Green-Duwamish River Watershed Water Quality Assessment (WQA) Microbial Source Tracking (MST) Sampling and Analysis Plan

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1 INTRODUCTION

The primary purpose of this study is to determine potential sources of bacteria (i.e., *Escherichia coli*) in the Green-Duwamish River Watershed.

1.1 Project Background

King County performed a detailed Water Quality Assessment (WQA) in Elliott Bay and the Duwamish River in 1999 (King County 1999). One component of this study was an assessment of bacteria (e.g., Fecal Coliform) in the Duwamish River. Additionally, King County, and previously Metro, has been sampling in the Green/Duwamish watershed for a variety of water quality parameters, including bacteria, since 1970. King County has been monitoring 14 stations in the Green River basin as part of this program since the mid-1970s. Under King County's program, the sampling frequencies and types of indicator bacteria measured have varied over the years, but samples have been collected consistently on at least a monthly basis.

Bacterial concentrations measured in the Green-Duwamish watershed have exceeded state standards. Numerous stream segments throughout the watershed are listed on the state's 1998 303(d) list of impaired water bodies for violations of water quality standards for fecal coliform bacteria (Ecology 1998; King County 2002). Section 303(d) of the Clean Water Act (CWA) requires the state to identify those water bodies that do not meet water quality standards, and to develop total maximum daily loads (TMDLs) to improve water quality in the affected reaches. Accordingly, King County is investigating approaches to address bacterial contamination in the watershed.

A recent study by the Massachusetts Water Resources Authority (MWRA) found that concentrations of bacteria generally tend to increase at Boston area beaches during wet weather (Coughlin and Stanley, unpublished manuscript). The MWRA study shows highly significant (i.e., P values <0.0001), yet weak relationships (R^2 values <=0.179) between rainfall and bacterial concentrations. These results suggest that while rainfall is one factor related to loadings, other, yet to be identified factors, are important in explaining variation in bacterial loadings to Boston area marine waters.

A preliminary review of a small portion of Green-Duwamish water quality data collected during storm events in 2001 and 2002 generally shows that loadings and concentrations of Fecal Coliform, *E. coli* and *Enterococcus* increase and decrease with storm flows (King County, unpublished results). This result suggests that precipitation and flows are related to bacterial concentrations and loadings. However, no clear quantitative relationship between flow-related variables and bacterial concentrations has been established to date. These preliminary findings, which are consistent with the MWRA findings, suggest that other unidentified factors may be associated with variation in bacterial concentrations in the Green River watershed.

Collectively, the findings described in this section generated an interest in improving the understanding of the sources and land uses contributing to bacterial loadings in the

watershed[1]. It is hypothesized that one of the primary factors associated with bacterial concentrations in surface waters is land use, which may determine the specific types of sources of bacterial loadings. It is plausible that land use/cover may be useful as a surrogate to predict these sources. Specific sources that may be related to land use include, but are not limited to, agricultural animals (pasture and agricultural land), septic systems (rural residential), pets (suburban areas) and wildlife/birds (forested and rural areas). In order to elucidate these potential relationships, it is necessary to identify the sources of bacteria in the Green River and its' tributaries and correlate them to land uses. This goal can be accomplished by microbial source tracking (MST).

Further, MST can be used to assist in setting, and evaluating progress in achieving, TMDLs for fecal coliforms in the mainstem reaches and streams that are on the 303(d) list. Affected creeks include Newaukum, Springbrook, and Soos (Ecology 2002). Finally, an improved understanding of the relevance of bacterial concentrations to human health and ecological conditions in the watershed is needed. The present microbial source tracking study, to be performed as part of the Green-Duwamish Water Quality Assessment (WQA), is designed to collect information on bacterial sources and land uses associated with bacterial populations. This will provide baseline information on human bacterial sources and bacterial concentrations generally, that may be used to focus future studies to address the human health and ecological implications.

Microbial Source Tracking (MST) is a methodology that can be used to determine the source of bacterial contamination in the environment. It involves identifying *E. coli* strains from water samples and matching them to genetically identical strains of known sources (e.g., domestic animals, humans, farms animals, etc...). *E. coli* isolates with matching genetic finger printings are more likely to originate from common sources, and therefore environmental isolates can be matched to that of known sources of origin. The *E. coli* strains that show specificity to one host are called Resident Clones and those strains that are found in related or unrelated sources are called Transient Clones. Only Resident Clones are used in the source tracking method.

King County Surface Water Management Division and the City of Seattle have conducted two MST studies on Little Soos and Pipers Creeks, respectively (King County 1995; City of Seattle 1993). The Pipers Creek study was done to identify the sources of fecal contamination in Pipers Creek. In the Pipers Creek study, ribotypes from *E. coli* isolated from water samples were compared to ribotypes derived from *E. coli* isolated from fecal sources of known origin in the Pipers Creek watershed. The results of this study showed that the source of fecal contamination in the Pipers Creek basin were of animal origin (primarily pets).

Similarly, the Little Soos Creek MST study compared water derived isolates to isolates of known origin. The results of this study showed that the majority of water derived isolates originated from cattle, horses, and dogs, with human septage as a minor component of the fecal contamination in Little Soos Creek.

The current study will build on these previous studies to accomplish more detailed goals described in Section 1.2.

[1] Determination of quantitative estimates of loadings from various sources is not a goal of this study. Semi-quantitative estimates may be possible. However, the primary goals are determining the types of sources and population structure associated with varying land-uses as described in Section 1.2.

1.2 Study Objectives

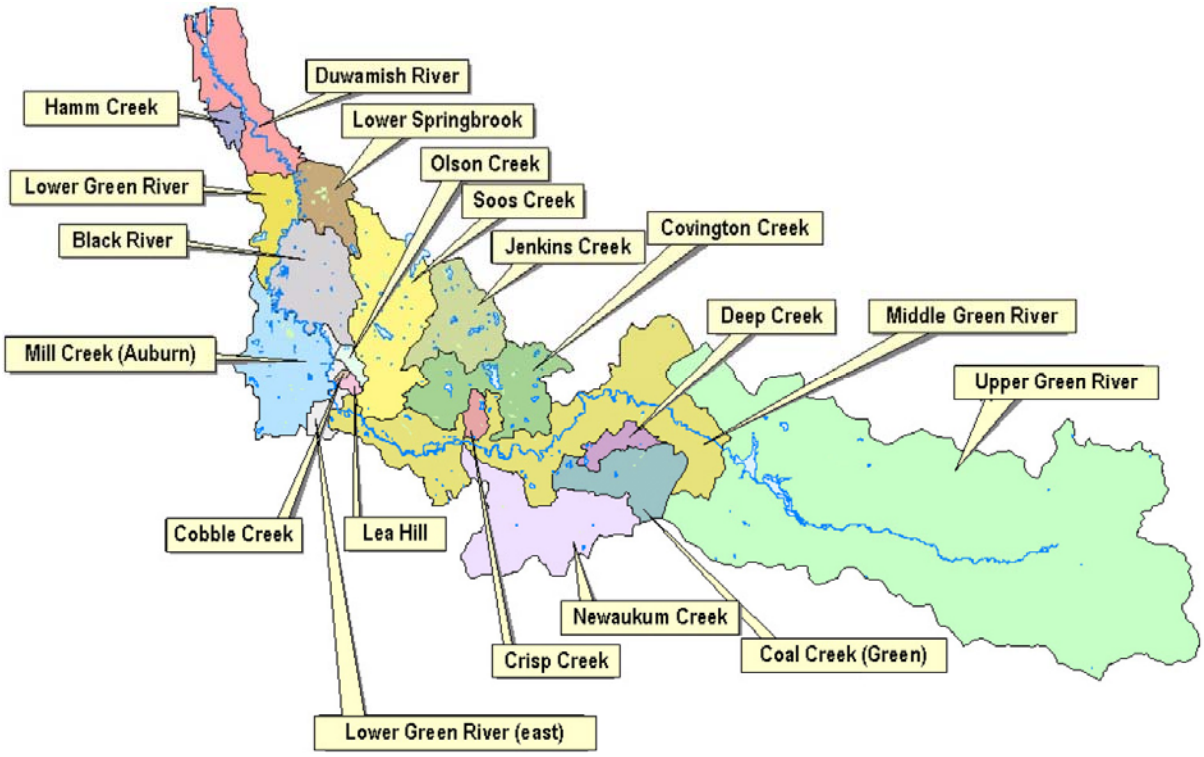
To address the general goals described in Section 1.1, the following objectives were defined for this study:

- Provide information that may be used to improve understanding of, and describe a relationship between land use, hydrology, season, and bacterial sources.
- Provide data that may be used to refine development of models used to simulate dynamics of *E. coli* populations in the Green River and that may be used to support health risk assessment in the future.
- Determine the relative contribution of human and animal sources from each of the selected tributaries to the mainstem Green.
- Improve our understanding of the types and sources of the *E. coli* population at various locations in the mainstem Green River that are attributable to types of land uses in representative subbasins.

1.3 Study Area

The Green-Duwamish Watershed includes a drainage area of varied terrain and land use from forested headwater areas at the crest of the Cascade Mountains to the industrial and port facilities of the Duwamish estuary. The project study area encompasses the Green-Duwamish River watershed from the Tacoma Diversion Dam at river mile 61 to the mouth of the Duwamish River at Elliott Bay, an area of 261 square miles. The upper Green River above the Tacoma Diversion Dam is not included in the study area (Figures 1 and 2, and Table 2).

Figure 1. Green-Duwamish River Watershed.



2 PROJECT TEAM AND RESPONSIBILITIES

Project team members and their responsibilities are summarized in Table 1. Unless otherwise noted, all team members are staff of the King County Department of Natural Resources Water and Land Resources Division.

Table 1. Project team members and responsibilities.

Name/Telephone	Title	Affiliation	Responsibility
John Brooker (206) 296-8390	Water Quality Planner	Science, Monitoring and Data Management	King County MST project manager
Rob Zisette (206) 441-9080	Environmental Consultant	Herrera Environmental Consultants, Inc.	Coordination of activities with IEH, report reviews
Mansour Samadpour (206) 522-5432	Environmental Consultant	Institute for Environmental Health, Inc	Ribosomal RNA analysis of *E. Coli* isolates & identification of isolates
Colin Elliott (206) 684-2343	Quality Assurance Officer	Environmental Laboratory	Overall laboratory QA/QC
Fritz Grothkopp (206) 684-2327	Laboratory Project Manager	Environmental Laboratory	Coordination of laboratory activities, laboratory QA/QC, and data reporting
Doug Henderson (206) 263-6317	Water Quality Planner	Science, Monitoring and Data Management	Manager for Green / Duwamish WQA
Greg Ma (206) 2684-340	Microbiology Lab Supervisor	Environmental Laboratory	Supervisor of KCEL microbiology laboratory activities
Lorin Reinelt (206) 296-1960	Senior Water Quality Planner	Science, Monitoring and Data Management	Assistant manager for Green / Duwamish WQA
Curtis Nickerson (206) 267-1405	Environmental Consultant	Taylor Associates, Inc.	Sample collection

The King County Department of Natural Resources Water and Land Resources Division will manage the study and coordinate work performed by others. Taylor Associates, Inc. will collect the water samples and deliver them to the King County Environmental Laboratory (KCEL). KCEL will prepare the sample isolates and coordinate delivery of cultures to the Institute for Environmental Health, Inc. (IEH). IEH will conduct the ribotyping analysis and provide data reports for the Green-Duwamish River Microbial Source Tracking Study. Herrera Environmental Consultants, Inc. will provide project management and report review services.

3 SCHEDULE

3.1 Sample Collection

Samples will be collected under storm and baseflow conditions during 2003 and possibly into early 2004. Criteria for storm and baseflow sampling are located in Section 4.2.

Samples will be collected during the daytime will be delivered to the King County Environmental Laboratory by 2 pm on the sampling day. A total of 45 samples will be collected during each sampling event.

3.2 Isolate Preparation

Fresh cultures of E. coli will be prepared by KCEL from the collected water samples within 24 hours of sample collection, and will be transported to the Institute for Environmental Health, Inc. (IEH) within three days of sample processing. In addition, pure isolates of E. coli will be developed and preserved (frozen) by KCEL, and will be transported to IEH when an agreement for services has been approved. The cultures will be accompanied by a chain-of-custody form, a listing of the sample numbers and plate dilutions sent, site locator number, date collected, and date processed.

3.3 Deliverables

Project status reports will be delivered to Herrera and the King County project manager by the Institute for Environmental Health, Inc. within 60 days of receiving the isolates.

A draft data analysis report will be delivered by the Institute for Environmental Health, Inc. to Herrera and the King County project manager 90 days after the final sample isolates are delivered to IEH.

King County and Herrera will review the draft report and any comments and/or requests for changes in the report will be provided back to Herrera within 30 days of receiving the report. Herrera will then coordinate with the IEH to make revisions and the final report will be delivered to King County by December 31, 2004.

4 STUDY DESIGN

This study will compare *E. coli* isolates collected near the mouths of five major tributaries (i.e., Hamm Creek, Springbrook Creek, Mill Creek, Soos Creek, and Newaukum Creek) to *E. coli* populations collected at four locations in the mainstem Green River.

To the extent that the subbasins can be categorized according to these land uses, *E. coli* population characteristics will be related to land use categories. Due to limitations in the existing land use/land cover databases, the mixed land uses in each subbasin, and the limited number of sampling stations (nine), this study will not produce definitive quantitative relationships between bacterial loadings and land use or cover. However it will provide qualitative baseline information as well as relative percent quantitative relationships on *E. coli* populations in the Green-Duwamish River watershed that can be used as a management tool and serve to focus and refine future studies on this question.

Representative samplings of waterborne *E. coli* will be collected at these locations and ribosomal RNA analyzed and compared to a library of ribosomal RNA patterns of *E. coli* from known sources. The library at IEH, Inc. contains over 50,000 known *E. coli* ribosomal patterns determined by the IEH protocol. These patterns have been found to be unique to the source group and have been found to maintain their uniqueness in studies across the geographic United States.

Quantitative *E. coli* data will also be collected from the nine MST study stations during each sampling period. These data will enable semi-quantitative attribution of the sources associated with the *E. coli* populations, as well as changes in the population distribution as the river moves downstream.

4.1 Site Location

The subbasins to be sampled are selected to represent the range of land uses and produce moderate geographic coverage within the Green-Duwamish River watershed. The various reaches of the Green River and subbasins that drain into those reaches, can generally be described to represent forested (upper reaches), agricultural/residential (Middle Green) and commercial/industrial (Lower Green-Duwamish) land uses.

The sampling sites are presented in Figure 2 and Table 2.

Figure 2. Microbial source tracking (MST) sampling locations.

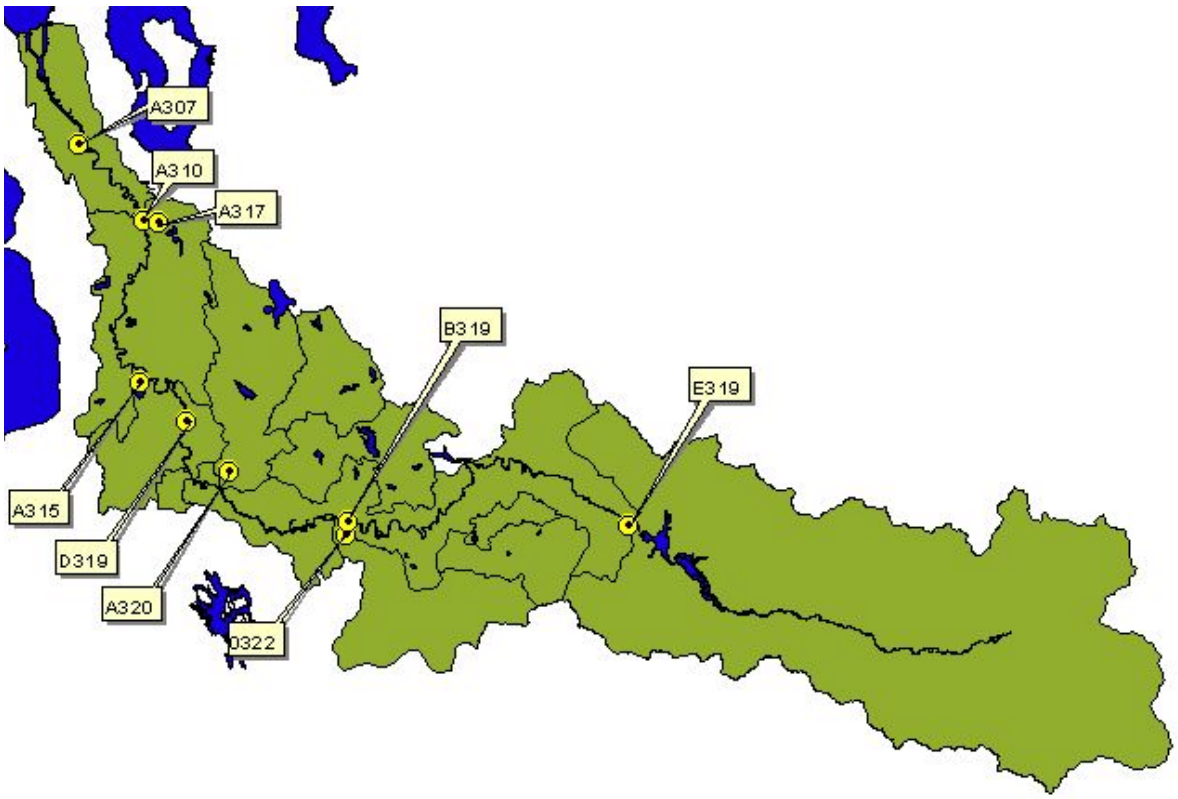


Table 2. Microbial source tracking (MST) sampling locations.

Locator	Description
A307	Hamm Creek-Lower Duwamish
A310	Mainstem Lower Green River
A317	Mouth Springbrook Creek
A315	Mouth Mill Creek
D319	Middle Mainstem Green River just below Olson Creek confluence
A320	Mouth of Soos Creek
B319	Middle Mainstem Green River near 277th street Bridge
0322	Mouth of Newaukum Creek
E319	Below Howard Hanson Dam (Tacoma Diversion Dam)

4.2 Sampling Criteria

4.2.1 Baseflow Event Criteria

The following criteria will be used to initiate a baseflow event. There will have been no measurable rain (<0.01 in.) with in 24 hour prior to a baseflow sampling event.

4.2.2 Storm Event Criteria

The following criteria will be used to initiate a storm event. Predicted rainfall for the study area should be at least 0.35 inches of rain for a 12-hour period. Sampling should be timed during the rainfall to collect the samples during the peak of the stream hydrograph.

5 SAMPLE COLLECTION

5.1 General Health and Safety Requirements

The following general health and safety guidelines have been provided in lieu of a project-specific Health and Safety Plan. These guidelines will be read and understood by all members of the sampling crew prior to any sampling activities.

- Sampling personnel will wear chemical-resistant gloves whenever coming into contact with samples.
- No eating, drinking, smoking, or tobacco chewing by sampling personnel will be allowed during active sampling operations.
- All accidents, "near misses," and symptoms of possible exposure will be reported to a sampler's supervisor within 24 hours of occurrence.
- All crewmembers will be aware of the potential hazards associated with chemicals used during the sampling effort.
- All crewmembers will be aware of the inherent dangers associated with sampling streams and rivers.

5.2 Collection Methods

Grab samples will be collected according to U.S. EPA standards (U.S. EPA, 1996) during baseflow and storm conditions. Samples will be collected while facing upstream to minimize contamination from field equipment. Sampling personnel will wear clean PVC gloves for personal protection and to prevent contamination of the samples.

The sample bottle will be filled by lowering the bottle, open with the neck faced down, into the stream to a depth of 1 to 3 inches. The bottle will be allowed to fill up just below the top shoulder of the bottle. If too much liquid is sampled, some may be "snapped" back into the stream (removing only the top portion in the bottle) to meet this filling requirement.

All samples containers will be placed in an ice chest, with plastic separating ice from bottles, until transported to the laboratory.

5.3 Replication

To adequately characterize *E. coli* populations at each sampling location, five replicate samples will be collected at each location during each sampling event. The samples will be collected a minimum of two minutes apart over a minimum of a ten minute period. This two-minute interval should allow sufficient flow of the waterbody between samples to ensure that each sample is distinct. It is acceptable to extend this total time period up to an hour. In this

way, the five samples collected can be interpreted as representing the sampling location and site specific characteristics, rather than any one particular loading event or time/flow influenced effect.

5.4 Sample Documentation

5.4.1 Sample Numbers and Labels

Unique sample numbers will be assigned to each sampling location for which stream water samples are collected for microbiological analysis. Sample numbers will be assigned prior to the sampling event and waterproof labels generated for each sample container.

5.4.2 Chain-of-Custody

A chain of custody (COC) form will accompany all samples documenting an unbroken custody path when transferring sample isolates from the KCEL to the IEH. Copies of the COC forms will be included in the data analysis report.

5.4.3 Field Notes

Field notes will be maintained for all field activities. Field notes will be kept on water-resistant paper and all field documentation will be recorded in indelible, black ink. Field notes will be recorded on pre-printed field sheets prepared specifically for this project. Information recorded on field notes should include:

- name of recorder,
- sample or station number,
- sample station locator information,
- date and time of sample collection,
- results for all field measurements (temperature, pH, dissolved oxygen, and conductivity),
- staff height.

Additional information recorded on the field sheets may include sampling methodology and any deviations from established sampling protocols. Additional anecdotal information pertaining to observations of unusual sampling events or circumstances may also be recorded on the field sheets.

6 SAMPLE HANDLING PROCEDURES

Consistent sample handling procedures are necessary to maintain sample integrity and provide high-quality defensible data. This section provides requirements for proper sample containers, labeling, preservation, and storage.

6.1 Sample Containers

All samples will be collected or split into sterile, laboratory-supplied 500 mL HDPE containers affixed with computer-generated labels. Information contained on sample labels will include a unique sample number; information about the sampling location; the collection date; and the requested analysis.

6.2 Sample Preservation

Samples will be held at 2º to 6º C during transport to the laboratory, and prior to analysis. No preservatives are required.

6.3 Sample Storage and Holding Time

Stream storm and baseflow samples will be stored at a temperature of 2º - 6º C. Samples will be processed within 24 hours of collection, if possible. If samples are received after laboratory hours, the samples will be processed at the earliest possible time.

7 MICROBIOLOGICAL LABORATORY PROCEDURES

The King County Environmental Laboratory (KCEL) will perform the initial analysis of all water samples. The ribotyping procedure will be performed under the direction of Dr. Mansour Samadpour at the Institute for Environmental Health, Inc.

7.1 Bacterial Culture and Isolation

Each water sample received will be processed using a qualitative membrane filtration method based on the EPA Total Coliform and E coli method (EPA 600-R-00-013). This medium will be incubated at 35º C for recovery of all *E. coli*, not only the thermotolerant strains. Dilutions of the river water will be estimated to provide targeted populations in the ideal countable range to optimize the recovery of a representative population of *E. coli*. The primary isolation medium, MI agar, may be refrigerated for up to seven days before being picked for target *E. coli* colonies (target colony morphologies for *E. coli* are not significantly affected on holding). Plates with the ideal ranges will be delivered to the ribotyping laboratory (IEH) within three days of completion.

Quantitative *E. coli* data will be collected on a periodic basis from the sampling stations added to the 2002 sampling program. These data will be recovered using the standard quantitative membrane filtration method (Standard Methods 9213D.3, mTEC agar) as has been used throughout the Green-Duwamish Study.

Up to ten target colonies will be picked from mTEC/urea substrate agar from each MST sampling station and transferred to nutrient type agar slants. Two of the ten isolates will be streaked for purification on MacConkey Agar (complete) and confirmed as *E. coli* by indologenesis and citrate non-utilization reactions. Other isolates from the ten will be purified and confirmed until two strains have been identified as *E. coli* for transfer to the Ribotyping laboratory. All remaining isolates from the initial picks will be archived as non-purified cultures until requested by the ribotyping laboratory (IEH) or until the end of the study.

7.2 Isolate Storage and Holding Time

Membranes incubated on MI agar will be stored at 4-10C for up to three days until transfer to the Ribotyping laboratory. Total holding time for inoculated MI plates should not exceed seven (7) days.

E. coli isolates from m-TEC agar will be stored at room temperature on Trypticase Soy Agar with or without Yeast Extract (TSA/TSAYE) slants for transport to the Ribotyping laboratory and archived in a -80º C freezer at KCEL and or IEH, Inc. Inoculated TSA slants may be held for up to two weeks before transfer to fresh medium is required.

At the end of the study, a collection of the strains identified and ribotyped by IEH will be delivered to the KCEL.

8 RIBOTYPING LABORATORY PROCEDURES

After the plates have been incubated by the KCEL, they are counted and the results are recorded onto an mFC log sheet and into a database.

The plates are then placed into plastic bags, maintained at 4-10 °C and shipped with the appropriate chain-of-custody forms to the Institute for Environmental Health using overnight mail or courier.

8.1 Sample Arrival and Login

1. Samples are delivered to the IEH laboratory by hand or through a courier or delivery service. The package is opened and the log sheet provided by the sender is removed.

2. Upon arrival all samples are removed from the package, confirmed to be on the provider's log sheet. Sample containers or microbiological plates are then inspected for damage and signs of contamination.

3. The date, time, delivery service name, tracking number, and person receiving the package are logged onto the Microbial Source Tracking Sample Log Sheet. In addition, the chain-of-custody section of the Sample Log Sheet is completed and the sample identities are checked against the chain-of-custody papers.

4. The samples, or their containers, are then tracked by provider ID until identified as *E. coli* and refrigerated until further processing.

5. The packing slip is removed from the package and attached to the log sheet.

8.2 Sample Rejection and Provider Notification

1. Samples that appear to be tampered with, damaged or adulterated are shown to the project manager, who will determine whether or not the samples should be accepted for processing.

2. If samples are not accepted for processing, the provider will be consulted to determine the sample(s) disposition. In addition, the provider will be notified if there are any missing samples, or if there are samples that are not logged onto the provider's log sheet.

8.3 Ribotyping

8.3.1 Isolation and purification of *E. coli* strains

Water samples are received in the form of MI agar plates. Two to five non-mucoid blue-green fluorescing (under longwave UV light) indigo-blue pigmented colonies are picked from MI plates corresponding to each water sample, and are plated on MacConkey agar for purification. At this stage, each of the colonies picked from a given sample bears the provider Sample ID number and an accession letter. A single well-isolated, non-mucoid colony is picked from each MacConkey plate and is plated on Triptic Soy Agar. After overnight incubation at 35 ° C, each culture is tested by Spot indole test using appropriate positive and negative controls. Indole-positive cultures are further tested for the ability to utilize citrate using the Simmon's Citrate media. Lactose-positive/Indole-positive/citrate-negative colonies are identified as *E. coli* and are assigned isolate numbers. At least two isolates of *E. coli* from each provider's Sample ID number will be identified.

8.3.2 Growing pure cultures of *E. coli* strains for freezing (long term storage) and isolation of DNA.

A portion of each *E. coli* strain isolated from the samples will be stored at -80° C, in nutrient broth plus 15% glycerol.

8.3.3 Restriction Endonuclease Digestion of *Escherichia coli* DNA

1. Sterile 1.5 ml microcentrifuge tubes are labeled with the isolate numbers of samples that will be digested on a given day.

2. A restriction digest cocktail is prepared according to the following formula (per reaction): 10 µl 25 mM $MgCl_2$, 3.5 µl reaction buffer (provided by the manufacturer of the restriction enzyme), 0.5 µl EcoRI restriction endonuclease, 0.5 µl Pvu restriction endonuclease, and 18 µl sterile water. The cocktail is held on ice until used.

3. 32.5 microliters of the digest cocktail is dispensed into each labeled microcentrifuge tube.

4. 2.5 microliters of lysed bacterial cells from 5b ix are added to the appropriate tube containing digest cocktail. The tubes are tightly closed and incubated at 35 degrees Celsius (± 0.5°C) for two hours.

8.3.4 Agarose Gel Electrophoresis of Restriction Digest Products

8.3.4.1 Preparation of 1% Agarose Gels

1. The number of gels that need to be run on a given day is determined by dividing the number of samples to be run by 20 and rounding this number up to the nearest whole number.

2. This number is multiplied by 200 to determine the amount of 0.5X TBE that will be used. (TBE is a buffer that allows the current to travel through the solution moving the cut DNA fragments through the gel.) It is multiplied by 2 to determine the amount of Agarose that will be used.

3. Example: One needs to run 200 samples, which is equivalent to 10 gels. 10 x 200 = 2000 ml of 0.5X TBE. 10 x 2 = 20 g of Agarose.

4. The total volume of gels that needs to be made is divided equally into volumes of 800 milliliters or less so that the mixture can be heated in one-liter bottles in a microwave.

5. The 1% gel mixture is ready when large bubbles form in a boil. This ensures a complete dissolving of the Agarose within the solution. The glass containers are then place in a water bath to cool to 55 degrees Celsius. The gels must not reach 50 degrees Celsius as they will begin to solidify.

6. The short gel mold is set on a level surface and arranged with a 20-lane comb.

7. Once the 1% Agarose gel mixture cools to 55 degrees Celsius, the mixture is poured into a gel mold. The correct amount of mixture is 190ml per mold. Any large bubbles that appear must be removed before the gel solidifies.

8. While the gel is cooling, 7.5µl of loading dye is added to each reaction tube. The loading dye is carefully mixed with the reaction cocktail without drawing the reaction cocktail into the pipet barrel.

9. Once the gel is at room temperature and solid the comb is removed and the gel is placed into a gel box containing enough 0.5x TBE to completely cover the surface of the gel, without creating air pockets. If storage is necessary adding TBE solution over the top of the gel enables storage in a refrigerator.

8.3.4.2 Loading Gel and Running Gel Electrophoresis

1. The electric field of a gel box is always calibrated before running electrophoresis. The milliampage must be at 13mA and the voltage must read 33V.

2. The gel lanes are loaded according to the gel sheet prepared, always in a left-to-right fashion. The size standard is sequenced so that it is in the succeeding lane in each succeeding gel.

3. The loading of the DNA cocktail and stop dye should be done with sterile gloves and a P20 pipetman.

4. The gel is run at 33 volts for 17 hours. The phosphate backbone of DNA is negatively charged causing DNA to migrate to the positive electrode when placed in an electric field. The gel acts as a sieve, hindering the larger pieces of DNA fragments and allowing smaller pieces to "run" further.

5. After the gel runs, it is removed from the gel box and gel mold and placed into a glass casserole dish containing 5µg ethidium bromide per milliliter distilled water.

8.3.4.3 Southern Blotting

Southern Blotting is performed following standard protocol to visualize DNA fragments.

9 QA/QC PRACTICES

9.1 Microbiology Lab

Quality assurance practices for the recovery of targeted *E. coli* and quantification of *E. coli* from river water will follow the practices routinely followed for quantitative membrane filtration methods by the microbiology laboratory at KCEL. Routine inter- and intra-analyst evaluations are performed monthly as a check on analyst reproducibility.

All materials and supplies used in the production of microbiological data will be sterile to preclude any introduction of bacterial or specifically, *E. coli* contamination. Routine procedures by the microbiology laboratory include challenge testing of sterilized glassware, media, and daily temperature recordings. Additionally, positive and negative controls and non-contamination controls (pre- and post-filtration blanks) are routinely incorporated into each batch of samples.

Duplicate samples (laboratory duplicates) will be performed on quantitative samples at the rate of 5% or 1 in 20 samples or at least one per batch. These may be incorporated with other samples of similar surface water matrix, and likely collected as part of this study. Evaluation of acceptability will be based on comparison with the precision calculation performed on the previous fifteen duplicate pairs performed on the same matrix condition type.

An experienced analyst will review each data set for microbiological acceptability and completeness. A narrative will document this evaluation for each sampling event.

9.2 Ribotyping

Genomic DNA is isolated from each *E. coli* strain using a standard protocol. All reagents and buffers are made according to formulas in the laboratory SOP. Reagents and buffers are tested for sterility. Every batch of restriction enzyme reaction contains two reactions with a positive control strain, which will be included on one lane per gel. Agarose gel electrophoresis is conducted under standard conditions, controlling for agarose gel concentration and volume, buffer straight, pH, milliamps (mA), voltage (V), and electrophoresis time. Each agarose gel is assigned a number, and when more than one gel is run, the position of the standard reference strain is changed in each gel remaining in sequence. For instance, the standard will be in the first lane in the first gel, the second lane in the next gel, and continue in succeeding gels.

After electrophoresis gels are stained in ethidium bromide, two individual gels are stained in a single container. Of the two gels placed in the same container, one corner of the gel with the higher number is clipped. The labels for each gel are also transferred to the staining container. Each gel is then photographed and a hard copy of the print is attached to the gel sheet. This hard copy contains the gel number, date, voltage, mA, gel strength, buffer strength, electrophoresis time, the isolate numbers loaded on each lane, and the enzyme used to cut the DNA.

Southern blotting is performed according to the protocol detailed in the IEH SOP. After photography, each gel is returned to the same staining container. Gels are denatured for Southern blotting in the same container. Each blotting apparatus is set in a separate container that is labeled with the gel number. Each membrane filter is labeled with the gel number, restriction enzyme designation, date, and technician's initials.

10 DATA ANALYSIS, RECORD KEEPING AND REPORTING

10.1 Data Interpretation

Photographic film records of rRNA profiles visualized by the Southern Blot method will be compared to rRNA profiles of *E. coli* strains stored in the IEH library of known source patterns. Each pattern has a distinctive numeric code depicting the elution pattern of the rRNA across the gel. Each isolate is evaluated against this library until all isolates have been categorized.

Tables summarizing the numbers of pattern profiles recovered, segregated by station and by source type over the sampling times will be produced. Associations with land uses (linked to sampling station) may be found by comparing source types with stations, and effects of seasonality or rainfall may be found by comparing source types with sampling dates. Isolates from samples collected in 2002 may be compared to isolates collected from 2003 at selected stations for analysis of seasonality, or annual variation.

Source population distributions may be established for each sampling station and for reaches of the watershed by combining the source patterns established for each station on a frequency basis and presented as per cent occurrence.

10.2 QA Reviews

Microbiology data will undergo standard QA review according to the King County Environmental Laboratory QA document. Data will be flagged accordingly. This level of QA review is necessary to provide the project and program managers with the level of information needed to correctly interpret the data.

10.3 Record Keeping

All sampling records, custody documents, raw laboratory data, data summaries and case narratives will be stored according to KCEL policy.

10.4 Reporting

Project data will be presented to the project and program managers in a format that will include the following:

- King County Environmental Laboratory Comprehensive Reports consisting of spreadsheets of microbiological parameters;

- A narrative of data including supporting QC documentation (provided by KCEL);
- A technical memorandum, summarizing field sampling, analytical results; interpretation of the results (provided by KCEL and Dr. Mansour Samadpour).

11 REFERENCES

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