pressure lines, could be used to allow the human eye to see all the low points, infiltration points, sand accumulation, and any areas of broken pipe. In some instances, pipe locations may not be known by the utility, homeowner, or state and local governments. In these cases, ground penetrating radar (GPR) may be used in locating the lines.

Section 6. Corrective Action Plan

6.1 Introduction

Once the source of contamination has been determined, a corrective action plan can be implemented. This section of the manual identifies examples of corrective actions to help reduce or eliminate fecal coliform bacteria within a tributary. Examples of corrective actions described in this manual mainly focus on the three principle sources of fecal coliform contamination identified in Duval County, not listed in any particular order: 1) sewer infrastructure; 2) septic tanks; and 3) stormwater (as a conveyance system) (Appendix F).

In Section 3 of the Manual, GIS mapping of data was obtained to perform site assessments in order to develop preliminary corrective action plans. Site assessments are made by conducting a spatial analysis of existing utility infrastructure (i.e., collection system lines, lift stations, stormwater pipes, and cross connection intersections between stormwater and collection system lines) locations with respect to deficient septic tank areas, failing infrastructure, small wastewater treatment facilities (i.e., package plants), and natural habitats that may harbor wildlife or agricultural areas used for livestock grazing (dairies, cow pastures, CAFOs, parks and recreational areas). Recommendations for various types and routes of interconnects from package plants or septic systems to existing sewer pipes can be developed from this mapping effort along with estimates of cost based on distances or linear feet of pipe required.

In Section 2 of the Manual, land use categories were identified as urban, suburban, and rural and examples of each category where provided. Any, all, or a combination of the categories could be contributors to the fecal coliform bacteria levels identified in the site assessment. Tables 6.1-6.3 (provided at the conclusion of Section 6) for each land use category provide generic corrective actions. In most cases, there may be some applications which have a common use for all three land use categories. Examples of general types of treatment options for each land use category as well as corrective actions as they pertain to more the more specific sources responsible for impacting Duval County are discussed below.

6.2 Urban Land Use

Potential corrective actions for the urban land use category may include any or a combination of the following:

- Rehabilitation of Existing Utility Infrastructure;
- Remediation of Onsite Wastewater Treatment and Disposal Systems;
- Stormwater Treatment;
- Centralized Advanced Wastewater Treatment Facilities;
- Upgrading Existing Secondary Wastewater Treatment Facilities to Advanced Secondary Treatment for Effluent Reuse; and
- Application of best management practices (BMPs) to certain areas.

6.2.1 Rehabilitation of Existing Utility Infrastructure

Severe and catastrophic utility infrastructure collapses are rare, but as the infrastructure ages, it starts to fail. In the past, sewer lines were constructed mainly of vitrified clay, brick, and concrete. Today, sewer lines are made of reinforced concrete, plastic, ductile iron, and steel which are projected to have a longer life expectancy than previously used pipe materials. Typical failures in the infrastructure are often associated with pipe material, difficult or corrosive soil conditions, large wastewater flows, adjacent utility impacts, traffic congestion, and deep excavations.

Ongoing rehabilitation of existing utility infrastructure is important in order to help reduce or eliminate sanitary sewer overflows (SSOs). Through proper preventative and predictive maintenance and identification of problem areas of the existing infrastructure, SSOs can be reduced. Since large-scale replacements of existing systems have large capital costs, it is necessary to routinely conduct Infiltration and Inflow studies to locate the severe problem areas. Examples of maintenance procedures also include regularly 1) using remote camera equipment to inspect the lines, 2) cleaning lines, 3) inspecting manholes, and 4) testing pumps at lift stations to ensure proper function. Local utilities should also use “mean time to failure” estimates to replace pumps at lift stations prior to failing as well as warning systems for failure of utilities in order to help minimize impacts when failures occur. In addition to performing remedial maintenance on an as-needed basis, a variety of corrective actions could be performed on a prioritized schedule. These include, but are not limited to:

- pipe bursting (to increase the carrying capacity of pipe);

- slip lining (to provide smoother surface within pipe);
- open cut (removal and replacement of pipe);
- fiberglass-line manholes;
- replace manholes;
- line wet wells;
- re-routing; and
- replace force mains.

Oftentimes, as is the case in Duval County, a single utility is not responsible for all sewer infrastructure throughout the area. Private lift stations, for instance, may be a potential source of fecal coliform contamination to area tributaries. It is suggested that in order to increase the accountability of these facilities, explicit testing and maintenance requirements be employed, a program be implemented to arrange for inspections and repair to be conducted by the same entity, and inspections be regulated by requiring that all reports be sent to the City.

6.2.2 Remediation of Onsite Wastewater Treatment and Disposal Systems

According to the United States Census Bureau (1997), onsite wastewater treatment systems, or septic tanks and their associated drain fields, are responsible for the collection, treatment, and release of approximately 4 billion gallons of treated effluent per day from a projected 26 million homes, businesses and recreational facilities across the nation. These systems work by providing primary treatment of wastewater, consisting of 1) the settling of the solids to the bottom where they are partially converted through biological processes to liquid form and 2) the trapping of greases, oils, and other floatable matter, followed by the discharge of liquid effluent into the drain field. Additional treatment transpires as the effluent percolates downward into the bio-mat, a micro-biologically active area that traps solids, metabolizes some nutrients and carbon, and typically controls the infiltration rate prior to contacting groundwater (Tri-State Water Quality Council, 2005). Properly designed, functioning, and maintained septic systems can effectively remove nearly all suspended solids, bio-degradable organic compounds, and fecal coliforms (EPA, 2002b; included as Appendix G), as opposed to other typical constituents of wastewater such as nitrogen and phosphorus (Tri-State Water Quality Council, 2005).

Old and poorly maintained septic systems are a major source of fecal coliform contamination in urban, suburban and rural settings throughout Duval County. Few programs exist to address onsite system operation and maintenance which result in failures leading to otherwise preventable costs and risks to public health and water resources (EPA, 2002b). One example program already underway in Duval County is lead by the Water and Sewer Expansion Authority (WSEA), established in 2003 as an independent authority by the City of Jacksonville, to provide a voluntary program through which property owners are given the opportunity to finance water and/or sewer infrastructure in their existing developed neighborhoods (http://www.coj.net/Departments/Independent+Boards+and+Agencies/Water+and+Sewer+Expansion+Authority/default.htm). Alternative programs include:

- a Department of Health-sponsored certification program for haulers, similar to the Fats, Oils and Grease (FOG) program run by JEA;
- a program in which septic tanks must be inspected when home ownership changes;
- a program to execute new maintenance and design requirements that are based on environmental conditions (e.g., location within an impaired watershed, unsuitable soil conditions, proximity to surface water) and are regulated by the City;
- a community-based onsite management system program in which clusters of conventional septic tanks are drained into centralized advanced wastewater treatment areas and are managed by a designated responsible party (e.g., local utility or health department) (National Environmental Services Center, 2006); and
- a subsidized septic tank-pumping program that could be used in conjunction with septic-tank phase out programs to help immediately alleviate impacts during the transition period.

In addition to these programs, a broad-ranging septic tank ordinance could be implemented to avoid future water quality impacts. Under this ordinance, a variety of tasks could be performed. For example:

- delineate areas where septic tanks are inappropriate and other options are required;
- determine critical habitat and environmentally sensitive areas where use of septic tanks should be restricted;
- develop and implement a mandatory septic system inspection and tracking program;
- encourage efforts to connect to the existing sanitary sewer system and wastewater treatment facilities; and
- require setbacks and minimum lot sizes for septic tank use.

6.2.3 Stormwater Treatment

Typical fecal coliform bacteria concentrations for untreated stormwater runoff are within a range from of 10,000 to 100,000 MPN/100mL (Metcalf & Eddy, Inc., 2003).

Corrective actions using stormwater treatment benefit a tributary by reducing the sediment, oil and grease, and nutrient and bacteria loadings discharged to the affected water body. When focusing corrective efforts on stormwater treatment, it is important to realize that reduction of the sediment load to a tributary is critical because bacteria can adsorb to sediment particles where they can survive, grow and be transported from one area to another (Gerba et al., 1976; LaLiberte and Grimes, 1982; Marino and Gannon, 1991; Davies et al., 1995).

Improvements to the stormwater system, including retrofits, and associated programs may already be underway in the community of interest and could be used to supplement additional, more focused efforts. If these programs are not already in place or are no longer functioning efficiently, implementation or reorganization may be a good place to start. Such programs may include, but are not limited to: 1) active illicit connection programs; 2) public outreach directed specifically towards homeowner’s associations (HOAs) that may include storm drain stenciling and distribution of informational door flyers (Appendix H); 3) citizen education programs on reclaimed water; 4) stormwater pond inspection programs aimed at preventing excessive sediment and turbidity in surface waters; 5) construction site inspection programs; and 6) effective street sweeping programs.

Additional stormwater-related corrective actions that should be considered include:

- Additional re-development and retrofit;
- Identification of stormwater utility responsible for pond maintenance, if unknown;
- Enforcement of zoning and management for proper pond design and creation;
- Use of flocculation via alum injection;
- Use of ultra-violet light disinfection or ozone treatment for small systems;
- Creation of wetland systems;
- Use of individual product-line developments;
- Increased sediment control;
- Increased use of catch basin inlets and coordinating maintenance program;

- Implementation of pet control practices; and
- Application of non-structural BMPs to the affected areas.

Please see below for a more detailed discussion of some of these corrective actions. All of the above, alone or in combination can be effective in reducing coliform loadings to surface waters; however, each must be evaluated based on the area in question, the cost, and public acceptance. It is recommended that analysis be conducted on a life-cycle cost basis and factors such as capital costs, operation and maintenance costs and regulatory issues be considered in order to determine the most feasible approach. More detailed stormwater treatment design is provided in Appendix I.

6.2.3.1 Flocculation via Alum Injection

Alum injection systems involve the addition of alum (an aluminum sulfate salt) solution to stormwater so as to cause fine particles to flocculate and settle out. These treatment systems are typically comprised of a flow-weighted dosing system that has been designed to fit within a storm sewer manhole, remotely located storage tanks where alum injection and mixing occur, and a downstream pond which acts as a settling basin and collects the “floc” or “alum sludge” (Kurz, 1999, http://www.stormwaterauthority.org/assets/Alum%20Injection.pdf). When this treatment technique, under semi-controlled conditions using jar tests, was compared to above-ground sand filtration and the use of a wet detention pond, alum coagulation proved to be the most efficient overall for removing total and fecal coliform bacteria, MS2 coliphage, and turbidity (Kurz, 1999). The greatest advantage of this method is the ability to effectively treat large volumes of water. Disadvantages that should be considered include: 1) potentially high costs which depend on watershed size and the number of outfalls that need to be treated; 2) the need for chemical storage and delivery; 3) a relatively high amount of maintenance as a result of being an ongoing operation that requires a trained on-site operator; and 4) the need to dispose of the floc layer which may contain bacteria and viruses that remain viable after coagulation. The floc layer can generally be disposed of using sump pumps to transport the sludge to a sanitary sewer or, for larger systems, a portable remote-control dredge could be utilized from the shoreline to pump the material to an upland drying area or sewer system. If an upland drying area is used, the resulting material could either be transported for land application or to a landfill, depending on the composition (e.g., presence of metals) of the original stormwater.

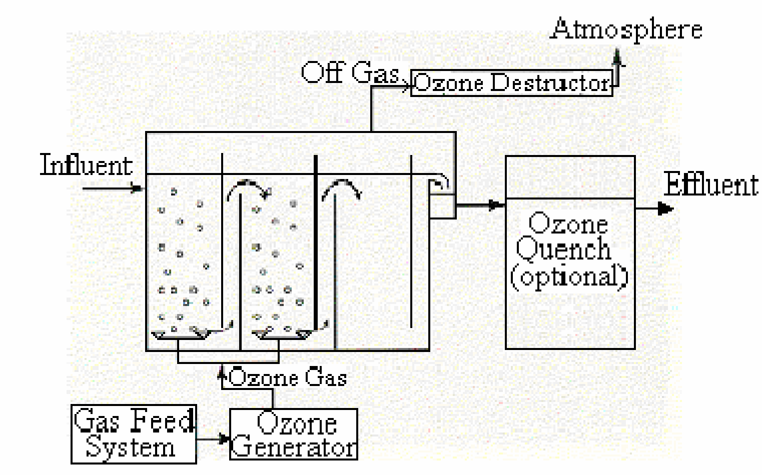
6.2.3.2 Ultra-Violet Light Disinfection

Given certain water quality characteristics of the tributary in question, ultra-violet (UV) light may be selected as the technique that would best remove fecal coliform bacteria and viruses from the surface waters. This method is generally selected because of the relatively low operation and maintenance costs and lack of negative environmental impacts associated with the treatment. Bacteria removal using UV light as treatment can be extremely high. For example, the City of Encinitas reported removal efficiencies from influent to effluent of 99.97% for total coliform, 99.80% for fecal coliform, and 99.65% for *Enterococcus* (City of Encinitas Clean Water Program, 2005). In addition, the use of UV light generally requires the use of relatively little mechanical equipment and therefore has smaller space requisites, does not involve any residual or disinfection by-products, and is suitable for tributaries with variable flow rates.

6.2.3.3 Ozone Disinfection

Ozone is very effective in water treatment for disinfection against bacteria. It is a powerful oxidant able to achieve disinfection with less contact time and concentration than all weaker disinfectants, such as chlorine, chlorine dioxide, and monochloramine (DeMers and Renner, 1992); however, ozone can only be used as a primary sanitizer because of the high decay rate of the residual and should therefore be coupled with one of the weaker decontamination agents for a for a complete disinfection system (EPA, 1999).

According to Caltrans (2004), an ozone treatment system has four basic components: a gas feed system, an ozone generator, an ozone contactor, and an off-gas destruction system (see figure below). The gas feed system provides a clean, dry source of oxygen to the generator. The ozone contactor transfers the ozone-rich gas into the water to be treated and provides contact time for disinfection (or other reactions). The final process step, off-gas destruction, is required as ozone is toxic in the concentration present in the off-gas. A quench chamber to remove ozone residual in solution may also be added to the treatment train.



Caltrans (2004)

Ozone systems are most applicable for continuous flow. For wet weather intermittent flow, a water sensor will be needed to start the ozone generator (the first flush of the runoff would not be treated unless an equalization/storage basin is provided).

An advantage to using ozone for disinfection is that the system only requires low doses to complete disinfection and has a limited number of by-products. The process does not provide residual ozone concentration in the effluent, which will minimize the impact to the receiving watershed. The systems are highly automated and have been proven to be very reliable.

When deciding on a treatment system, the very high energy requirement and initial cost of the equipment associated with ozonation technology should be considered. Other disadvantages include: 1) a relatively high level of maintenance and operator skill are required; 2) ozone is highly corrosive and toxic; 3) some ozonation by-products may be harmful to the receiving water; 4) in the presence of many of the compounds commonly encountered in water treatment, ozone decomposition forms hydroxyl free radicals; and 5) ozone escaping into the atmosphere may contribute to air pollution problems.

6.2.3.4 Bioretention Systems, Constructed Wetlands, and Wet Ponds

Bioretention systems, constructed wetland systems, and wet ponds all have demonstrated effectiveness in removing stormwater-associated bacteria. Several physical, chemical, and biological processes combine to contribute to bacterial reduction in these systems. Removal processes include filtration, adsorption, solar irradiation, sedimentation, aggregation, oxidation, antibiosis, predation, and competition. Constructed wetlands have reported removal rates ranging from 76% (fecal coliform colony counts, Birch et al., 2004) to over 90% (coliform bacteria, Kadlec and Knight, 1996). In a comparison study, Davies and Bavor (2000) reported that constructed wetlands performed significantly better than water pollution control ponds in reducing bacterial loading to recreational waters. Survival studies indicated that survival rates were also higher in the pond sediments and that predation was a major factor influencing bacterial survival.

The diverse vegetation, variations in stream flow and depth, and bacterial predators in the wetland system all combine with physical processes of sedimentation, adsorption to clays and organic matter, and variations in redox regimes to enhance the reduction of coliform bacterial concentrations (Rusciano and Obropta, 2005; Davies and Bavor, 2000). Constructed bioretention systems reduce bacterial loads from similar processes. A bioretention system is a constructed structural BMP used to treat stormwater runoff, often in parking lots. It includes grassed sides sloping to a ponding area enriched with native vegetation. There is generally a soil planting layer, underlain by a sand layer. In some designs, the sand layer drains to an underdrain fitted to the sewer system. In others, it recharges shallow groundwater systems. Rusciano and Obrpota (2005) reported total suspended solid removal averaging 92.3% and fecal coliform count reductions ranging from 54.7 to 99.7%. In conjunction with the use of a leachate pH of 4.7, acidic conditions were attributed as a primary removal mechanism, along with filtration, adsorption, and predation.

The use of constructed wetland and bioretention systems are highly effective BMPs in treating stormwater and wastewater effluents. These systems can be incorporated into new development designs or can be retrofitted to existing landscapes. These BMPs may be particularly effective for bacterial contamination when placed adjacent to or downstream from septic systems and septic drain fields and/or used as vegetative filters intercepting runoff to recreational waters.

6.2.4 Centralized Advanced Wastewater Treatment

While urban areas generally use primary and secondary wastewater treatment facilities, studies show that constructing centralized advanced wastewater treatment facilities, depending on the level of additional treatment, can actually decrease the fecal coliform pollutant loading to the water bodies. This is primarily the result of eliminating less efficient and less reliable package treatment plants. Increasing the treatment levels to advanced treatment, includes the addition of effluent filtration which removes suspended, colloidal, biological, and dissolved constituents. The removal of residual total suspended solids also results in better effluent disinfection required to meet more stringent discharge and reuse requirements. When the organic matter (particulate) is not adequately removed from the waste stream, this matter may shield the bacteria during the disinfection process. As an additional benefit, advanced treatment reduces the nutrient levels to surface waters.

6.2.5 Advanced Secondary Treatment

The major differences between advanced waste treatment and advanced secondary treatment are that the latter does not remove nutrients and costs significantly less to implement. As with advanced treatment, centralized facilities are preferred in order to eliminate less efficient and less reliable package treatment plants.

Existing secondary wastewater treatment facilities can be redesigned to meet effluent criteria for reclaimed water or reuse of reclaimed water. Thus, a secondary wastewater treatment facility currently discharging to a water body may be equipped to produce effluent for reuse by adding effluent filtration and high level disinfection.

Reuse facilities benefit the watershed via the removal of the discharge from the tributary or water body, thus eliminating any discharge of constituents and by the use of highly treated reclaimed water instead of pulling irrigation water out of wells and thus reducing the demand on the shallow aquifer.

6.2.6 BMPs

Other examples of corrective actions for the urban areas would be to apply structural BMPs to concrete batch plants, marinas, industrial facilities, parks and recreational areas. Since BMPs typically apply to each land use category, they are discussed in more detail later in this section of the manual.

6.3 Suburban Land Use

Many of the corrective actions described above for the urban land use category can also be utilized in suburban areas. Septic tanks and associated drain fields are typically used in suburban settings to treat the domestic wastewater flows due to location and costs associated with construction of a centralized wastewater treatment facility. As a result, those corrective actions described in Section 6.2.2 are relevant in addition to the construction of centralized wastewater treatment facilities and the application of BMPs. It is crucial that BMPs be applied to the suburban areas since it usually is not economically feasible to construct these large-scale treatment systems.

Additionally, non-structural BMPs, primarily including educational programs, have proven to be highly effective in these areas. This type of BMP provides the typical homeowner with information that can help control pollutant discharges and is described in more detail in Section 6.4.1.

6.4 Rural Land Use

For the rural land use category, BMPs are the most beneficial and economical type of treatment. Examples of the types of BMPs that could be employed include those for agricultural, forestry, dairies, CAFOs, and biosolids facilities. An excellent resource to help implement BMPs in rural areas is the Natural Resources Conservation Service (NRCS) and the University of Florida Institute of Food and Agricultural Services (IFAS). Both provide the landowner with resources for planning, implementation, and financing.

BMPs are determined based on community and environmental factors and can be divided into two main types; structural and non-structural. Since non-structural BMPs focus on preventative or source-control practices, whenever possible, these procedures should be employed and used in conjunction with structural BMPs (treatment practices) when necessary. Source control practices are primarily implemented through education, management, planning and infrastructure maintenance. Included in this category are:

- public education and outreach (e.g., storm drain stenciling, newsletters distributed with water bills);
- elimination of illicit connections and discharges;
- spill prevention, control and cleanup;
- materials handling;
- street/storm drain maintenance (e.g., street sweeping, catch basin cleaning);
- illegal dumping controls;
- promotion of Low Impact Development (e.g., minimize impervious surfaces, protect wetlands and riparian buffers); and
- stormwater reuse.

A variety of treatment practices were described in detail above and include, but are not limited to, the use of ponds, vegetative biofilters, constructed wetlands, filters, chemical treatment, and additional treatment schematics. BMPs which have been approved by Federal and State agencies for the above mentioned corrective actions can be found in Appendix J.

Table 6.1: Generic Corrective Actions for Urban Land Use

URBAN	POTENTIAL SOLUTION
Auto Parking Facilities	Stormwater Treatment
Bus and Truck terminals	Stormwater Treatment
Commercial and Services	Stormwater Treatment
Commercial and Services under construction	Stormwater Treatment, BMPs
Communications	Stormwater Treatment
Electrical Power Facilities	Stormwater Treatment
Electrical Power Transmission Lines	Stormwater Treatment
Governmental	Stormwater Treatment
High Density under construction	Stormwater Treatment, BMPs
Industrial	Stormwater Treatment, Centralized AWT
Industrial under Construction	Stormwater Treatment, BMPs
Institutional	Stormwater Treatment
Oil and Gas Processing	Stormwater Treatment
Other Heavy industrial	Stormwater Treatment
Other Light Industry	Stormwater Treatment
Parks and Zoos	Stormwater Treatment, BMPs
Port Facilities	Stormwater Treatment, BMPs
Railroads	BMPs
Residential, High density	Stormwater Treatment, Centralized AWT
Roads and highways	Stormwater Treatment
Sewage Treatment Plants	Stormwater Treatment, BMPs, CMOM
Ship building and repair	Stormwater Treatment, BMPs
Stadiums*	Stormwater Treatment
Transportation under construction	Stormwater Treatment, BMPs

Table 6.2: Generic Corrective Actions for Suburban Land Use.	
SUBURBAN	**SOLUTION**
Airports	Stormwater Treatment
Cemeteries	BMPs
Community Recreational Facilities	BMPs
Disturbed Land	BMPs
Food Processing	Stormwater Treatment
Golf Course	Stormwater Treatment, BMPs
Marinas and Fish Camps	BMPs, dump stations
Medium Density under construction	BMPs
Military	Stormwater Treatment, BMPs
Other Recreational	BMPs
Racetracks	Stormwater Treatment, BMPs
Recreational	Stormwater Treatment, BMPs
Residential, Medium Density	Stormwater Treatment

Table 6.3: Generic Corrective Actions for Rural Land Use.	
RURAL	**SOLUTION**
Residential, Low Density	Stormwater Treatment, BMPs
Residential, Rural	Septic Tanks, BMPs
Rural Land****	BMPs
Sand and Gravel Pits	Stormwater Treatment, BMPs
Sand other than beaches	BMPs
Solid Waste Disposal	Stormwater Treatment, BMPs
Spoil Areas	Stormwater Treatment, BMPs
Timber Processing	Stormwater Treatment, BMPs
Upland Forests	BMPs
Upland Nonforested	BMPs
Water	Stormwater Treatment, BMPs
Water supply plants	Stormwater Treatment, BMPs
Wetlands	STAs
Abandoned Mining Lands	BMPs
Agriculture	BMPs
Borrow areas	Stormwater Treatment, BMPs
Extractive	BMPs
Holding Ponds	BMPs
Inactive Land**	BMPs
Low Density under construction	BMPs
Open Land	BMPs
Oil and Gas Storage*****	Stormwater Treatment, BMPs
Prestressed Concrete Plants	Stormwater Treatment, BMPs
Reclaimed Lands	Stormwater Treatment, BMPs

*Stadiums: Those facilities not associated with High Schools, Colleges, or Universities

** Inactive Land: Identified as lane with a street pattern but without visible structures

*** Auto Parking Facilities: Parking Facilities not directly related to other land uses

**** Rural Land: Land in transition without positive indicators of intended activity

***** Oil and Gas Storage: Tank storage excluding those areas associated with industrial use or manufacturing use
Note: Stormwater Treatment refers to the stormwater management practices available for each individual category. BMPs are Best Management Practices individualized to meet the specific pollutants associated with each category.

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Web Resources

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http://cfpub.epa.gov/npdes/stormwater/menuofbmps/illi_1.cfm

http://esetappsdoh.doh.state.fl.us/irm00beachwater/default.aspx

http://arcimspub.sjrwmd.com

http://esetappsdoh.doh.state.fl.us/irm00beachwater/default.aspx

http://www.neiwpcc.org/Index.htm?sanitarysurveys.htm~mainFrame

http://www.stormwaterauthority.org/assets/Alum%20Injection.pdf

http://www.coj.net/Departments/Independent+Boards+and+Agencies/Water+and+Sewer+Expansion+Authority/default.htm

http://www.stormwater.net (included as Appendix I)