EPA New England Bacterial Source Tracking Protocol
Draft – January 2012

Purpose

This document provides a common framework for EPA New England ("EPA-NE") staff to develop and implement bacterial source tracking sample events, and provides a recommended approach to watershed association, municipal, and State personnel. Adopted from Boston Water and Sewer Commission ("BWSC") (2004), Pitt (2004), and based upon fieldwork conducted and data collected by EPA-NE, the protocol relies primarily on visual observations and the use of field test kits and portable instrumentation during dry and wet weather to complete a screening-level investigation of stormwater outfall discharges or flows within the drainage system. When necessary, the addition of more conclusive chemical markers may be included. The protocol is applicable to most typical Municipal Separate Storm Sewer Systems ("MS4s") and smaller tributary streams. The smaller the upstream catchment area and/or more concentrated the flow, the greater the likelihood of identifying an upstream wastewater source.

Introduction

The protocol is structured into several phases of work that progress through investigation planning and design, laboratory coordination, sample collection, and data evaluation. The protocol involves the concurrent collection and analyses of water samples for surfactants, ammonia, total chlorine, and bacteria. When more precise confirmation regarding the presence or absence of human sanitary sewage is necessary, and laboratory capacity is available, the additional concurrent collection of samples for select Pharmaceutical and Personal Care Product ("PPCP") analysis is advised. When presented with a medium to large watershed or numerous stormwater outfalls, the recommended protocol is the screening of all outfalls using the surfactant, ammonia, total chlorine, and bacterial analyses, in addition to a thorough visual assessment. The resulting data and information should then be used to prioritize and sample a subset of outfalls for all parameters, including PPCP compounds and additional analyses as appropriate. Ideally, screening-level analyses can be conducted by state, municipal, or local watershed association personnel, and a prioritized sub-set of outfalls can be sampled through a commercial laboratory or by EPA-NE using more advanced confirmatory techniques.

Step I – Reconnaissance and Investigation Design

Each sample event should be designed to answer a specific problem statement and work to identify the source of contamination. Any relevant data or reports from State, municipal, or local watershed associations should be reviewed when selecting sample locations. Aerial photography, mapping services, or satellite imagery resources are available free to the public through the internet, and offer an ideal way to pre-select locations for either field verification or sampling.

Sample locations should be selected to segregate outfall sub-catchment areas or surface waters into meaningful sections. A common investigative approach would be the identification of a specific reach of a surface water body that is known to be impaired for bacteria. Within this specific reach, stormwater outfalls and smaller tributary streams would be identified by desktop reconnaissance, municipal outfall mapping, and field investigation when necessary. Priority outfalls or areas to field verify the presence of outfalls should be selected based on a number of factors, including but not limited to the following: those areas with direct discharges to critical or impaired waters (e.g. water supplies, swimming beaches); areas served by common/twin-invert manholes or underdrains; areas with inadequate levels of sanitary sewer service, Sanitary Sewer Overflows ("SSOs") or the subject of numerous/chronic sanitary sewer customer complaints; formerly combined sewer areas that have been separated; culverted streams, and; outfalls in densely populated areas with older infrastructure. Pitt (2004) provides additional detailed guidance.

When investigating an area for the first time, the examination of outfalls in dry-weather is recommended to identify those with dry-weather flow, odor, and the presence of white or gray filamentous bacterial growth that is common (but not exclusively present) in outfalls contaminated with sanitary sewage (see Attachment 1 for examples). For those outfalls with dry-weather flow and no obvious signs of contamination, one should never assume the discharge is uncontaminated. Sampling by EPA-NE staff has identified a number of outfalls with clear, odorless discharges that upon sampling and analyses were quite contaminated. Local physical and chemical conditions, in addition to the numerous causes of illicit discharges, create outfall discharges that can be quite variable in appearance. Outfalls with no dry-weather flow should be documented, and examined for staining or the presence of any obvious signs of past wastewater discharges downstream of the outfall.

As discussed in BWSC (2004), the protocol may be used to sample discreet portions of an MS4 sub-catchment area by collecting samples from selected junction manholes within the stormwater system. This protocol expands on the BWSC process and recommends the concurrent collection of bacteria, surfactant, ammonia, and chlorine samples at each location to better identify and prioritize contributing sources of illicit discharges, and the collection of PPCP compounds when more conclusive source identification is necessary.

Finally, as discussed further in Step IV, application of this sampling protocol in wet-weather is recommended for most outfalls, as wet-weather sampling data may indicate a number of illicit discharge situations that may not be identified in dry weather.

Step II – Laboratory Coordination

All sampling should be conducted in accordance with a Quality Assurance Project Plan ("QAPP"). A model QAPP is included as Attachment 2. While the QAPP details sample collection, preservation, and quality control requirements, detailed coordination with the appropriate laboratory staff will be necessary. Often sample events will need to be scheduled well in advance. In addition, the sampling team must be aware of the strict holding time requirements for bacterial samples – typically samples analysis must begin within 6 hours of sample collection. For sample analyses conducted by a commercial laboratory, appropriate coordination must occur to determine each facility's respective procedures and requirements. The recommendations in this protocol are based on the use of a currently unpublished EPA-NE modification to *EPA Method 1694 – Pharmaceuticals and Personal Care Products in Water,* *Soil, Sediment, and Biosolids by HPLC/MS/MS*. Several commercial laboratories may offer Method 1694 capability. EPA-NE recommends those entities wishing to utilize a contract laboratory for PPCP analyses ensure that the laboratory will provide quantitative analyses for acetaminophen, caffeine, cotinine, carbamazepine, and 1,7-dimethylexanthine, at Reporting Limits similar to those used by EPA-NE (See Attachment 3). Currently, the EPA-NE laboratory has limited capacity for PPCP sampling, and any proposed EPA-NE PPCP sample events must be coordinated well in advance with the appropriate staff.

Step III – Sample Collection

Once a targeted set of outfalls has been selected, concurrent sampling and analyses for surfactants, ammonia, and total chlorine (which can all be done through the use of field kits), in addition to bacteria (via laboratory analysis) should be conducted. When numerous outfalls with dry-weather flow exist, sample locations should be prioritized according to the criteria mentioned above. In addition, field screening using only the field kits may occur during the field reconnaissance. However, it must be emphasized that the concurrent sampling and analyses of bacteria, surfactant, ammonia, and total chlorine parameters is the most efficient and cost-effective screening method.

When first observed, the physical attributes of each outfall or sampling location should be noted for construction materials, size, flow volume, odor, and all other characteristics listed on the data collection form (Attachment 4). In addition, GPS coordinates should be collected and a photograph of the sample location taken. Whenever possible, the sampling of storm drain outfalls should be conducted as close to the outfall opening as possible. Bacterial samples should be collected first, with care to not disturb sediment materials or collect surface debris/scum as best possible. A separate bottle is used to collect a single water sample from which aliquots will be analyzed for surfactants, ammonia, and total chlorine. A sample for PPCP analysis is recommended to be collected last, as the larger volume required and larger bottle size may cause some sediment disturbance in smaller outfalls or streams. If necessary, a second smaller, sterile and pre-cleaned sampling bottle may be used to collect the surface water which can then be poured into the larger PPCP bottle. Last, a properly calibrated temperature/specific conductance/salinity meter should be used to record all three parameters directly from the stream or outfall. When flow volume or depth is insufficient to immerse the meter probe, a clean sample bottle may be utilized to collect a sufficient volume of water to immerse the probe. In such instances, meter readings should be taken immediately.

As soon as reasonably possible, sample aliquots from the field kit bottle should be analyzed. When concurrent analyses are not possible, ammonia and chlorine samples should be processed first, followed by surfactant analysis, according to each respective Standard Operating Procedure as appropriate based on the particular brand and type of field test kit being used. All waste from the field test kits should be retained and disposed of according to manufacture instructions. Where waste disposal issues would otherwise limit the use of field kits, EPA-NE recommends that, at a minimum, ammonia test strips with a Reporting Limit below 0.5 mg/L be utilized. Such test strips typically are inexpensive and have no liquid reagents associated with their use. Results should be recorded, samples placed in a cooler on ice, and staff should proceed to the next sample location.

Upon completion of sampling and return to the laboratory, all samples will be turned over to the appropriate sample custodian(s) and accompanied by an appropriate Chain-of-Custody ("COC") form.

Step IV – Data Evaluation

Bacterial results should be compared to the applicable water quality standards. Surfactant and ammonia concentrations should be compared to the thresholds listed in Table 1. Evaluation of the data should include a review for potential positive results due to sources other than human wastewater, and for false negative results due to chemical action or interferences. In the EPA-NE region, field sampling has indicated that the biological breakdown of organic material in historically filled tidal wetlands may cause elevated ammonia readings, as can the discharge from many landfills. In addition, salinity levels greater than 1 part per thousand may cause elevated surfactant readings, the presence of oil may likewise indicate elevated levels, and fine suspended particulate matter may cause inconclusive surfactant readings (for example, the indicator ampule may turn green instead of a shade of blue). Finally, elevated chlorine from leaking drinking water infrastructure or contained in the illicit wastewater discharge may inhibit bacterial growth and cause very low bacterial concentrations. Any detection of total chlorine above the instrument Reporting Limit should be noted.

Table 1 – Freshwater Water Quality Criteria, Threshold Levels, and Example Instrumentation [1]

Analyte/ Indicator	Threshold Levels/ Single Sample[3]	Instrumentation
E. coli [2]	235 cfu/100ml	Laboratory via approved method
Enterococci [2]	61 cfu/100ml	Laboratory via approved method
Surfactants (as MBAS)	$\geq$ 0.25 mg/l	MBAS Test Kit (e.g. CHEMetrics K-9400)
Ammonia (NH_3)	$\geq$ 0 .5 mg/l	Ammonia Test Strips (e.g. Hach brand)
Chlorine	> Reporting Limit	Field Meter (e.g. Hach Pocket Colorimeter II)
Temperature	See Respective State Regulations	Temperature/Conductivity/Salinity Meter (e.g. YSI Model 30)

[1] The mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. EPA
[2] 314 CMR 4.00 MA - Surface Water Quality Standards - Class B Waters.
[3] Levels that may be indicative of potential wastewater or washwater contamination

Once dry-weather data has been examined and compared to the appropriate threshold values, outfalls or more discreet reaches of surface water can be selected for sampling or further investigation. Wet-weather sampling is also recommended for all outfalls, especially for those that did not have flow in dry weather or those with dry-weather flow that passed screening thresholds. Wet-weather sampling will identify several situations that would otherwise pass unnoticed in dry weather. These wet-weather situations include, but are not limited to the following: elevated groundwater that can now cause an exchange of wastewater between cracked or broken sanitary sewers, failed septic systems, underdrains, and storm drains; increased sewer volume that can exfiltrate through cracks in the sanitary piping; increased sewer volume that can enter the storm drain system in common manholes or directly-piped connections to storm drains; areas subject to capacity-related SSO discharges, and; illicit connections that are not carried through the storm drain system in dry-weather.

Step V – Costs

Use of field test kits and field instruments for most of the analytical parameters allows for a significantly reduced analytical cost. Estimated instrument costs and pro-rated costs per 100 samples are included in Table 2. The cost per 100 samples metric allows averaged costs to account for reagent refills that are typically less expensive as they do not include the instrument cost, and to average out the initial capital cost for an instrument such as a temperature/ conductivity/salinity meter. For such capital costs as the meters, the cost over time will continue to decrease.

Table 2 – Estimated Field Screening Analytical Costs [1]

Analyte/ Indicator	Instrument or Meter [2]	Instrument or Meter Cost/No. of Samples	Cost per Sample (Based on 100 Samples) [3]
Surfactants (as MBAS)	Chemetrics K-9400	$77.35/20 samples ($58.08/20 sample refill)	$3.09
Ammonia (NH_3)	Hach brand 0 – 6 mg/l	$18.59/25 samples	$0.74
Total Chlorine	Hach Pocket Colorimeter II	$389/100 samples ($21.89 per 100 sample refill)	$3.89
Temperature/ Conductivity/ Salinity	YSI	$490 (meter and cable probe)	$4.90

1 Estimated costs as of February 2011
2 The mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. EPA
3 One-time meter costs and/or refill kits will reduce sample costs over time

From Table 2, the field analytical cost is approximately $13 per outfall. Typical bacterial analyses costs can vary depending on the analyte, method, and total number of samples to be performed by the laboratory. These bacterial analyses costs can range from $20 to $60. Therefore, the analytical cost for a single outfall, based on the cost per 100 samples, ranges from $33 to $73. As indicated above, these costs will decrease slightly over time due to one-time capitals costs for the chlorine and temperature/conductivity/salinity meters.

Step VI – Follow-Up

Once all laboratory data has been reviewed and determined final in accordance with appropriate quality assurance controls, results should be reviewed with appropriate stakeholders to determine next steps. Those outfalls or surface water segments that fail to meet the appropriate water quality standard, and meet or exceed the surfactant and ammonia threshold values, in the absence of potential interferences mentioned in Step IV, indicate a high likelihood for the presence of illicit connections upstream in the drainage system or surface water. Whereas illicit discharges are quite variable in nature, the exceedance of the applicable water quality standard and only the ammonia or surfactant threshold value may well indicate the presence of an illicit connection. When available, the concurrent collection and analyses of PPCP data can greatly assist in confirming the presence of human wastewater. However, such data will not be available in all instances, and the collective data set and information regarding the physical characteristics of each sub-catchment or surface water reach should be used to prioritize outfalls for further investigation. As warranted, data may be released to the appropriate stakeholders, and should be accompanied by an explanation of preliminary findings. Release of EPA data should be fully discussed with the case team or other appropriate EPA staff.

References Cited

Boston Water & Sewer Commission, 2004, *A Systematic Methodology for the Identification and Remediation of Illegal Connections.* 2003 Stormwater Management Report, chap. 2.1.

Pitt, R. 2004 *Methods for Detection of Inappropriate Discharge to Storm Drain Systems. Internal Project Files*. Tuscaloosa, AL, *in* The Center for Watershed Protection and Pitt, R., *Illicit Discharge Detection and Elimination: A Guidance Manual for Program Development and Technical Assessments*: Cooperative Agreement X82907801-0, U.S. Environmental Protection Agency, variously paged. Available at: http://www.cwp.org.

Instrumentation Cited (Manufacturer website URL):

MBAS Test Kit - CHEMetrics K-9400:
https://www.chemetrics.com/index.php?route=product/category&path=59_76
Portable Colorimeter – Hach Pocket Colorimeter II: http://www.hach.com/
Ammonia (Nitrogen) Test Strips: http://www.hach.com/
Portable Temperature/Conductivity/Salinity Meter: YSI Model EC300A:
https://www.ysi.com/EC300A

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Sampling of Stormwater Outfalls Using
Stormwater Toolbox Test Kits

Quality Assurance Project Plan (QAPP)
Month/Year

Organization Name

Org Name Project Manager: Name

Signature: ________________________________ Date: _____________

EPA Toolbox Coordinator: Shannon Shea

Signature: ________________________________ Date: _____________

EPA Project Officer: Todd Borci

Signature: ________________________________ Date: _____________

EPA QA Officer: Emily Ambeliotis

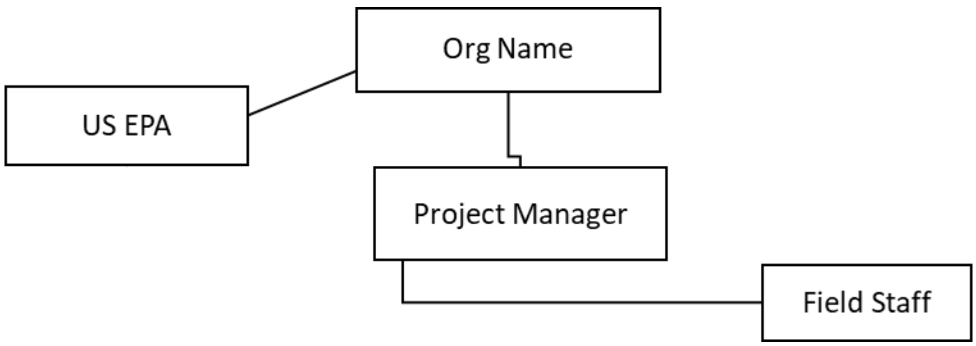
Signature: ________________________________ Date: _____________

1.0 Background

U.S. EPA Administrative Order 5360.1 requires that "all projects involving environmental monitoring performed by or for the U.S. EPA shall not be undertaken without an adequate Quality Assurance Project Plan (QAPP)." The purpose of this document is to describe the process used to develop, select, manage, and finalize stormwater monitoring projects. In describing this process, quality assurance goals and methods will be established, thus ensuring that the overall program and each monitoring project will meet or exceed EPA requirements for quality assurance.

The objective of these projects will be to collect data that is usable by EPA enforcement staff for enforcement actions and information requests. The primary focus of this project will be on urban water stormwater outfalls in the New England Region watersheds.

2.0 Project Organization and Responsibilities



Name	Title	Description of Responsibility
####	Project Manager	QAPP development and overall project coordination
####	Field Staff	Conducts sampling at selected outfall locations
Emily Ambeliotis	EPA QA Officer	Reviews QAPP
Shannon Shea	EPA Toolbox Coordinator	Trains field staff on use of the toolbox
Todd Borci	EPA Project Officer	Coordinates MS4 location targeting

3.0 Project Description

Org Name has been granted a loan of the Stormwater Toolbox from the EPA to conduct stormwater outfall sampling. The Stormwater Toolbox, developed by EPA for the purpose of identifying illicit connections to municipal separate storm sewer systems (MS4s), will be used by Org Name to analyze stormwater outfall samples for ammonia, chlorine, and surfactants, as well has water quality parameters temperature, salinity, and conductivity (specific conductance). The data collected as part of this project will be used for screening purposes only and is intended to help identify outfalls that may need additional investigation.

3.1 Data Quality Objectives

Accuracy and precision values for each parameter are listed below (Table 1). The values are to be considered as goals because some specific compounds are known outside these goals. Field duplicate will be collected at a rate of 1 per 20 samples.

Table 1: Quality Control Goals

Parameter	Reporting Limits	Water Quality Criteria or Guidelines (MA or EPA)	Quality Assurance Goals		
			Precision	Accuracy	Completeness
Temperature	0 to +40ºC	28.3ºC	0.1ºC	± 0.3ºC	≥90%
Specific Conductance	0 to 100 mS/cm	N/A	5 µS/cm	±10% cal std (µS/cm)	≥90%
Salinity	0 to 10 ppt	1.0 ppt	0.1 ppt	0.1 ppt	≥90%
Surfactants	0.25 mg/L[1]	0.25 mg/L	Field dup 30% RPD	±0.25 mg/L[2]	≥90%
Ammonia	0.25 mg/L[1]	1.0 mg/L	Field dup 30% RPD	Varies[2]	≥90%
Chlorine	0.02 mg/L	4.0 mg/L (drinking water)	Field dup 30% RPD	See Hach Manual	≥90%

[1]Needs field verification to confirm
[2]Colorimetric method sometimes yields variable values and results that fall between intervals can be estimated.

3.2 Data Usage

Data will be used to determine if further investigation of stormwater outfall locations may be necessary. Data generated during this project is to be used for screening purposes only. No samples will be submitted for lab analysis. Site observations, documentation, and results of sampling during these events will be submitted to EPA.

4.0 Sampling Plan and Locations

Site locations will be determined from field or desktop reconnaissance by project staff. Sample analyses will be predetermined based on conditions known about the sampling location prior to sampling. These may include data from previous sampling or from data collected from Mass DEP or local watershed associations.

When first observed, the physical attributes of each outfall or sampling location should be noted for construction materials, size, flow volume, odor, and all other characteristics listed on the data collection form (Attachment 1). In addition, location coordinates will be collected and a photograph of the sample location taken. The sampling of storm drain outfalls should be conducted as close to the outfall opening as possible.

Sample aliquots from the field kit bottle should be analyzed immediately after collection. Ammonia and chlorine samples should be processed first, followed by surfactant analysis, according to each respective Standard Operating Procedure as appropriate based on the particular brand and type of field test kit being used. All waste from the field test kits should be retained and disposed of according to manufacture instructions.

Last, a properly calibrated temperature/specific conductance/salinity meter should be used to record all three parameters directly from the stream or outfall. When flow volume or depth is insufficient to immerse the meter probe, a clean sample bottle may be utilized to collect a sufficient volume of water to immerse the probe. In such instances, meter readings should be taken immediately.

4.1 Sampling Procedures

Samples will be collected as grab samples. Stormwater samples will be collected under guidance of the following EPA Standard Operating Procedures:

- Measuring Ammonia using Ammonia 0 – 6.0mg/L (Nitrogen) Hach® 0 – 6.0mg/L Test Kit (EIASOP-SWTestKits#)

- Measuring Pocket Colorimeter Analysis System Low Range (0.0 – 2.00 mg/L) - Free and Total Chlorine High Range (0.0 – 4.5 mg/L) - Total Chlorine (EIASOP-SWTestKits#)
- Measuring Detergents using Detergents CHEMets 0-3 ppm Test Kit (EIASOP-SWTestKits#)
- Ambient Water Sampling (ECASOP-Water#)
- Water quality meter (Instruction Manual)

See Table 1 below for details on preservation and holding times for each sampling parameter.

Table 1: Parameter Specifications

Parameter (field equipment)	Preservation	Holding time
Temperature (°C)	None	Immediate
Specific Conductivity (µS/cm or mS/cm)	None	Immediate
Salinity (ppt)	None	Immediate
Surfactants (mg/L)	None	Immediate
Ammonia (mg/L)	None	Immediate
Chlorine (mg/L)	None	Immediate

4.2 Documentation and Reporting

All required information will be recorded on the field datasheets provided by the EPA (see Attachment 1). Datasheets, photographs, and field kit testing results will be submitted to EPA upon project completion. No formal report will be written for this project.

4.3 Calibration

The water quality meter will be properly calibrated by EPA prior to the start of the project. Conductivity calibration checks will be completed daily prior to the start of sampling (sampling days only). All other test kits are factory calibrated and do not require additional calibration. If any equipment requires maintenance beyond a simple fix, Org Name will coordinate with EPA to ensure equipment issues are properly addressed.

5.0 Performance and Systems Audits

All samplers will be trained by EPA on how to use the stormwater toolbox field test kits. No sampling assessments will be performed beyond initial training.

Field forms and log sheets will be checked for completeness and accuracy. If a discrepancy is found, it will be flagged and reported to EPA.

6.0 Data Evaluation

All data will be submitted to EPA for evaluation. Surfactant and ammonia concentrations should be compared to the thresholds listed in Table 2. Evaluation of the data should include a review for potential positive results due to sources other than human wastewater, and for false negative results due to chemical action or interferences. In the EPA New England region, field sampling has indicated that the biological breakdown of organic material in historically filled tidal wetlands may cause elevated ammonia readings, as can the discharge from many landfills. In addition, salinity levels greater than 1 part per thousand may cause elevated surfactant readings, the presence of oil may likewise indicate elevated levels, and fine suspended particulate matter may cause inconclusive surfactant readings (for example, the indicator ampule may turn green instead of a shade of blue). Finally, elevated chlorine from leaking drinking water infrastructure or contained in the illicit wastewater discharge may inhibit bacterial growth and cause very low bacterial concentrations. Any detection of total chlorine above the instrument reporting limit should be noted.

Table 2: Freshwater Water Quality Criteria, Threshold Levels, and Example Instrumentation

Analyte/Indicator	Threshold Levels/ Single Sample	Instrumentation
Surfactants (as MBAS)	0.25 mg/l	MBAS Test Kit (e.g. CHEMetrics K-9400)
Ammonia (NH_3)	0.5 mg/l	Ammonia Test Strips (e.g. Hach brand)
Total Chlorine (Cl_2)	4.0 mg/L (drinking water)	Field Meter Kit (e.g. Hach Pocket Colorimeter II)
Temperature/Conductivity/Salinity	See Respective State Regulations	Temperature/Conductivity/Salinity Meter (e.g. YSI Model 30)

Instrumentation Cited (Manufacturer URLs)
MBAS Test Kit - CHEMetrics K-9400:
https://www.chemetrics.com/index.php?route=product/category&path=59_76

Portable Colorimeter – Hach Pocket Colorimeter II: http://www.hach.com/

Ammonia (Nitrogen) Test Strips: http://www.hach.com/

Portable Temperature/Conductivity/Salinity Meter: YSI Model EC300A: https://www.ysi.com/EC300A

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