Regional Water Quality Control Board
North Coast Region

Russian River Pathogen Indicator Bacteria TMDL – Supplemental Sampling Plan

Quality Assurance Project Plan

Version 1.0

Originated by:

Steve Butkus

North Coast Regional Water Quality Control Board

November 16, 2011

Group A: Project Management

A1: Title and Approval Sheet

Project Title: Russian River Pathogen Indicator Bacteria TMDL – Supplemental Sampling Plan

Lead Organization: North Coast Regional Water Quality Control Board
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Effective Date: July 1, 2011

Approvals

Steve Butkus, Project Manager – Data Manager – Contract Manager
North Coast Regional Water Quality Control Board

See Appendix 11: Approval Sheet Signatures

Signature Date

Rich Fadness, Project QA Officer
North Coast Regional Water Quality Control Board

See Appendix 11: Approval Sheet Signatures

Signature Date

William Ray, Board Quality Assurance Officer
California State Water Resources Control Board

See Appendix 11: Approval Sheet Signatures

Signature Date

Gary Anderson, Lab Director
Lawrence Berkeley National Laboratory (LBNL)

See Appendix 11: Approval Sheet Signatures

Signature Date

Yeggie Dearborn, Lab Director
Cel Analytical Laboratories

See Appendix 11: Approval Sheet Signatures

Signature Date

Michael Ferris, Sonoma County Public Health Laboratory, Lab Director
County of Sonoma

See Appendix 11: Approval Sheet Signatures

Signature Date

A2: Table of Contents

Group A: Project Management

Group B: Data Generation and Acquisition

Group C: Assessment and Oversight

Group D: Data Validation and Usability

List of Tables

List of Figures

List of Appendices

.

A3: Distribution List

Table 1: QAPP Distribution List Primary Contact Information

Contact Information	Organization's Mailing Address
Project Manager; **Data Manager;** **Contract Manager** Main Contact: Steve Butkus Phone: 707-576-2834 Email: SButkus@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Project Quality Assurance Officer; Main Contact: Rich Fadness Phone: 707-576-6718 Email: RFadness@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Lawrence Berkeley National Laboratory; **Laboratory Director** Main Contact: Gary Anderson Phone: 510-495-2795 Email: glandersen@lbl.gov	Lawrence Berkeley National Laboratory Ecology Department One Cyclotron Road, MS 70A-3317 Berkeley, CA 94720
Cel Analytical Laboratories; **Laboratory Director** Main Contact: Yeggie Dearborn Phone: 415-882-1690 Email: yeggie@celanalytical.com	Cel Analytical, Inc. 82 Mary Street, Suite #2 San Francisco, CA 94103
Sonoma County Public Health Laboratory - **Laboratory Director** Main Contact: Michael Ferris Phone: 707-565-4711 Email: MFERRIS@sonoma-county.org	County of Sonoma Department of Public Health Services 3313 Chanate Road Santa Rosa, CA 95404

A4: Project/Task Organization

A monitoring study has been initiated by the North Coast Regional Water Quality Control Board. This QAPP serves as a supplemental sampling plan for the *Russian River Pathogen Indicator Bacteria TMDL Monitoring Plan.* Additional water samples for laboratory analysis will be collected in conjunction with the Russian River Pathogen Indicator Bacteria TMDL sampling efforts. The North Coast Regional Water Board will be responsible for the collection of samples for the analysis of *Bacteroides*, stable isotope analyses of nitrate for relative source differences in oxygen (δ18O) and nitrogen (δ15N), and fast DNA microarray (Phylochip) samples.

Sonoma County Public Health Laboratory will be responsible for the analysis of *Bacteroides* samples. Cel Analytical Laboratories will be responsible for the analysis of stable isotope analyses of nitrate samples. Lawrence Berkeley National Laboratory (LBNL) will be responsible for the analysis of Phylochip samples.

Table 2 identifies all personnel involved with this study. Descriptions of each person's responsibilities follow the table. Figure 1 shows relationships between personnel.

Table 2. Personnel responsibilities.

Name	Project Title	Organizational Affiliation	Contact Information (Telephone number, fax number, email address.)
Steve Butkus	Project Manager; Data Manager; Contract Manager	North Coast Regional Water Board	(707)-576-2834 (707)-523-0135 sbutkus@waterboards.ca.gov
Rich Fadness	Project QA Officer	North Coast Regional Water Board	(707)-576-6718 (707)-523-0135 rfadness@waterboards.ca.gov
Gary Anderson	Contract Lab Director	Lawrence Berkeley National Laboratory	510-495-2795 510-486-7152 glandersen@lbl.gov
Yvette Piceno	Contract Lab QA Officer	Lawrence Berkeley National Laboratory	510-486-4498 510-486-7152 ympiceno@lbl.gov
Yeggie Dearborn	Contract Lab Director	Cel Analytical Laboratories	415-882-1690 415-882-1685 yeggie@celanalytical.com
Steven Tan	Contract Lab QA Officer	Cel Analytical Laboratories	415-882-1690 415-882-1685 steven@celanalytical.com
Michael Ferris	Contract Lab Director	Sonoma County Department of Public Health Services	(707)-565-4711 (707)-565-7839 mferris@sonoma-county.org
Lisa Critchett	Contract Lab QA Officer	Sonoma County Department of Public Health Services	(707)-565-4711 (707)-565-7839 mferris@sonoma-county.org

North Coast Regional Water Board

Project Manager - Data Manager – Contract Manager

Steve Butkus - He is responsible for managing the project team, project oversight, and interactions with the contracted laboratories. He will provide complete oversight of the project including supervision of field-related data collection tasks, training of field personnel, data management, and reporting.

Project Quality Assurance (QA) Officer

Rich Fadness - His role is to establish the quality assurance and quality control procedures found in this QAPP. He will review and assess all procedures during the life of the project against the QAPP requirements. He will report all findings to the Project Manager, including all requests for corrective action. He may stop all actions, including those conducted by contracted laboratories, if there are significant deviations from required practices or if there is evidence of a systematic failure. At his discretion, he will be responsible for various project audits in order to ensure the Monitoring Plan and QAPP directives are met.

Field Personnel

North Coast Regional Water Board staff will conduct all field sampling and data collection activities.

Lawrence Berkeley National Laboratory (LBNL)

Ecology Department - Laboratory Director

Gary Anderson – He will be responsible for ensuring that microbiological samples sent to this contract Laboratory are processed in accordance with the method and QA assurance requirements found in the Phylochip Manual (see Appendix 5) and the LBNL QAP (Appendix 8).

LBNL Ecology Department - Quality Assurance Officer

Yvette Piceno - She will be responsible for the QA/QC procedures found in this QAPP as part of the sampling analysis. She will also work with Mr. Anderson, the Laboratory Director at Lawrence Berkeley National Laboratory Ecology Department, by communicating QA/QC issues contained in this QAPP.

Cel Analytical Laboratories, Inc.

Commercial Laboratory Director

Yeggie Dearborn – She will be responsible for ensuring that water samples sent to this contract Laboratory are processed in accordance with the method and QA assurance requirements found in the stable isotope analysis Standard Operating Procedure (see Appendix 6) and the Cel Analytical Laboratories QAP (Appendix 9).

Commercial Laboratory Quality Assurance Officer

Steven Tan - He will be responsible for the QA/QC procedures found in this QAPP as part of the sampling analysis. She will also work with Ms. Dearborn, the Labortory Director at Cel Analytical Laboratories, by communicating QA/QC issues contained in this QAPP.

County of Sonoma

Sonoma Department of Health Services- Public Health Services Laboratory Director

Michael Ferris – He will be responsible for ensuring that microbiological samples sent to this contract Laboratory are processed in accordance with the method and QA assurance requirements found in the Sonoma County Public Health Laboratory Standard Operating Procedure (see Appendix 7) and the Sonoma County Public Health Laboratory QAP (Appendix 10).

Public Health Services Laboratory Quality Assurance Officer

Lisa Critchett - She will be responsible for the QA/QC procedures found in this QAPP as part of the sampling analysis. She will also work with Mr. Ferris, the Laboratory Director at Sonoma Department of Health Services- Public Health Services Laboratory, by communicating QA/QC issues contained in this QAPP.

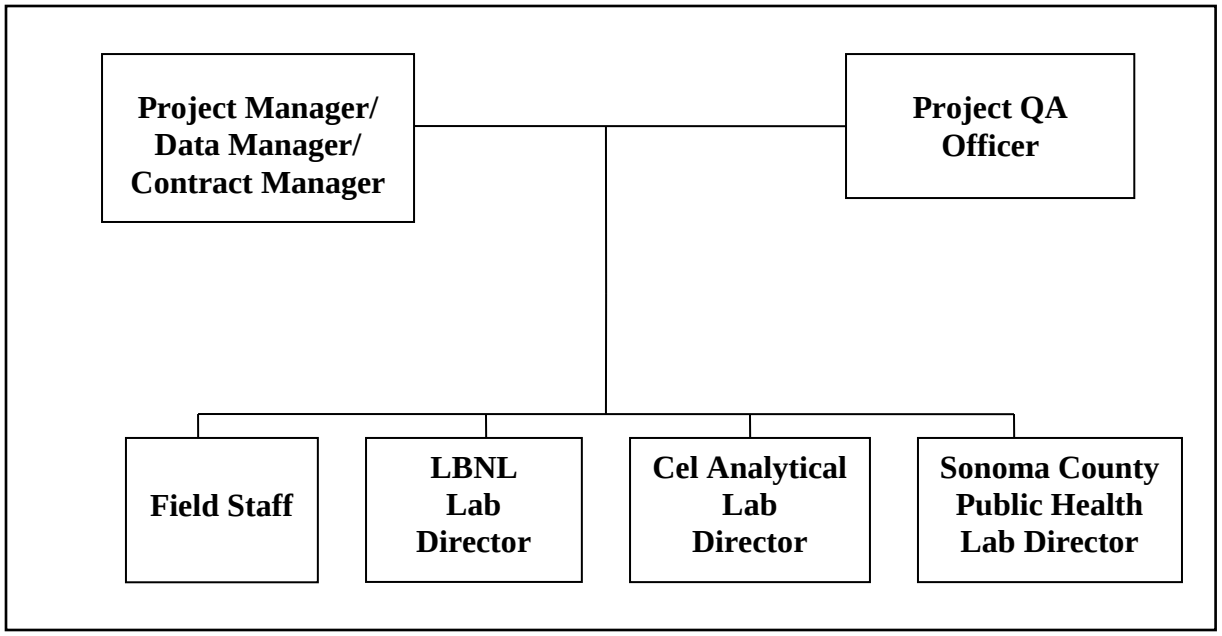


Figure 1. Project Organizational Chart

A5: Problem Definition/Background

Problem Statement.

The North Coast Regional Water Board staff are developing the Russian River Total Maximum Daily Loads (TMDLs) for pathogen indicators to identify and control contamination. Potential pathogen contamination has been identified in three areas of the lower and middle Russian River watershed (Hydrologic Units 114.10 and 114.20). This has led to the placement of waters within these areas on the federal Clean Water Act Section 303(d) list of impaired waters. The contamination identified has been linked to impairment of the contact recreation (REC-1) and non-contact recreation (REC-2) designated beneficial uses. Health advisories have been published and/or posted by Sonoma County and the City of Santa Rosa authorities.

The 2008/2010 Section 303(d) lists the following waters as impaired for REC-1 use (Figure 2):

1. Russian River from the railroad bridge upstream of Healdsburg Memorial Beach to the highway 101 crossing;
2. Russian River from Fife Creek to Dutch Bill Creek (i.e., Monte Rio reach)
3. Santa Rosa Creek watershed.
4. Laguna de Santa Rosa watershed
5. Green Valley Creek watershed
6. An unnamed tributary (Stream 1) at Fitch Mountain

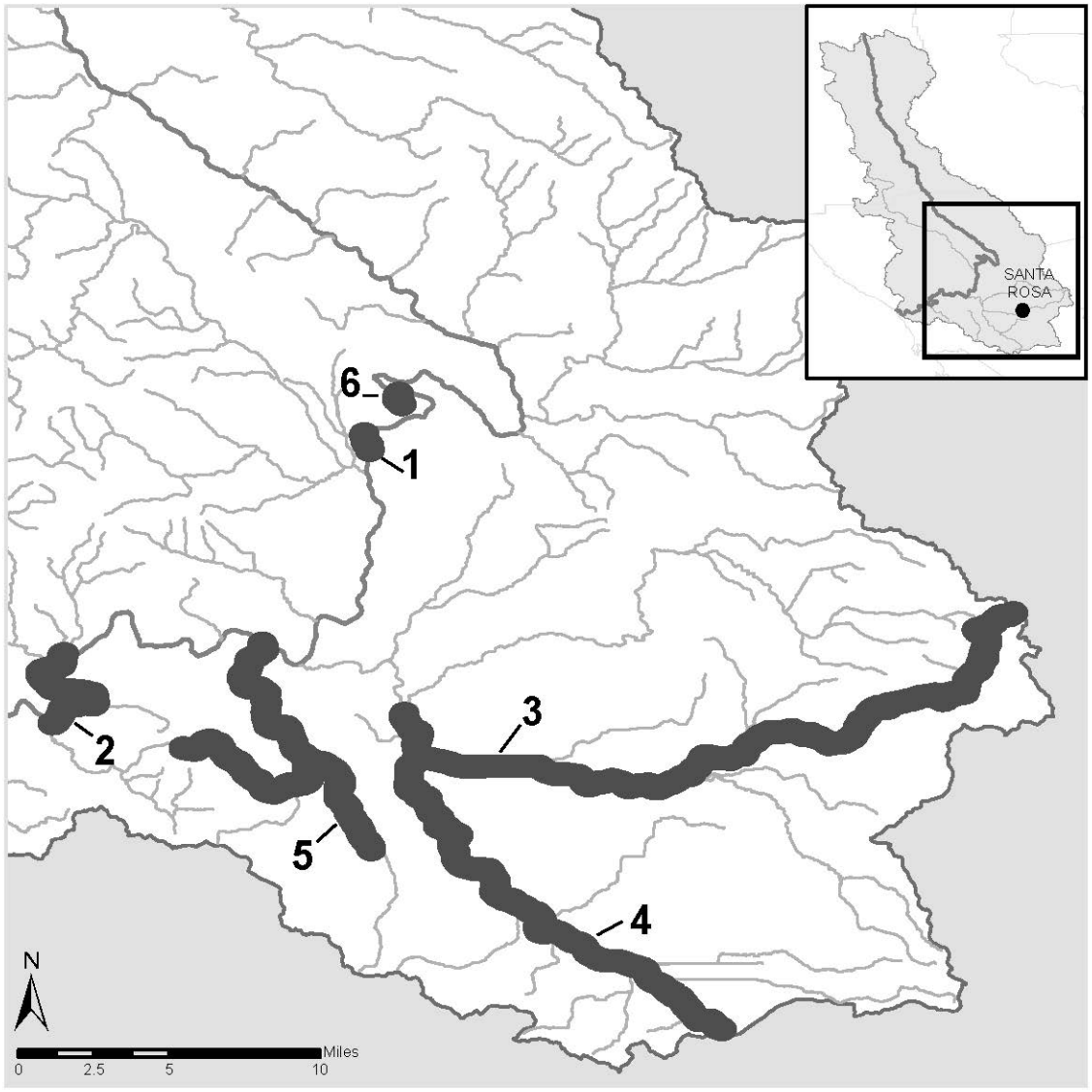


Figure 2. Russian River Watershed Surface waters impaired for REC-1

The key pollutant sources are not readily identifiable without further investigation. Potential sources include:

- Homeless and itinerant worker encampments along listed waterbodies, which lack sanitary disposal facilities;
- Residences lacking proper or fully functioning septic disposal;
- Improperly connected sewer lines;
- Direct discharges of waste from residential/commercial/long-term camping facilities;
- Leaks and spills of wastewater from permitted facilities, (including Healdsburg, Windsor, Santa Rosa Regional, and Forestville).
- Dairy pond failure;
- Runoff from landscape applications of manure;

- Irrigation creating direct runoff to surface waters;
- Intensive human contact use (and potential for direct discharge) at bathing beaches;
- Wildlife, livestock, and pets.

In addition to the discharge of waste, the fate and transport of pathogen indicating organisms may complicate determination of their origin. For example, some bacterial growths that occur are associated with vegetation and algae, and not from pathogenic sources. These reservoirs of bacteria can develop upstream of where exceedances are measured and appear only after vegetative matter dies and breaks loose.

Working hypotheses regarding causes of the impairments:

- A combination of reduced flows, in-stream impoundments, and channel geometry modified by excessive sediment loads may all contribute to conditions ideal for growth of bacteria during warm weather.
- Increased human presence and recreation in the water and riparian areas is known to occur seasonally. Adequate numbers of facilities are not provided for sanity disposal,
- Resort areas and golf courses using recycled wastewater for irrigation which may inadvertently discharge into storm drains and the river.
- Many residences and facilities along the watercourse are not supplied with sanitary sewers, and may have inadequate waste disposal capacity.
- Riparian watering of livestock is known to occur throughout the watershed introducing manure to the waterways.

Russian River Pathogens Pilot Study – Findings and Recommendations

A pilot study was conducted in collaboration with UC Davis Aquatic Ecosystems Analysis Laboratory (Shilling et al. 2009; Viers et al. 2009). UC Davis researchers prepared a compilation of existing methods for examining pathogen contamination in the environment, all available site specific data, physical definition of the local settings, and potential investigative methods for source assessment and speciation of source organisms. They used a weight-of-evidence approach to identify and quantify fecal contamination, potential sources, and trends. Methods evaluated included the use of real time quantitative polymerase chain reaction (PCR), coupled with stable isotope analysis (SIA) of nitrate, traditional pathogen indicator bacteria, and spatial land use analyses.

The study made several recommendations for monitoring in support of TMDL development which were incorporated into this QAPP's monitoring design:

- The pathogenic indicator bacteria commonly measured (i.e., total coliform, fecal coliform, E. coli, *Enterococcus* do not make ideal indicators of recent fecal material input since they may survive for months after introduction into the environment (Whitman et al. 2003). *Bacteroides* make better indicators of recent fecal material input since they do not survive long in the environment. A combination of E. coli, *Enterococcus*, and *Bacteroides* sampling and identification may provide answers to multiple questions about long-term fecal matter loading, host organisms, and recent fecal matter inputs.

- Stable isotope analysis (SIA) should be used to evaluate nitrate for relative source differences in oxygen (δ18O) and nitrogen (δ15N) will help identify the source of the water associated with the bacteria samples.
    - Samples with δ18O measures above 15 ‰ are largely runoff processes.
    - Samples with δ15N measures below 5 ‰ are typically ammonium from *in situ* processes such as wastewater treatment.
    - Samples with δ15N measures above 5 ‰ are manure and septic waste.

Currently, there is insufficient understanding concerning the composition of the overall microbial population (microbiome) and variations therein to accurately assess the risk to the bathing public from the presence of pathogens using the current indicator organism methodology. This lack of understanding and other issues also make it difficult to assess the effectiveness of pathogen reduction by pollution control projects.

A major problem facing the regulators is that there is lack of information regarding the microbial ecology of recreational waters, especially from non-point source pollution. There is currently little understanding of the impact of source microbiomes such as stormwater or sewage treatment plant outfalls on the overall microbiome of the receiving waters. Current indicator bacteria tests do not identify the potential sources of contamination, thus making it impossible to ascertain the source of pathogen indicator bacteria causing exceedance of water quality objectives.

A new technology is available that greatly improves microbial source identification. The Phylochip was originally developed by the Lawrence Berkeley National Laboratory using U.S. Department of Homeland Security funding to detect airborne bacteria for bioterrorism surveillance. The Phylochip can quantify over 50,000 bacterial species in a single sample. The Phylochip can quantify concentrations of the human pathogens, including quantifying microbiomes associated with the measured pathogens.

Decisions or Outcomes.

Goals of the Russian River Pathogen Indicator TMDL Monitoring Plan include:

- Collection of the principal data needs required to understand sources of pathogenic indicator organisms.
- Understanding the microbiological transport mechanisms.
- Advise the TMDL Allocation Process for developing mitigation strategies for reduction in pathogenic indicator organisms.

Monitoring tasks were identified for the following four management questions:

1. What is the spatial variability of the microbiome community?
2. What is the temporal variability of the microbiome community?
3. Do land uses influence the variability of the microbiome community?
4. Do recreational beach areas influence the variability of the microbiome community?

This monitoring plan is organized into tasks to collect information to address these management questions. This monitoring plan is organized into tasks to collect information to address these

management questions. The data collected will be assessed with a number of statistical methods to help answer the monitoring questions. The samples will be collected concurrently with the pathogenic indicator bacteria sampling resulting for the Russian River Pathogen Indicator TMDL Monitoring Plan. The data collected will be assessed with a number of statistical methods to help answer the monitoring questions. Non-parametric statistical methods will be used for all assessments. The Mann-Whitney U Test is a non-parametric test for assessing whether two samples of observations come from the same distribution. The Kruskal-Wallis Test is a one-way analysis of variance conducted using ranked data. The non-parametric methods will be used for testing equality of population medians among groups.

Water Quality or Regulatory Criteria

The Basin Plan (NCRWQCB, 2007) promulgates specific Water Quality Objectives (WQOs) for pathogenic indicator bacteria in the Middle and lower Russian River watershed. These WQOs are established to protect REC-1 beneficial use. The Basin Plan includes both narrative and numeric WQOs as described below:

"The bacteriological quality of waters of the North Coast Region shall not be degraded beyond natural background levels. In no case shall coliform concentrations in waters of the North Coast Region exceed the following: In waters designated for contact recreation (REC-1), the median fecal coliform concentration based on a minimum of not less than five samples for any 30-day period shall not exceed 50/100 ml, nor shall more than ten percent of total samples during any 30-day period exceed 400/100 ml."

The Basin Plan also establishes indicator bacteria WQOs for the protection of shellfish that may be harvested for human consumption (SHELL). The Basin Plan identifies some of the subareas of the Russian River Hydrologic Unit have to potential SHELL beneficial use. The Basin Plan includes numeric WQOs as described below:

"At all areas where shellfish may be harvested for human consumption (SHELL), the fecal coliform concentration throughout the water column shall not exceed 43/100 ml for a 5-tube decimal dilution test or 49/100 ml when a three-tube decimal dilution test is used (National Shellfish Sanitation Program, Manual of Operation)."

At the present time the scope of the Russian River Pathogen TMDL will not address impairment to potential SHELL beneficial use. However, the North Coast Regional Water Board may conduct a Use Attainability Analysis to evaluate whether the SHELL classification is correctly designated in subareas of the Russian River basin. The outcome of the UAA will determine whether TMDL allocations need to be modified in the future.

The Policy (SWRCB, 2004) for assessing information for placing waters on the Section 303(d) list allows the use of other evaluation guidelines beyond the legally promulgated Basin Plan. For the 2008/2010 list, the North Coast Regional Water Board used the California Department of Health Services draft criteria for posting beaches (CDHS, 2006). These criteria are as follows:

Beach posting is recommended when indicator organisms exceed any of the following single sample levels:

- Total coliforms: 10,000 per 100 ml
- Fecal coliforms: 400 per 100 ml
- *E. coli*: 235 per 100 ml
- *Enterococcus*: 61 per 100 ml

Additional sanitary surveys are recommended when indicator organisms exceed any of the following, based on the log mean of at least 5 equally spaced samples in a 30-day period:

- Total coliforms: 1,000 per 100 ml
- Fecal coliforms: 200 per 100 ml
- *E. coli*: 126 per l00 ml
- *Enterococcus*: 33 per 100 ml

A TMDL is the loading capacity of a pollutant that a water body can accept while protecting beneficial uses. Usually, TMDLs are expressed as loads (mass of pollutant calculated from concentration, multiplied by the volumetric flow rate), but in the case of indicator organisms, it is more logical and standard practice for TMDLs to be based only on concentration. TMDLs can be expressed in terms of either mass per time, toxicity, or other appropriate measure [40 CFR 130.2(1)]. A concentration based TMDL is appropriate for indicator bacteria because the public health risks associated with recreating in contaminated waters increases as pathogen concentration increases. Additionally, pathogens are not readily controlled on a mass basis. Therefore, the North Coast Regional Water Board will likely establish a concentration-based TMDL for pathogen indicator bacteria in the Russian River.

Recent studies conducted by the Southern California Coastal Water Research Project show little to no epidemiological connection between conventional indicator bacteria and regulatory criteria. There are also likely to be many cases of exceedances of the State standards that are caused by natural sources, especially in geographic areas with no storm drains, or sewage systems. Analysis of the entire microbiome community with samples collected in conjunction with the *Russian River Pathogen Indicator Bacteria TMDL Monitoring Plan* will help advise the TMDL Allocation Process for developing mitigation strategies for reduction in pathogenic indicator organism.

A6. Project/Task Description

Work Statement and Produced Products.

This project will focus on microbiological source identification in the middle and lower Russian River watershed. It will consist of dry and wet weather water sample collection and laboratory analyses. The project will provide data sets after each sampling event and the production of a final monitoring data report at the end of the project. The monitoring report will be used to advise allocation of loads in the development of the TMDL.

Constituents To be Monitored and Measurement Techniques.

Analysis of water samples for the microbime community using the Phylochip will be conducted by Lawrence Berkeley National Laboratory (LBNL). Analysis of samples for stable isotopes of nitrate will be conducted through Cel Analyitcal Laboratories. Analysis of water samples for *Bacteroides* will be conducted by Sonoma County Public Health Laboratory.

These data will be collected in accordance with the Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in SWAMP (SWAMP, 2007).

Project Schedule

Table 3 outlines the project schedule, including initiation and completion dates for the major tasks, required deliverables, and due dates.

Table 3. Project Schedule Timeline.

Activity	Date		Deliverable	Deliverable Due Date
	Anticipated Date of Initiation	Anticipated Date of Completion		
Collect and process water samples	May 2011	August 2012	Lab Data Reports	Continuous
Draft Monitoring Plan Data Report	January 2012	November 2012	Draft Report	November 2012
Final Monitoring Plan Data Report	November 2012	January 2013	Final Report	January 2013

Geographical setting

The study area is the middle and lower Russian River watershed bounded by the confluence of Commisky Creek (upstream of Cloverdale) to the mouth at the Pacific Ocean (near Jenner). (see Figures 3, 4 & 5 below)

Constraints

Water samples will be collected during wet and dry weather conditions. Wet period sampling will take place during or following storm events that are predicted to generate 0.10 inch or greater of rainfall and are preceded by 72 hours of dry weather (less than 0.10 inch of rainfall). Dry period sampling must be preceded by 72 hours of dry weather.

Physical constraints include safe access to the sampling locations. Some locations may become flooded or otherwise unsafe during wet period monitoring. If this occurs, the sample will be collected at an alternative time when safe sampling is possible. Additional samples will be collected to achieve the data quality objective for completeness shown in Table 4.

A7: Quality Objectives and Criteria for Measurement Data

This section contains the measurement quality objectives of this study and includes analyses both in the field and in the laboratory. Data quality indicators for this study will consist of the following:

Measurement Quality Objectives (MQOs) are statements about how good the measurements need to be in order to be useful as inputs to the decision process. MQOs are often reduced to statements about the acceptable values of Data Quality Indicators (DQIs).

There are four quantitative DQIs: accuracy, precision, completeness, and sensitivity. Accuracy and precision are monitored by the use of Quality Control (QC) samples. Completeness is a calculated value. Sensitivity is monitored through instrument calibration and the determination of method detection limits (MDLs) and reporting limits. The three qualitative DQIs, bias, representativeness and comparability, are assessed through the sample design process and selection of methods. The DQIs are defined below.

Accuracy

Accuracy is determined by the degree of agreement between a reported value and the true or expected value. Accuracy includes a combination of random error (precision) and systematic error (bias) components that are due to sampling and analytical operations.

Laboratory accuracy will be determined by following the policy and procedures provided in the laboratory's Quality Assurance Plan. These generally employ estimates of percent recoveries for known internal standards, matrix spikes and performance evaluation samples, and evaluation of blank contamination.

Precision

Precision is defined as the measure of agreement among repeated measurements of the same property under identical or substantially similar conditions. It is usually expressed as Relative Percent Difference (RPD). The calculation for RPD is:

$$((X_1 - X_2) / ((X_1 + X_2)/2))*100,$$

with the result expressed as a percent, where X_1 represents the first sample measurement and X_2 represents the second sample measurement. Only samples with a ±25% relative percent difference (RPD) will be considered as valid. Laboratory precision of lab duplicates will be determined by following the policy and procedures provided in the individual laboratory's Quality Assurance Plan. This typically involves analysis of same-sample lab duplicates. Only samples with a ±25% relative percent difference (RPD) will be considered as valid.

Completeness

Completeness refers to the amount of acceptable quality data collected as compared to the amount needed to ensure that the uncertainty or error is within acceptable limits. It is expressed as a percentage of the number of valid measurements that should have been collected. Data quality objectives require 90 percent completeness as shown in Table 4.

Sensitivity

Sensitivity is the ability of the test method or instrument to discriminate between measurement responses. Sensitivity is addressed primarily through the selection of appropriate analytical methods, equipment and instrumentation. The specifications for sensitivity are unique to each analytical instrument and are typically defined in laboratory Quality Assurance Plans (QAP) and Standard Operating Procedures (SOPs). This is assessed through instrument calibrations, calibration verification samples and the analysis of procedural blanks with every analytical batch.

Method sensitivity is dealt with by the inclusion of the required SWAMP Target Reporting Limits, where such values exist, and by the application of the definition of a Minimum Level as provided by the Inland Surface Water and Enclosed Bays and Estuaries Policy. The purpose of this comparison is to establish that the reporting limits of the analytical techniques used to measure pollutants are sufficiently low to conclude that a non-detect is below the applicable and relevant criteria. As presented in Table 4, the method detection limits are below the SWAMP reporting limits in accordance with the DQOs for nitrate-N. SWAMP reporting limits have not been identified for the other constituents measured.

Bias

Bias is defined as the systematic or persistent distortion of a measurement process that causes errors in one direction. Bias of sample collection will be controlled using best professional judgment to obtain representative samples that reflect field conditions.

Representativeness

"Representative" is a qualitative term that expresses "the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition" (ANSI/ASQC, 1994). This is addressed primarily in the sampling program design, through the selection of sampling sites and procedures which ensure that the samples taken reflect the goals of the project and represent typical field conditions at the time and location of sampling. Representativeness in the laboratory is ensured through the proper handling, homogenizing, compositing, and storage of samples and through the analysis of samples within specified holding times so that sample results reflect the environmental conditions form which the samples were collected as accurately as possible.

Comparability

Comparability is a measure of the extent to which the data from one study can be compared to that of another. In the field, this is addressed primarily through The use of standardized sampling and analytical methods, units of reporting, and site selection procedures.

In the laboratory, comparability is ensured through the use of comparable analytical procedures and ensuring that project staff are trained in the proper application of the procedures. Within-study comparability is assessed through analytical performance (QC sample analyses).

Table 4. Data quality objectives for laboratory measurements.

Parameter	Method	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Microbiome Community	Fast DNA microarray (Phylochip)	Spike-in hybridization controls, positive and negative control probes	Precision will not be assessed.	-	0.001% 16S rRNA gene PCR product	-
Bacteroides	Quantitative PCR	Proper positive and negative response	Matching duplicates on 10% of samples	-	-	90%
Nitrate-N	SM 4500 NO3 E	Standard Reference Material within 95% CI stated by provider of material.	Laboratory split sample 25% RPD	Matrix spike 80% - 120%	0.01 mg/L	90%
δ18O and δ15N	Stable isotope analysis	Check standards fall within precision limits of calibrated value (relative to IAEA)	15N: <0.4 per mL 18O: <0.8 per mL	-	1uM NO3	-

A8: Special Training and Certification

Specialized Training or Certifications.

No specialized training or certifications are required for this project. All staff involved in sample collection will be fully trained in the aseptic technique of water sample collection and procedures. Staff trainings will be conducted for proper field sampling and sample-handling techniques prior to any sampling activities. If necessary, additional training will be provided by the Project Manager, and only those staff with proficiency will be permitted to conduct field work. The Project Manager will provide training for all field personnel and retain in administrative files documentation of all training

Laboratory personnel training will include the review of proper laboratory procedures and sample-handling techniques, including receiving, handling/storage, and chain-of-custody procedures, prior to conducting any sample analysis, and only those staff with proficiency will be permitted to conduct laboratory analysis. The contract laboratory directors (Table 2) will provide training for all laboratory personnel and retain in administrative files documentation of all training.

Training and Certification Documentation.

Training records for the North Coast Regional Water Board staff are maintained at the North Coast Regional Water Board office. Laboratory safety manual and safety training records are maintained by each of the contract laboratories (Table 2).

A9: Documents And Records

Documents and records generated from this project will be organized and stored in compliance with this QAPP. This will allow for future retrieval, and to specify the location and holding times of all records.

QAPP Updates and Distribution

A QAPP is a document that describes the intended technical activities and project procedures that will be implemented to ensure that the results will satisfy the stated performance or acceptance criteria.

All originals of the first and subsequent amended QAPPs will be held at the North Coast Regional Water Board office by the Project Manager. The Project Manager under the direction, supervision, and review of the QA Officer, will be responsible for distributing an updated version of the QAPP. Copies of the QAPP will be distributed to all parties involved with the project directly or by mail (see Table 1). Any future amended QAPPs will be held and distributed in the same fashion.

Standard Operating Procedures

Field crews will review and collect samples as outlined in the most recent version of the Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (SWAMP, 2007).

Laboratory personnel will conduct all analysis and sampling handling as outlined in each of the Laboratories SOPs (see Appendices 5, 6 & 7).

Documentation of Data Collection (Field) Activities

Records are maintained for each data collection activity. The Project Manager will document and track the aspects of the sample collection process, including the generation of fieldsheets at each sampling site and COC forms (see Appendices 2 & 3) for the samples collected. COC forms will accompany water samples to the appropriate laboratories for analysis. An individual field sheet is used for each station per sampling event.

Typical information required on the water quality field sheets includes, but is not limited to:

- Site name and watershed location
- Station Description
- Station Access Information

- Sample Name and ID #
- Personnel on-site performing the sampling
- Dates and times of sample collection
- Site observations and any aberrant sample handling comments
- Sample QA collection information
- Sample collection information (sample collection methods and devices, sample collection depth, sample preservation information, sample analysis, matrix sampled, etc.).

Certain information that will not change can be pre-filled out prior to the survey to save time in the field. Other information is time-, location- and condition-specific, and should be filled out at the station ONLY. Completion of appropriate field documentation and forms for each sample is the responsibility of the Project Manager.

Documentation of Analytical (Laboratory) Activities

Documentation of all water quality samples to be analyzed by the individual Laboratories is critical for tracking data and evaluating the success of any activity. Each laboratory is required to provide the Project Manager with a current QAPP or equivalent (see Appendices 5, 6 and 7).

Laboratory Records

Each laboratory Lab Director will be responsible for documenting and tracking the aspects of samples receipt and storage, analyses, and reporting. Upon completion of laboratory analysis, laboratory data review, and data validation, the laboratory will issue a report in an electronic format describing the results of analysis for each sample submitted. Prior to issuance of the laboratory report, the laboratory's QA manager will review and approve the report. To assure that water quality information will be available in a time frame that will allow public health advisories to be issued in a timely manner, preliminary laboratory results should be transmitted to the Project Manager within 24 hours.

Components of the laboratory report include:

- A short summary sheet discussing the sampling event and results
- Sample information: sample site name and location, sample identifiers, date and time collected
- Parameter name (i.e., total coliforms or enterococci), and method reference
- Enumeration result
- Laboratory reporting limit
- Date and time of sample analysis
- Quality control information relevant for the analysis (i.e., field blank and duplicate results)
- Chain of Custody
- Holding times met or not
- Case Narrative of deviations from methods, procedural problems with sample analysis, holding time exceedances, and any additional information that is necessary for describing the sample; this narrative should explain when results are

outside the precision and accuracy required, and the corrective actions taken to rectify these QC problems.

- Explanation of data abnormalities

Chain of Custody

The original COC form will accompany the sample to the laboratory (see Appendix 3). Each transfer of the sample will be indicated on the COC form. The person listed on the COC form should have full sight or control of the sample at all times until the COC is relinquished by that person and received by the next party signed on the COC. A copy of the COC form will be included with the final laboratory report.

Electronic Data

The Project Manager will maintain a localized centralized database of information collected during this project. The database will include all analytical results. Data from contract laboratories are kept exactly as received and are copied onto the hard disk for editing as needed, based on error checking and verification procedures. After verification and final database establishment, the raw data files and databases are copied onto the North Coast Regional Water Board Network for storage on-site and off-site. Electronic data will also be copied to CD media for backup storage in public files at the North Coast Regional Water Board's offices. The original datasheets and reports produced are accumulated into project-specific files maintained at the North Coast Regional Water Board's offices for a minimum of five years.

GROUP B: DATA GENERATION AND ACQUISITION

B1: Sampling Process Design

The Monitoring Plan is organized into four individual tasks and sampling plans to collect information which will address the identified management questions. Task 1 evaluates the temporal and spatial variability of indicator bacteria at high use public recreation beaches. Tasks 2 and 3 evaluate the influence of land use and beach recreational use on pathogen indicator concentrations.

Task 1: Site Variability

Task 1 is designed to answer the following management questions:

1. What is the spatial variability in the middle and lower Russian River of microbiome community?
2. What is the temporal variability in the middle and lower Russian River of microbiome community between wet and dry seasons?

The Russian River Pathogens Pilot Study (see Element A5) identified the need to expand bacteria monitoring over a range of climatic conditions and to sites other than public beaches.

Wet weather runoff, or storm flow runoff, events occur episodically, are short in duration, and are characterized by rapid wash-off and transport of high bacteria loads from all land use types to receiving waters. During these events the residence times for bacteria are very short due to the high velocity of flows. Dry weather runoff is not generated from storm events. Rather, flow during dry weather is typically more uniform than wet weather storm flow, is not uniformly linked to every land use, and has lower flows, lower loads, and slower transport. This makes the bacteria die-off and/or re-growth processes more important.

To assess across site variability, sampling will be conducted during both wet and dry periods. :

Dry Season

Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan will be collected on a weekly basis beginning in May 2011 and ending September 2011. One of these sampling events will be chosen for collection of samples for analysis of *Bacteroides*, stable isotopes of nitrate, and Phylochip analyses.

Wet Season

Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan will be collected will be collected during wet periods October 2011 through April 2012. Wet periods are defined by federal regulation (40 CFR 122.21(g)(7)(ii)) and the USEPA Storm Water Sampling Guidance Document (USEPA, 1992) as greater than 0.1 inch and at least 72 hours from the previously measurable (greater than 0.1 inch

rainfall) storm event. One of these sampling events will be chosen for collection of samples for analysis of *Bacteroides*, stable isotopes of nitrate, and Phylochip analyses.

Sample Collection

Assessment of the spatial and temporal variability within the middle and lower Russian River will be conducted by collecting samples at each of the listed stations in Table 5 and mapped in Figure 3. Field crews will locate these sampling locations with the use of the reach maps in Appendix 4, or by GPS if needed.

Table 5. Sampling Location for Task 1

Station ID	Station Name	Location	Latitude	Longitude
114RR4234	Alexander Valley Campground	Alexander Valley Road	38.658672	-121.170433
114RR3119	Camp Rose	Camp Rose Road	38.613511	-121.167928
114RR2940	Memorial Beach	Old Redwood Hwy	38.604650	-121.122922
114RR2036	Steelhead Beach	Old River Road	38.500311	-121.100561
114RR1898	Forestville Access Beach	River Drive	38.510331	-121.078803
114RR1325	Johnson's Beach	Church Street	38.499389	-121.001972
114RR0898	Monte Rio Beach	Bohemian Hwy	38.466258	-122.990628
114RR6273	Commisky Station	Hwy 101	38.882508	-122.944231
114RR5748	Cloverdale River Park	Crocker Road	38.823144	-123.009458
114RR4751	Geyserville Bridge	Highway 128	38.712922	-121.104519
114DB0147	Dutch Bill Creek	Fir Road	38.463314	-122.990083
114RR0066	Jenner	Jenner Boat Ramp	38.449431	-123.115608
114SR6158	Santa Rosa Creek	Los Alamos Road	38.458314	-121.368450
114SR3260	Santa Rosa Creek	Railroad Street	38.434813	-122.719683
114LG3411	Laguna de Santa Rosa	Sebastopol Community Center	38.407926	-122.818068
114GV2455	Green Valley Creek	Martinelli Road	38.480444	-121.091008

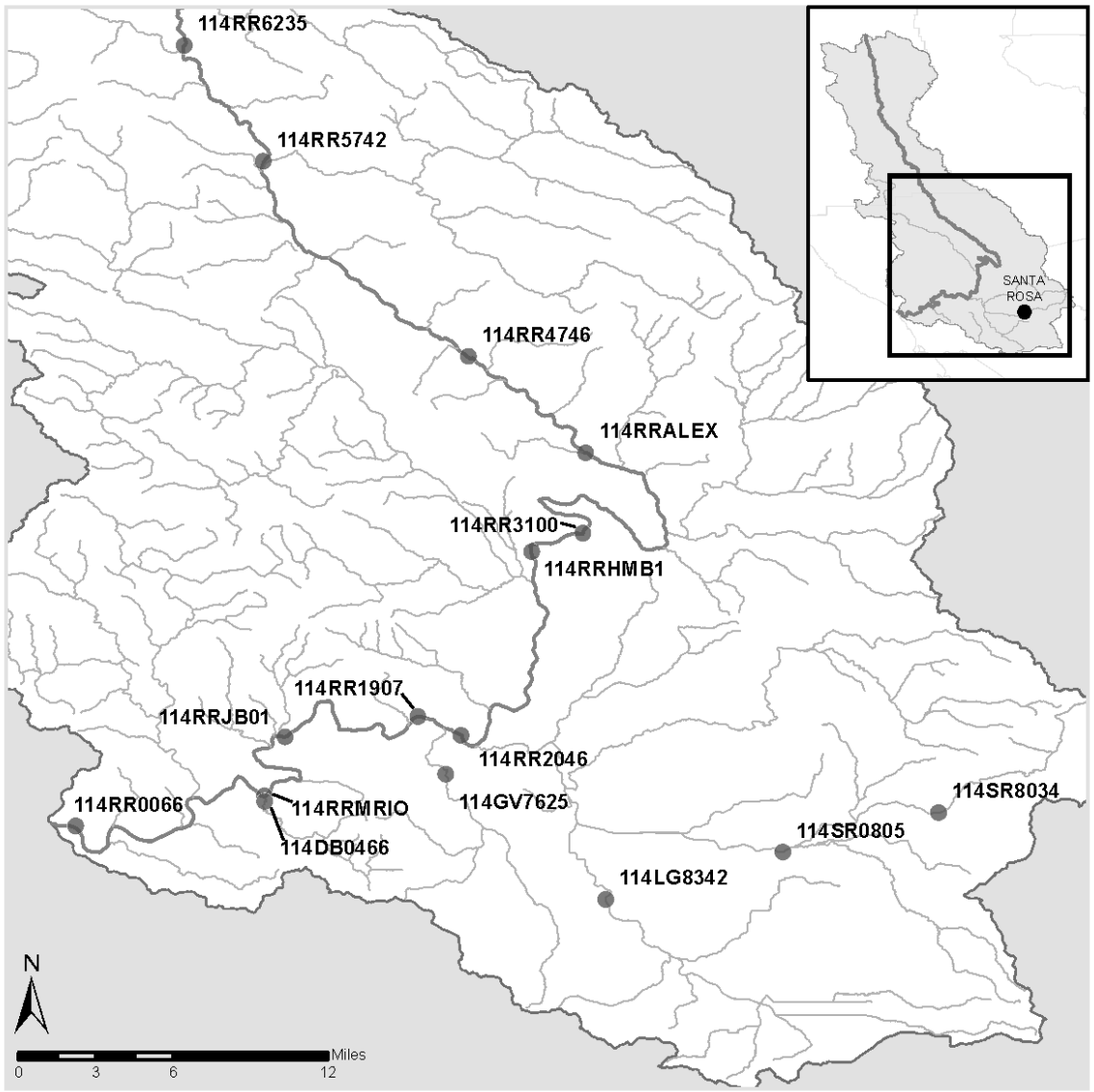


Figure 3: Sampling Locations for Task 1

The resulting sample size will be a total of 16 dry season samples and 16 wet season samples. For the *Bacteroides* samples, sterile water will be poured into the sample container in the field. For the stable isotope analysis samples, deionized water will be poured into the sample container in the field. For the Phylochip samples, sterile water will be poured into the sample container in the field and filtered in the North Coast Regional Water Board laboratory. Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 34 samples to Lawrence Berkeley National Laboratory (LBNL) for the analysis of the microbiome community with the Phylochip. 16 samples collected during 2 sampling events.
- 34 samples to Cel Analyitcal Laboratories for the analysis of stable isotope analyses of nitrate samples. 16 samples collected during 2 sampling events.
- 34 samples to Sonoma County Public Health Laboratory for the analysis of *Bacteroides*. 16 samples collected during 2 sampling events.

Task 2: Land Use Variability

Task 2 is designed to answer the following management questions:

1. What is the variability of the microbiome community between different land covers?
2. What is the temporal variability of the microbiome community between wet and dry seasons?
3. Do land uses influence the variability of the microbiome community?

In the Russian River Pathogens Pilot Study (see Element A5), it was determined that runoff from different land uses exhibited different bacteria levels. The objective of this task is to assess the relative magnitude and variability of bacteria in waters draining from each of the major land uses in the middle and lower Russian River watershed.

Target Population

The target population is the major land cover categories in the middle and lower Russian River watershed (Figure 2). The subpopulations are the specific land uses selected to represent the majority of the watershed. Based on the land cover spatial data acreage within the study area (Fry et al. 2011) and Urban Service Areas (PRMD, 2010), five land cover categories were chosen for sources assessment:

1. Forest Land
2. Rangeland
3. Agriculture
4. Urban & Residential Sewered areas
5. Residential Non-sewered areas.

Sample Frame

Several factors must be evaluated to find sample locations representing the specific land use categories:

- A large fraction of the upstream drainage must represent one of the selected land use categories.
- Sampling locations must be publically accessible (e.g., Bridge crossings).
- Watershed size should be large enough to generate sample flow during dry weather periods.
- Stream flow should not be regulated (i.e., Dry Creek).

Spatial data was used to delineate 20 subbasin watershed drainage areas in the study area. These subbasin areas were intersected with the land cover spatial data (Fry et al. 2011). For each of the selected land use categories, the three (3) subbasins with the greatest percentage of that land use were identified as the sample frame.

To assess land use variability, sampling will be conducted during both wet and dry periods:

Dry Season

Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan will be collected from October 2011 through June 2012. One of these sampling events will be chosen for collection of samples for analysis of *Bacteroides*, stable isotopes of nitrate, and Phylochip analyses.

Wet Season

Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan will be October 2011 through June 2012. One of these sampling events will be chosen for collection of samples for analysis of *Bacteroides*, stable isotopes of nitrate, and Phylochip analyses. Wet periods are defined by federal regulation (40 CFR 122.21(g)(7)(ii)) and the USEPA Storm Water Sampling Guidance Document (USEPA, 1992) as greater than 0.1 inch and at least 72 hours from the previously measurable (greater than 0.1 inch rainfall) storm event.

Sample Collection

Assessment of the spatial and temporal variability within the middle and lower Russian River will be conducted by collecting samples at each of the listed stations in Table 6 and mapped in Figure 4. Field crews will locate these sampling locations with the use of the reach maps in Appendix 4, or by GPS if needed.

Table 6. Sampling Locations for Task 2

Station ID	Watershed	Site Location	Land Use Category	Latitude	Longitude
114PI0729	Piner	Fulton Road	Developed Sewered	38.448439	-122.769552
114CO0655	Copeland	Commerce Drive	Developed Sewered	38.343216	-122.712096
114FO3662	Foss	Matheson Street	Developed Sewered	38.610756	-122.871743
114XX1675	Irwin	Sanford Road	Developed Onsite Septic	38.430035	-122.825181
114UL3960	Limerick	Old Redwood Highway	Developed Onsite Septic	38.588091	-122.849275
114UT3915	Turner	Daywalt Road	Developed Onsite Septic	38.352362	-122.767381
114UR3927	Woolsey	River Road	Agriculture	38.489806	-122.802845
114UW0048	Abramson	Willowside Road Levy	Agriculture	38.445692	-122.803044
114UD0000	Lambert	Lambert Bridge Road	Agriculture	38.653973	-122.927398
114CR3673	Crane	Snyder Lane	Shrubland/Herbaceous	38.355143	-122.685734
114GO0351	Gossage	Gilmore Ave	Shrubland/Herbaceous	38.337063	-122.734803
114BL1999	Blucher	Lone Pine Road	Shrubland/Herbaceous	38.365517	-122.786515
114PA3647	Palmer	Palmer Creek Road	Forest Land	38.574354	-122.954499
114UM0355	Mays	Neeley Road	Forest Land	38.498416	-122.995001
114VB0410	van Buren	St. Helena Road	Forest Land	38.512635	-122.637307

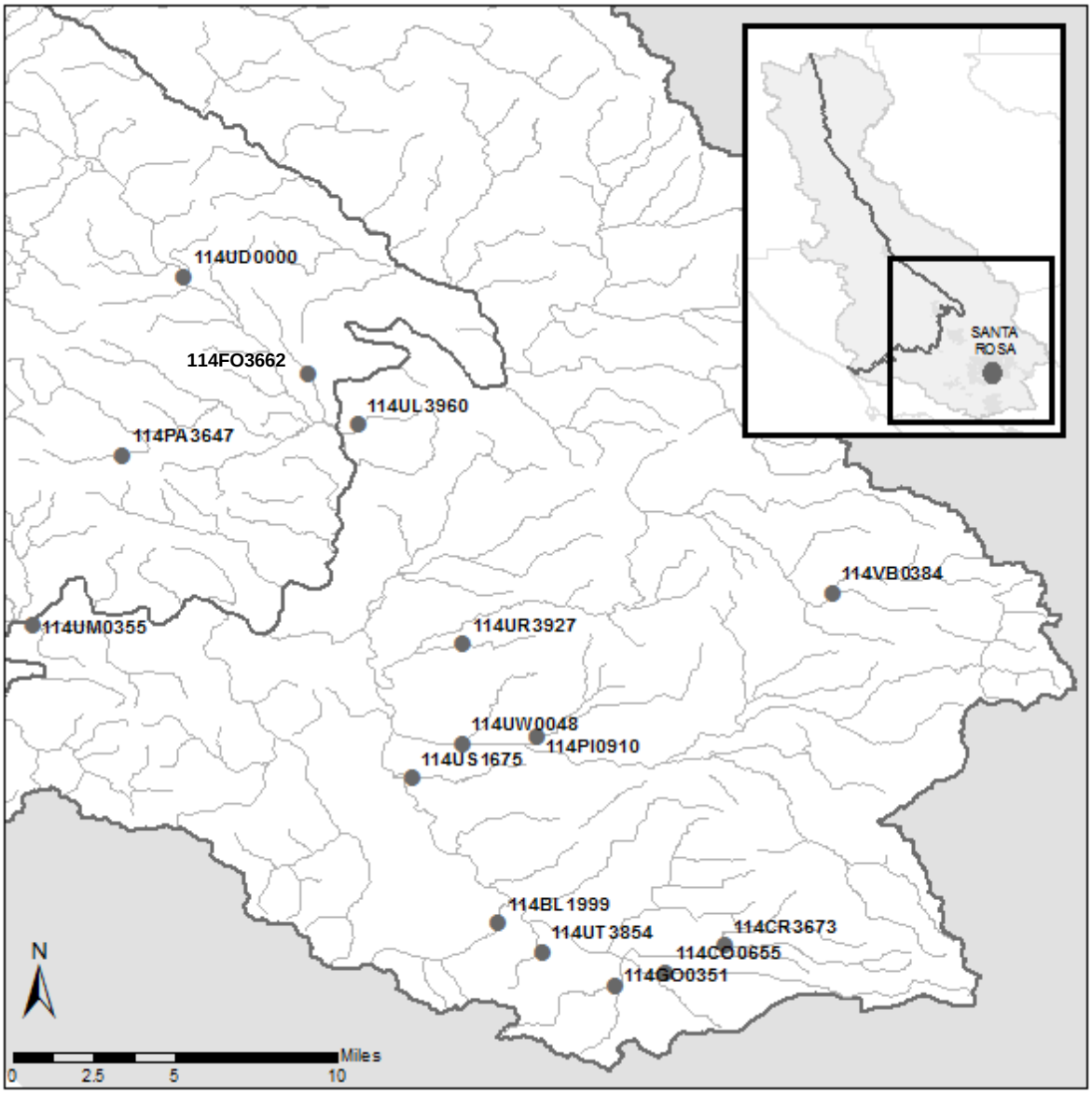


Figure 4: Sampling Locations for Task 2

The sample locations in Table 6 will be sampled during both wet and dry periods. One field blank sample will be collected for each sampling event. For the *Bacteroides* samples, sterile water will be poured into the sample container in the field. For the stable isotope analysis samples, deionized water will be poured into the sample container in the field. For the Phylochip samples, sterile water will be poured into the sample container in the field and filtered in the North Coast Regional Water Board laboratory along with the ambient water samples.

The resulting sample size will be a total of 16 dry season samples and 16 wet season samples. Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 32 samples to Lawrence Berkeley National Laboratory (LBNL) for the analysis of the microbiome community with the Phylochip. 15 samples collected during 2 sampling events.

- 32 samples to Cel Analytical Laboratories for the analysis of stable isotope analyses of nitrate samples. 15 samples collected during 2 sampling events.
- 32 samples to Sonoma County Public Health Laboratory for the analysis of *Bacteroides*. 15 samples collected during 2 sampling events.

Analysis

The data for each land use will be combined resulting in 6 samples per land use category. Estimates of flow will be made for each sample based on the nearest downstream stream flow gage by applying proportional watershed areas for each site or applying the Rational Method. Load duration curves will be derived and compared between each land use. Load duration curves are a useful tool to better characterize the pollutant problems over the entire flow regime.

Task 3: Recreational Use Variability

Task 3 is designed to answer the following management questions:

1. Do recreational beach areas influence the variability of the microbiome community?

The Russian River Pathogens Pilot Study (see Element A5) identified the need to conduct a more robust assessment of the variability of indicator bacteria measurements. The objective of this task is to assess the relative magnitude and variability of indicator bacteria levels that may be associated with increased human recreation use at public beach areas.

Sample Collection

Intensive sampling events will be conducted to assess the local impact of recreational activities on indicator bacteria levels at public beaches. Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan will be conducted at Johnson's Beach and Monte Rio Beach. Field crews will locate these sampling locations with the use of the reach maps in Appendix 4, or by GPS if needed.

Table 7. Sampling Locations for Task 3

Station ID	Station Name	Location	Latitude	Longitude
114RR1325	Johnson's Beach	Church Street	38.499389	-121.001972
114RR0898	Monte Rio Beach	Bohemian Hwy	38.466258	-122.990628

Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan will be collected each day for 8 days (Wednesday, September 21, 2011 through Wednesday, September 28, 2011) to assess daily variability. Sample collection dates bracket the Russian River Jazz & Blues Festival (Johnson's Beach) and the Russian River Cleanup (at numerous locations along the river) to capture the possible variability in indicator bacteria concentrations due to the elevated recreational use. One (1) sample will be collected for each sampling day and location. No sample field blanks will be collected.

The resulting sample size will be a total of 16 samples. Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 16 samples to Lawrence Berkeley National Laboratory (LBNL) for the analysis of the microbiome community with the Phylochip. Blank lab samples will be filtered each day. 16 samples collected during 8 sampling events.
- 16 samples to Cel Analyitcal Laboratories for the analysis of stable isotope analyses of nitrate samples. 16 samples collected during 8 sampling events.
- 16 samples to Sonoma County Public Health Laboratory for the analysis of *Bacteroides*. 16 samples collected during 8 sampling events.

B2: SAMPLING METHODS

Samples will be collected by North Coast Regional Water Board staff in aseptic containers prepared by the manufacturer. Samples will be collected according to a combination of: a) Standard Operating Procedures as described in the SWAMP Quality Assurance Management Plan, Appendix 4, Field Protocols and b) Appendix E, SWAMP SOPs and recommended Methods for Field Data Measurements and c) Standard Methods for the Examination of Water and Wastewater 20th Ed., which describe the appropriate sampling procedures for collecting samples for water chemistry and microbiology.

Personnel safety is a concern during wet weather events. Sample collection will be made using grab sample devices (i.e., poles fitted with sample bottles) from a safe location near the water's edge. Under no circumstances will personnel enter the water during a storm event.

Field Preparation

Field run preparation will consist of preparing field sheets (see Appendix 2), chain of custody forms (see Appendix 3), sample labels, and sample collection bottles. Field crews will be responsible for preparing all forms and obtaining sample bottles for sample collection from the contract laboratories.

Sample Volume and Bottle Type

Samples collected for *Bacteroides* analysis will be collected in sterile 110 mL polypropylene container. The container will be filled to the 100mL mark on the bottles. Total sample volumes will be approximately 100 mL.

Samples collected for stable isotope analysis will be collected in 100 mL polypropylene containers. Total sample volumes will be approximately 100 mL.

Samples collected for *nitrate-N* analysis will be collected in 500 mL polypropylene container. Total sample volumes will be approximately 500 mL.

Samples collected for Phylochip analysis will be collected in three separate 125 mL sterile plastic containers. The container will be filled to the 100mL mark on the bottles. Total sample volumes will be approximately 300 mL.

Sample Preservation and Holding Times

All samples to be analyzed in the lab will be preserved on ice at 6°C and transported in coolers (darkness) to the analytical labs at the end of the field run. The labs will process the samples within the specified holding time after the first sample was collected.

Responsible Person

The Project Manager is ultimately responsible for coordinating field activities. However It is the combined responsibility of the members of the field crew to determine if the performance requirements of the specific sampling method have been met and to collect an additional sample if required. Any deviations from field protocols defined in the project QAPP will be reported to the Project Manager immediately.

Any issues that cannot be readily corrected should be brought to the attention of the Project Manager, who is responsible for investigating and resolving all issues, and noted on the corresponding field sheet.

B3: Sample Handling and Custody

Samples will be considered to be in custody if they are in the custodian's possession or view or retained in a secured place (under lock) with restricted access. The principal documents used to identify samples and to document possession will be COC records and fieldsheets. COC procedures will be used for samples throughout the collection, transport, and analytical process.

Maximum Holding Times

Field parameters do not have a holding time since the results will be determined on site. For all samples, the samples will be immediately placed on ice in a cooler for transport to the laboratories. All samples will be delivered at the end of the field run. Analysis will begin within the holding time specified in Table 8.

Sample Handling

Identification information for each sample, including the project name, site location, date and time of collection, and lab analyses to be conducted, will be recorded on the label on the plastic sample bottles when the sample is collected. Sample identification is addressed below. Subsequently, identification information for each sample will be recorded on the laboratory data sheet (see Appendix 3) before submission to the contract laboratories.

All samples will be handled so as to minimize bulk loss, analyte loss, contamination or degradation. Sample containers will be clearly labeled. All caps and lids will be checked for tightness prior to transport. Samples will be placed in the ice chests with enough ice, or other packing to completely fill the ice chest. Chain of custody forms will be placed in an envelope and taped to the top of the ice chest. Samples will be handled using aseptic technique so as to minimize chance for contamination.

The following sampling technique will be used for collection of *Bacteroides* samples.

Sample Container Preparation
Sterile 100-mL irradiated nuclease-free plastic containers for water samples will be supplied by the Sonoma County Public Health Laboratory. The containers are enclosed in a heat-sealed plastic bag.

Ziploc® (or other brand) plastic bags will be used to store the sample containers after collecting the water sample. The inside of the Ziploc® bags will be decontaminated at the Regional Water Board lab by spraying the inside with *DNA AWAY*™ and wiping it dry with Kimwipes®.

Sample Collection

Water samples will be collected with the following strict "clean hands" aseptic technique, which is more precise than required by the SDRWQCB Surface Water Ambient Monitoring Program (SWAMP) protocols.

First, the exterior of the decontaminated Ziploc® bag will be labeled with sample identification, sample location, sample date, sample time, using black, waterproof ink.

Two samplers will be needed to collect a *Bacteroides* sample. A clean pair of nitrile gloves will be put on both of the sampler's hands and sprayed with 70% ethanol and air-dried. The gloves will then be sprayed with *DNA AWAY*™, a DNA destabilizing reagent, and wiped dry with Kimwipes®.

The first sample will tear open the plastic bag with the sterile 100-mL plastic containers, and hold the bag open without removing the container. The second sampler will remove the container without touching the outside of the plastic bag.

The second sampler will collect the water sample. The sample bottle will be carefully opened and the cap held carefully face down to prevent aerial contamination. The sampling container will be inverted and allowed to fill and then capped and held in one hand. Excessive water will be removed from the exterior of the sample container using Kimwipes®. The second sampler will spray the sample bottle with *DNA AWAY*™ and wipe dry with Kimwipes®.

Each sample container will be placed in a separate, sealed Ziploc® bag. The first sampler will unseal the decontaminated Ziploc® bag and will hold open the bag while the second sampler places the sample bottle into the Ziploc® bag without touching the exterior of the bag. The first sampler will then seal the Ziploc® bag only touching the exterior of the bag. The sealed Ziploc® bags containing the *Bacteroides* samples will be placed in a cooler with blue-ice. The gloves are then removed and discarded.

These steps will be performed for each sample collected. Gloves will be used only once. During sampling, if gloved hands touch anything other than the sampling bottle or Ziploc® bag, the gloves will be discarded, and the procedure will be repeated.

One field blank will be performed during each sampling event to ensure sterile techniques. The same sampling techniques as described above will be used. For the blank, nuclease-free water will be substituted for the creek water sample.

Table 8: Sampling and Preservation

Analyte	Units	Container	Sample Volume	Preservation	Maximum Holding Time
Phylochip Analysis	cells/mL	Three 125mL sterile, plastic containers	300 mL	Sodium thiosulfate is pre-added to the containers in the laboratory for chlorine elimination. Refrigerate at 4°C Samples to be filtered and frozen	6 hours
Bacteroides	cells/mL	110mL	100mL	Freeze at -80°F Samples to be filtered and frozen	6 hours
Nitrate-N	mg/L	Polypropylene	500 mL	Refrigerate at 4°C	48 hrs
Stable isotope analysis	δ18O and δ15N	Polypropylene	30 mL	Temperature and filtration. Samples to be filtered (min 0.2um nylon) and frozen	60 days

Chain of Custody Procedures

Field measurements do not require specific custody procedures since they will be conducted on site at the sample collection location. All bacteria samples will be accompanied by chain of custody forms (see Appendix 3). At the time samples are transferred, both the person receiving and relinquishing the samples should verify that all samples collected are reflected on the chain of custody forms. The condition of the samples will also be noted and recorded by the receiver. COC records will be included in the final administrative record as prepared by the analytical laboratories. Any deviations should be explained on the field sheets and chain of custody forms, as needed.

Transport

Samples received by the contract laboratories will be processed immediately upon receipt and within the specified holding time.

Bacteroides samples placed in the cooler with blue-ice will be transported to the Sonoma County Public Health Laboratory within the 6-hours from the first sample time collected. The sample will be stored at the Sonoma County Public Health Laboratory in a -80C freezer until the *Bacteroides* analysis is performed. Samples to be analyzed for *Bacteroides* will be delivered to:

Sonoma County Public Health Laboratory
3313 Chanate Road
Santa Rosa, CA 95404
Tel: 707-565-4711

Samples to be analyzed for nitrate-nitrogen and stable isotope analyses will be transferred via courier to:

Cel Analytical, Inc.
82 Mary Street, Suite #2
San Francisco, CA 94103

Samples to be analyzed for Phylochip analyses of nitrate will be filtered at the North Coast Regional Water Board laboratory. Sample filters will be delivered for storage at -80ºF to:

Sonoma County Public Health Laboratory
3313 Chanate Road
Santa Rosa, CA 95404
Tel: 707-565-4711

When all sample filters for Phylochip analysis have been collected, sample filters will be delivered in a dark cooler on dry ice to:

Lawrence Berkeley National Laboratory Ecology Department
One Cyclotron Road, MS 70A-3317
Berkeley, CA 94720

Field crews will deliver samples and required documentation to laboratory staff designated to receive samples. Samples collected will be verified against field sheets and chain of custody forms. Discrepancies and any additional notes, such as holding time exceedances, incorrect sample identification information, inappropriate sample handing, or missing/inadequate field equipment calibration information, will be noted on the field sheets and chain of custody forms, as needed by the staff receiving the samples.

Responsible Individuals

The Project Manager and Project QA Officer will have ultimate responsibility for ensuring samples are properly handled and transferred. However, it is also the responsibility of the persons collecting, relinquishing, and receiving samples to initially verify correct sample handling and transfer.

B4: Analytical Methods and Field Measurements

The laboratory analytical methods to be used for this project to analyze water samples in the laboratory are listed in Table 9.

Table 9. Laboratory Analytical Methods.

Analyte	Laboratory / Organization	Project Action Limit	Project Quantitation Limit	Achievable Laboratory Limits	
				Analytical Method/ SOP	MDLs
Bacteroides	Sonoma County Public Health Lab	None	50% detection efficiency	Appendix 7	50% detection efficiency
Nitrate-N	Cel Analytical Inc.	None	0.01 mg/L	SM 4500 NO3 E	0.01 mg/L
Stable Isotopes	Cel Analytical Inc./UC Davis Stable Isotope Facility	None	0.1 %	Appendix 6	0.1 %
Microbiome Community	LBNL	None	None	Appendix 5	None

Corrective Actions

When failures in the laboratory occur, the individual Laboratory Managers will each be responsible for corrections in their respective laboratories. All failures will be documented on the field sheet with the data report, along with the corrective action that was made. Additionally, corrections will be annotated in any applicable maintenance logs.

B5: Quality Control

QA/QC for sampling processes begins with proper collection of the samples in order to minimize the possibility of contamination. Water samples will be collected in laboratory-certified, contaminant-free bottles. For this project, sterile, bacteria-free containers will be used.

Appropriate sample containers and sampling gear are transported to the sample site. Water samples are collected and put on ice for transport to the appropriate laboratories. This section describes the various laboratory and field quality control activities and samples to be used in this study.

Quality Control Samples

Quality control samples shall be collected according per sampling event. Specific quality control sample types are described below.

Collection of Water Samples

Field crews will ensure that sampling bottles are filled properly. Filled sample bottles will be kept on ice during the sampling event and placed into coolers along with completed COC for transfer to the analytical laboratories. A fieldsheet will be completed at each site. The fieldsheets will include empirical observations of the site and water quality characteristics. Replicate sampling as described for each task will be conducted to assess variability of results. Field blanks will be used to assess possible sample contamination

Field Blank

Field blanks provide bias information for field handling, transport, and storage operations. They will be collected at a minimum of one sampling location during each sampling event. Field blanks are used to ensure that no contamination originating from the collection, transport, or storage of environmental samples occurs.

A field blank consists of analyte-free water that is poured into the sample collection device and sub-sampled for analyses to verify that field sampling procedures are adequate and sample handling and transportation does not introduce any analytes of interest. Field blanks will be preserved, packaged, and sealed exactly like the surface water samples and will be submitted to the lab. The lab results must be less than the MDL of the target analytes to be acceptable. Field blanks will be collected and analyzed for all analyses during each sampling event for Tasks 1 and 2.

Laboratory Blank

Laboratory blanks (also known as method blanks) provide bias information on possible contaminants for the entire laboratory analytical system. The laboratory will process laboratory blanks through the laboratory sample handling, preparation and analytical processes. These blanks will be made from sterile purified water that is known to have no detectable levels of the target analytes. They will be processed at a minimum of one laboratory blank during each sampling event. Laboratory blanks will be analyzed along with the project samples to document background contamination of the analytical measurement system. The lab results must be less than the MDL of the target analytes to be acceptable.

Laboratory Duplicates

Laboratory duplicates provide precision information on the analytical methods with the target analytes. The laboratory will generate the duplicate samples by splitting one sample into two parts, each of which will be analyzed separately. They will be processed at a minimum of one laboratory blank during each sampling event. No special sampling considerations are required.

The Phylochip samples will not require duplicate lab samples. The creation of the PhyloChip represents a significant investment of time and resources that results in what is now a mature technology capable of detailed measurements of microbial community composition in a high-throughput and reproducible manner. Key features that set the PhyloChip apart from other similar technologies are the use of multiple oligonucleotide probes for every known category of prokaryotic organisms for high confidence detection and the pairing of a mismatch probe for every perfectly matched probe to minimize the effect of non-specific hybridization. A strong linear correlation has been confirmed between microarray probe set intensity and concentration of OTU specific 16S rRNA gene copy number, allowing quantification in a wide dynamic range. Validation experiments have demonstrated that intensity-based quantification of environmental samples is highly reproducible with variation between replicate chips being less than 9%.

The microarray has been extensively validated and successfully used on a number of complex environmental samples and the resulting findings have been confirmed by additional methods, including qPCR and 16S rRNA gene clone libraries (Mendes et al. 2011, Hazen et. al 2010, Brodie et al., La Duc et al. 2009). LBNL studies using split-samples have confirmed that >90% of all 16S rRNA sequence types identified by the more expensive clone library method are also identified by the PhyloChip. In addition, the PhyloChip has demonstrated several-fold increases in detected microbial diversity over the clone library method. One of the reasons for this is the high sensitivity of the PhyloChip, with the ability to detect organisms present at a fraction below 10^{-4} abundance of the total sample (DeSantis et al. 2007). Numerous validation experiments using sequence-specific PCR have confirmed that taxa identified by the microarray but not clone libraries were indeed present in the original environmental samples (DeSantis et al. 2007, La Duc et al. 2009). Although each sample analysis by the PhyloChip provides detailed information on microbial composition, the highly parallel and reproducible nature of this array allows tracking community dynamics over time and treatment. With no prior knowledge, specific microbial interactions may be identified that are key to particular changing environments.

The PhyloChip is a mature, validated technology based on the successful GeneChip® hardware platform (Affymetrix, Inc., Santa Clara, CA). Results are reproducible and reliable, owing to the standardization of lab protocols, internal standards, and embedded controls. PhyloChip scans are assessed to attribute the observed fluorescence intensity at each of $>10^6$ array positions with presence or absence of over 59,000 bacterial and archaeal taxa. Due to the low variation in technical replicates between PhyloChip assays, an average of over 40 probe-pairs used, in combination, to identify each of the specific OTUs, and multiple controls including the use of standardized spike mixture for each sample to assay chip-to-chip variation, it is our experience that biological replicates are of greater value than technical replicates.

B6: Instrument/Equipment Testing, Inspection, and Maintenance

Microbiological sample bottles will be provided by the contract laboritories prior to the sampling events. The North Coast Regional Water Board Laboratory maintains sufficient quantities of fresh calibration standards for the instruments that will be used for field activities.

Laboratory equipment will be inspected, calibrated, and maintained according to the individual laboratories QAP (see Appendices 5, 6 and 7).

If an instrument fails to meet calibration or perform properly, an initial examination is made to determine the cause. If possible, repairs are made and the instrument is calibrated and examined for operational status. All repair activities are recorded in the Calibration and Maintenance Log. If an instrument fails to respond after initial attempts at repair, the equipment will be taken out of use and sent to the manufacturer for servicing.

Persons Responsible for Testing, Inspection and Maintenance

The Project QA Officer is responsible for ensuring equipment relevant to the project is properly tested, inspected and maintained. Staff may be delegated the responsibilities of carrying out these tasks.

B7: Instrument/Equipment Calibration and Frequency

No field measurements will be collected so no equipment and instruments are operated.

B8: Inspection/Acceptance of Supplies and Consumables

The Project Manager, Laboratory Directors, and Project QA Officer are each responsible for the inspection and acceptance of supplies and consumables used during this project. The actual inspection may be delegated to lab staff.

Upon receipt and prior to use, all reagents and commercially prepared media will be inspected by the laboratory staff for broken seals and to compare the age of each reagent to the manufacturer's designated shelf life. All manufacturer-supplied specifications, which may include shelf life, storage conditions, sterility, performance checks, and date, are kept by the laboratories.

Microbiological sample bottles will be provided by the manufacturer. They will be shipped to and stored at the North Coast Regional Water Board laboratory prior to use for sampling. Confirmation that sample bottles are laboratory-certified clean will be made when received from the manufacturer.

Staff responsible for the equipment ordering will inspect the supplies and consumable materials for quality, and will report any that do not meet acceptance criteria to the appropriate Laboratory Director and Project QA Officer. Upon receipt of materials, a designated employee receives and signs for the materials. The items are reviewed to ensure the shipment is complete, and they are then delivered to the proper storage location. Supplies are dated upon receipt, stored

appropriately, and are discarded on expiration date. Confirmation that sample bottles are laboratory certified clean will be made when received and prior to use in the field.

B9: Non-Direct Measurements

Non-direct measurements (also referred to as secondary data) are data previously collected under an effort outside this project. There will be no data obtained for this project that are derived from non-direct measurement sources, with the exception of meteorological data.

The National Weather Service Quantitative Precipitation Forecast will be reviewed from the internet on a daily basis for the purpose of documenting weather conditions within the project area for sampling conditions. The National Weather Service website provides a website with diel maps of precipitation forecasts over the entire study area. The acceptance criteria for determining wet or dry period will be based on Santa Rosa weather station shown on the website. The data from this weather station is assumed to be a conservative representation for the entire study area. Precipitation in areas of higher elevation is likely to be larger than measured in Santa Rosa. The National Weather Service Quantitative Precipitation Forecast website address is: http://www.cnrfc.noaa.gov/precipForecast.php?cwa=MTR&day=1&img=5

B10: Data Management

Data will be maintained as established in Element 9 above. All data from this study will be managed in accordance with the SWAMP Data Management Plan (2009) and SWAMP Standard Operating Procedures (SOPs).

The Project Manager maintains overall responsibility for proper data handling; however specific tasks may be delegated to other staff. The Project Manager will maintain hard copies of all original monitoring related project documents in project-specific files that are maintained at the North Coast Regional Water Board office. Monitoring related documents include: the Monitoring Plan (MP), the Quality Assurance Project Plan (QAPP), field sheets, COC forms and laboratory reports.

Data entry oversight will be the responsibility of the Project Manager. The Project Manager will document and track the aspects of the sample collection process, including the generation of field sheets and COC forms for the samples collected. COC forms will accompany all water samples to the appropriate laboratory for analysis. The laboratories will document and track the aspects of sample receipt and storage, analyses, and reporting.

Data/Information Handling and Storage

North Coast Regional Water Board staff will prepare field sheets prior to the field run to include sample run and sample location identification information. The sheets will be printed on waterproof paper – one per site. Field crews will record observations and field measurement data at the sampling locations. Prior to leaving the field site, field sheets will be checked for completeness and accuracy.

Computerized Information System Maintenance

Official electronic files will be maintained by the Project Manager once the data reports are received from the contract laboratory QA Officers. The files will be located on the North Coast Regional Water Board network. The North Coast Regional Water Board Information Technology unit performs backup nightly on all network drives.

GROUP C: Assessment and Oversight

C1: Assessments & Response Actions

Assessment and oversight involves both field and laboratory activities to ensure that the QAPP is being implemented as planned and that the project activities are on track. By implementing proper assessment and oversight, finding critical problems toward the end of the project is minimized, when it may be too late to apply corrections to remedy them.

Project Assessments

Readiness reviews will be conducted prior to each sampling run by the Project Manager. All sampling personnel will be given a brief review of the sampling procedures and equipment that will be used to achieve them. Field measurements will be collected as part of the concurrent sampling for the *Russian River Pathogen Indicator TMDL Monitoring Plan*. Details of the field sampling effort can be found in the Russian River Pathogen Indicator TMDL Monitoring Plan QAPP.

Readiness reviews will consist of the following activities:

Supply Checks

Adequate supplies of all necessary supplies will be checked before each field event to make sure that there are sufficient supplies to successfully support each sampling event.

Paperwork Checks

All field activities and measurements will be properly recorded in the field. Therefore, prior to starting each field event, necessary paperwork such as fieldsheets, chain of custody record forms, etc. will be checked to ensure that sufficient amounts are available during the field event.

Two types of assessments may be used in this project: field activity audits and laboratory audits.

Field Activity Audits

Field activity assessments are held to assess the sample collection methodologies, field measurement procedures, and record keeping of the field crew in order to ensure that the activities are being conducted as planned and as documented in this QAPP.

Annual assessments of field crews will be conducted to ensure that field sampling procedures outlined in this QAPP are followed. Prior beginning any field sampling activities, the Project Manager or Quality Assurance Officer will verify that proper equipment is available for all field personnel. This includes sampling equipment, safety equipment, and field measurement

equipment. It will also be verified that all personnel involved in field activities have received sufficient training and are able to properly use the equipment and follow procedures. The Project Manager or Quality Assurance may also verify the application of procedures and equipment periodically. If the Project Manager or Quality Assurance Officer finds any deficiencies, corrective actions will be put in place and reported, and follow-up inspections will be performed to ensure the deficiencies have been addressed. Field assessments will include:

- Readiness reviews to verify field teams are properly prepared prior to starting field activities;
- Field activity audits to assess field team activities during their execution; and
- Post sampling event reviews to assess field sampling and measurement activities methodologies and documentation at the end of all events or a selected event.

Post sampling event reviews will be conducted by the Project Manager following each sampling event in order to ensure that all information is complete and any deviations from planned methodologies are documented. Activities include reviewing field measurement documentation in order to help ensure that all information is complete.

Laboratory Audits

Initially, laboratory performance will be assessed through the use of a laboratory intercomparison study, and analysis of split samples. Laboratory assessments may involve two types of activities:

- Data reviews of each data package submitted by a laboratory; and
- Audits of laboratory practices and methodologies.

Laboratory audits will include sample submission for a proficiency test for each sampling run. The results of the lab's analysis will be compared to the known analytes (e.g. lab blanks) or acceptable ranges (e.g. lab duplicates)

Laboratory data review will be conducted by the QA Officer upon receipt of data from each lab. Data will be checked for completeness, accuracy, specified methods were used, and that all related QC data was provided with the sample analytical results.

Corrective Action

If an audit of any field sampling or laboratory operation discovers any discrepancy, the Project Manager will discuss the observed discrepancy with the appropriate person responsible for the activity. The discussion will begin with whether the information collected is accurate, what were the cause(s) leading to the deviation, how the deviation might impact data quality, and what corrective actions might be considered. The results of the resolution of the discrepancy will be documented in writing on the fieldsheet and on a separate log that will be kept in the project file.

Problems regarding field data quality that may require corrective action will be documented in the fieldsheets. Deficiencies that cannot be immediately corrected will be noted on the fieldsheets and brought to the attention of the Project Manager and Project QA Officer.

Individual laboratory data quality will be reviewed by the Laboratory Director and Laboratory QA Officer for their respective labs. Deficiencies and corrective actions taken will be noted on the laboratory data sheets as well as documented on the Excel spreadsheets to which the data will be transferred. Overall laboratory data quality will be reviewed by the Project QA Officer.

The Project Manager and the Project QA Officer have the authority to issue stop work orders to stop all sampling and analysis activities until the discrepancy can be resolved.

C2: Reports to Management

Interim and Final Reports

The Project Manager will review draft reports to ensure the accuracy of data analysis and data interpretation. The contract Lab Directors will report data to the Project Manager after quality assurance has been reviewed. Every effort will be made to submit reports to the Project Manager in a timely manner. Draft and final reports will be issued by the Project Manager according to the schedule in Table 10.

Table 10 – Report Due Dates

Report Type	Frequency	Responsible Party	Schedule
Data Report	Per Batch Delivered	LBNL Lab Director	On-going
Data Report	Per field run	Cel Analytical Lab Director	On-going
Data Report	Per field run	Sonoma Public Health Lab Director	On-going
Draft Monitoring Plan Data Report	N/A	Project Manager	April 2012
Final Monitoring Plan Data Report	N/A	Project Manager	June 2012

Quality Assurance Reports

Separate quality assurance reports will not be generated. Quality assurance information annotated on field and lab sheets will be included with the Data reports.

Group D: Data Validation and Usability

D1: Data Review, Verification, and Validation Requirements

Data review, verification, and validation procedures help to ensure that project data will be reviewed in an objective and consistent manner. Data review is the in-house examination to ensure that the data have been recorded, transmitted, and processed correctly.

Checking for Typical Errors

In-house examination of the data produced from the project will be conducted to check for typical types of errors. This includes checking to make sure that the data have been recorded, transmitted, and processed correctly. The kind of checks that will be made will include checking for data entry errors, transcription errors, transformation errors, calculation errors, and errors of data omission.

Checking Against DQIs

Data generated by project activities will be reviewed against Data Quality Indicators (DQIs). This will ensure that the data will be of acceptable quality and that it will be SWAMP-comparable with respect to minimum expected DQIs.

Checking Against QA/QC

QA/QC requirements were developed and documented in Elements B3, B4, B5, B7, and B8, and the data will be checked against this information. Checks will include evaluation of field and laboratory duplicate results; and field and laboratory blank data pertinent to each method and analytical data set. This will ensure that the data will be SWAMP-comparable with respect to quality assurance and quality control procedures.

Data Checking

Lab data consists of all information obtained during sample analysis. Initial review of laboratory data will be performed by the individual lab's Laboratory Director in accordance with the lab's internal data review procedures. Upon receipt of the completed data packages from the microbiological laboratories and fieldsheets from the field crews, the Project QA Officer and Project Manager will review all data, fieldsheets and field notes to verify that the QAPP was followed. Items reviewed will include:

- Comparison of the scheduled sampling plan with fieldsheets and custody forms to assure that planned samples were collected.
- Review of fieldsheets and data to assure that information specified in the QAPP was collected.
- Review of custody forms, including checks for breaches of custody, sample temperature upon receipt at the laboratory, and any anomalies noted on custody form.
- Review of laboratory data packages to verify that holding times were met.

- Review of the data package to verify that it was complete, and review of the QA/QC laboratory sheets.
- Analysis of RPD between each set of duplicate field samples.

Any problems noted will be brought to the attention of the appropriate laboratory manager and/or field crew. As any sample for microbial enumeration is perishable, serious problems in data quality may require resampling. This will occur at the discretion of the Project Manager.

Data Verification

Data verification is confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. Data verification is the process of evaluating the completeness, correctness, and conformance/compliance of a specific data set against the methodology, procedural, or project requirements. Data verification will be conducted as described in Element D2 to ensure that the data is complete, correct, and conforms to the minimum requirements set forth in this QAPP.

Data Validation

Data validation is an analyte- and sample-specific process that evaluates the information after the verification process (i.e., determination of method, procedural, or contractual compliance) to determine analytical quality and any limitations. Data validation is the process whereby data are filtered and accepted or rejected, based on a set of criteria. It is a systematic procedure of reviewing a body of data against a set of criteria to provide assurance of its validity prior to its intended use. The data are checked for accuracy and completeness. The data validation process consists of data generation, reduction, and review (see Element D2).

Data Separation

Data will be separated into three categories for use with making decisions based upon it. These categories are:

1. Data meeting all data quality objectives,
2. Data meeting failing precision criteria, and
3. Data failing to meet accuracy criteria.

Data falling in the first category is considered usable by the project. Data falling in the last category is considered not usable. Data falling in the second category are data meeting all data quality objectives, but with failures of quality control practices. These data will be set aside until the impact of the failure on data quality is determined. Once determined, if sufficient evidence is found supporting data quality for use in this project, the data will be moved to the first category, but will be flagged with a "J" as per EPA specifications, or not used if the data fail to meet precision and accuracy criteria.

Responsible Individuals

The Project Manager will be responsible for data review. This includes checking that all technical criteria have been met, documenting any problems that are observed and, if possible, insuring that deficiencies noted in the data are corrected.

D2: Verification and Validation Methods

Defining the methods for data verification and validation helps to ensure that project data are evaluated objectively and consistently. Information on these methods is provided below.

After each sampling event, the fieldsheets are checked for completeness and accuracy by the Project Manager. If there are any questions, clarification from the field crew is obtained as soon as possible. Fieldsheets are then placed into project-specific files maintained by the Project Manager.

All data records will be checked visually and will be recorded as checked by the checker's initials as well as with the dates on which the records were checked.
All of the laboratory's data will be checked as part of the verification methodology process. At least 10% of the laboratory's data will be independently checked as part of the validation methodology.

Data that is discovered to be incorrect or missing during the verification or validation process will be reported to the Project Manager immediately. If the errors involve laboratory data then this information will also be reported to the appropriate Laboratory Director.

If there are any data quality problems, the Project Manager and Project QA Officer will identify whether the problem is a result of project design issues, sampling issues, analytical methodology issues, or QA/QC issues (from laboratory or non-laboratory sources). If the source of the problems can be traced to one or more of these basic activities then the person or people in charge of the areas where the issues lie will be contacted and efforts will be made to immediately resolve the problem. If the issues are too broad or severe to be easily corrected then the appropriate people involved will be assembled to discuss and try to resolve the issue(s) as a group. The Project Manager has the final authority to resolve any issues that may be identified during the verification and validation process.

D3: Reconciliation with User Requirements

Information from field data reports (including field activities, post sampling events, corrective actions, and audits), laboratory data reviews (including errors involving data entry, transcriptions, omissions, and calculations and laboratory audit reports), reviews of data versus MQOs, reviews against Quality Assurance and Quality Control (QA/QC) requirements, data verification reports, data validation reports, independent data checking reports, and error handling reports will be used to determine whether or not the project's objectives have been met.

The Project Manager will be responsible for reporting project reconciliation. This will include measurements of how well the project objectives were met. Data from all monitoring measurements will be summarized in tables. There are no known limitations that are inherent to the data to be collected for this study. Explanations will be provided for any data determined unacceptable for use or flagged for QA/QC concerns.

The project will provide data for the selected analytes described in Element A5. All data will be readily available to the public. The data generated will also be useable for comparative purposes by other water monitoring projects and programs within the various components of the State and Regional Water Boards.

Appendix 1: Citations

California Department of Health Services. (CDHS) 2006. Draft Guidance for Freshwater Beaches. Last Updated May 8, 2006.

Fry, J., Xian, G., Jin, S., Dewitz, J., Homer, C., Yang, L., Barnes, C., Herold, N., and Wickham, J., 2011. Completion of the 2006 National Land Cover Database for the Conterminous United States, *PE&RS*, Vol. 77(9):858-864.

NCRWQCB, 2007. Water Quality Control Plan for the North Coast Region. North Coast Regional Water Quality Control Board. Santa Rosa, CA.

PRMD, 2010. Sonoma County General Plan. Permit and Resource Management Department, Sonoma County, Santa Rosa.CA. http://www.sonoma-county.org/prmd/docs/gp/98gp-02.htm.

Shilling, F.M., Viers, J.H. and M.L. Johnson. 2009. Russian River Pathogen TMDL Monitoring Design: A Technical Report to the North Coast Regional Water Quality Control Board. Project Report 06-428-110 dated September 2009. Aquatic Ecosystems Analysis Laboratory, U.C. Davis.

State Water Resources Control Board (SWRCB), 2004. Water Quality Control Policy for Developing California's Clean Water Act Section 303(d) List. State Water Resources Control Board, Sacramento, CA.

SWAMP 2007. Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (SWAMP). Marine Pollution Studies Laboratory – Department of Fish and Game (MPSL-DFG) Field Sampling Team. MPSL-DFG_FieldSOP_v1.0. dated 15 October 2007. Available at: http://swamp.mpsl.mlml.calstate.edu/wp-content/uploads/2009/04/swamp_sop_field_measures_water_sediment_collection_v1_0.pdf

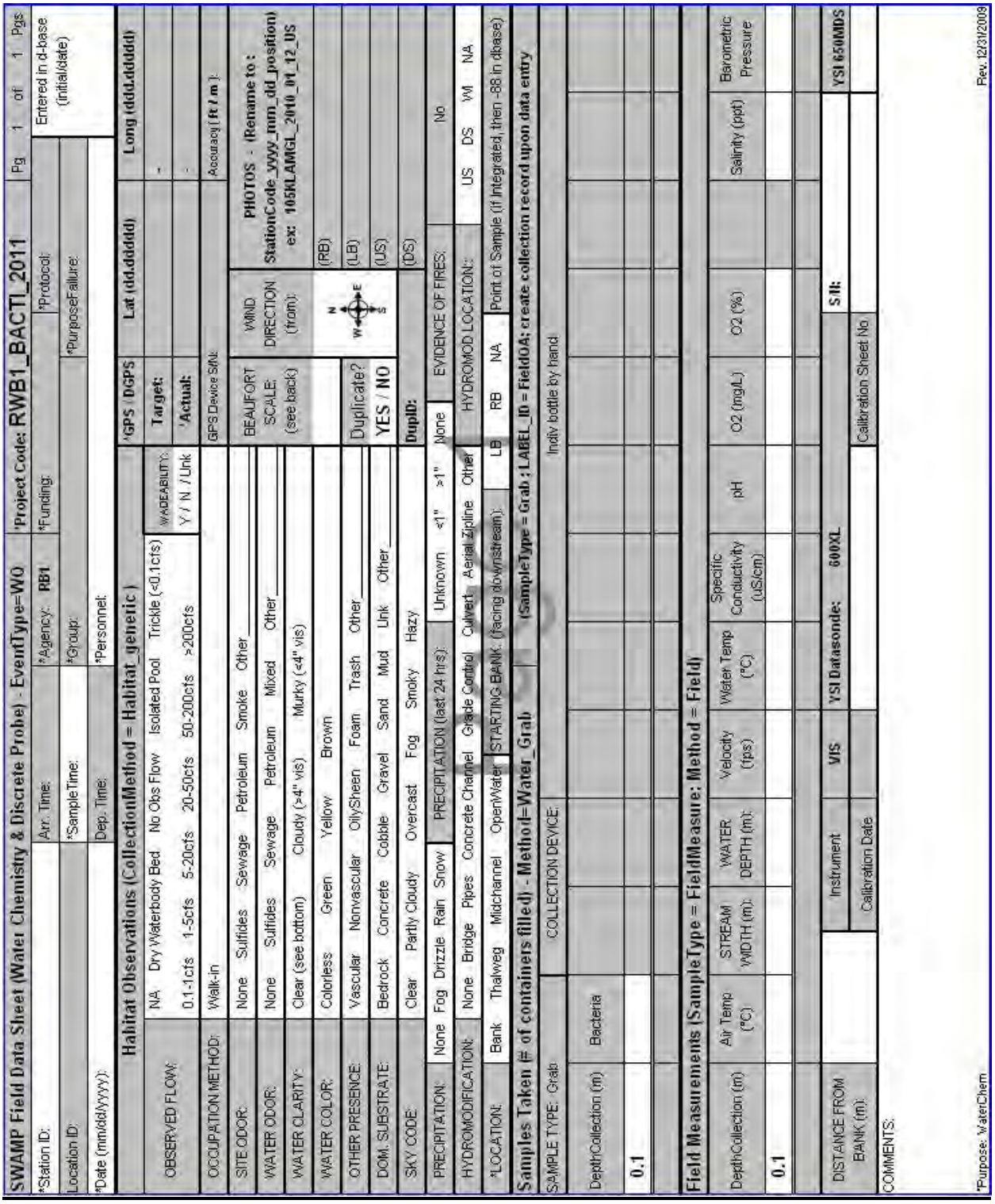
U.S. Environmental Protection Agency. (USEPA) 1986. Bacteriological Ambient Water Quality Criteria for Marine and Fresh Recreational Waters. EPA440/5-84-002. Washington, D.C.

U.S. Environmental Protection Agency. (USEPA) 1992. NPDES Stormwater Sampling Guidance Document. EPA 833-B-92-001. Washington, D.C.

Viers, J.H.. Shilling, F.M., Johnson, M.L., Bowen, L. Hutchinson, R.A., Calanchini, H., Wehrman, A. and H. Schott. 2009. Russian River Pathogen TMDL Monitoring Design: A Summary Report to the North Coast Regional Water Quality Control Board. Project Report 06-428-110 dated September 2009. Aquatic Ecosystems Analysis Laboratory, U.C. Davis.

Whitman, R.L., D.A. Shively, H. Pawlik, M.B. Nevers, and M.N. Byappanahalli. 2003. Occurrence of Escherichia coli and Enterococci in Cladophora (Chlorophyta) in nearshore water and beach sand of Lake Michigan. Appl. Environ. Microbiol., 69(8): 4714-4719.

Appendix 2: Field Data Sheet



SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ | *Project Code: RWB1_BACTI_2011 | Pg 1 of 1 Pgs

*Station ID: | Arr. Time: | *Agency: RB1 | *Funding: | *Protocol: | Entered in d-base (initial/date)

Location ID: | *SampleTime: | *Group: | *PurposeFailure:

*Date (mm/dd/yyyy): | Dep. Time: | *Personnel:

Habitat Observations (CollectionMethod = Habitat_genetic) | WADEABILITY: Y / N / Unk | 'GPS / DGPS | Lat (dd.ddddd) | Long (ddd.ddddd)

OBSERVED FLOW: NA Dry Waterbody Bed No Obs Flow Isolated Pool Trickle (<0.1cfs) / 0.1-1cfs 1-5cfs 5-20cfs 20-50cfs 50-200cfs >200cfs | Target: | - | -

OCCUPATION METHOD: Walk-in | 'Actual: | GPS Device S/N: | Accuracy (ft / m):

SITE ODOR: None Sulfides Sewage Petroleum Smoke Other | BEAUFORT SCALE: (see back) | VMND DIRECTION (from): | PHOTOS - (Rename to : StationCode_yyyy_mm_dd_position) ex: 105KLAMGL_2010_01_12_US

WATER ODOR: None Sulfides Sewage Petroleum Mixed Other | (RB)

WATER CLARITY: Clear (see bottom) Cloudy (>4" vis) Murky (<4" vis) | Duplicate? | (LB)

WATER COLOR: Colorless Green Yellow Brown | YES / NO | (US)

OTHER PRESENCE: Vascular Nonvascular OilySheen Foam Trash Other | DupID: | (DS)

DOM. SUBSTRATE: Bedrock Concrete Cobble Gravel Sand Mud Unk Other

SKY CODE: Clear Partly Cloudy Overcast Fog Smoky Hazy

PRECIPITATION: None Fog Drizzle Rain Snow | PRECIPITATION (last 24 hrs): | Unknown <1" >1" | None | EVIDENCE OF FIRES: | No

HYDROMODIFICATION: None Bridge Pipes Concrete Channel Grade Control Culvert Aerial Zipline Other | HYDROMOD LOCATION: | US DS WM NA

*LOCATION: Bank Thalweg Midchannel OpenWater | STARTING BANK: (facing downstream): | LB RB NA | Point of Sample (if Integrated, then -88 in dbase)

Samples Taken (# of containers filled) - Method=Water_Grab | (SampleType = Grab ; LABEL_ID = FieldOA; create collection record upon data entry

SAMPLE TYPE: Grab | COLLECTION DEVICE: | Indiv bottle by hand

DepthCollection (m)	Bacteria																		
0.1																			

Field Measurements (SampleType = FieldMeasure; Method = Field)

DepthCollection (m)	Air Temp (°C)	STREAM WIDTH (m):	WATER DEPTH (m):	Velocity (fps)	Water Temp (°C)	Specific Conductivity (uS/cm)	pH	O2 (mg/L)	O2 (%)		Salinity (ppt)	Barometric Pressure
0.1												

DISTANCE FROM BANK (m): | Instrument | V/S | YSI Datasonde: 600XL | S/N: | YSI 650MDS

Calibration Date | Calibration Sheet No.

COMMENTS:

*Purpose: WaterChem | Rev. 12/31/2009

Appendix 3: Chain of Custody Forms

82 Mary Street Suite #2
San Francisco, CA 94103
Tel: (415) 882-1690 ; Fax: (415) 882-1685

cel analytical, inc.
water, wastewater & sludge Microbiology

Types of Water Matrices: Drinking water (DW)____; Wastewater(WW)___, Recreational water (RW)
Source(S)_____
Routine(R)___Repeat(R2)___

Custody and Sample Information - Print ALL information. Put N/A (not applicable) in blanks.

Lab ID#:

Custody and Sample Information - Print ALL information. Put N/A in blanks not applicable.

Report to: | **Bill to:** | **Project #:** | **Client:** | **Turn around Time:**

Sampled by (signature): | **# samples** | **Date:** | **Indicate Analysis Needed**

Work Activitiy:

Item No.	Sample Number	Station Location / Description / Name	MATRIX: Water (SpecifyType)	Sludge	Other	Date	Preservatives: Yes	No	chlorinated							
1																
2																
3																
4																
5																
6																
7																
8																
9																
10																

Released By: (Signature)	Date / Time Released:	Received By: (Signature)	Shipped Via:
			Sample condition: Accepted Rejected

Instructions/Comments::

BASIC LABORATORY CHAIN OF CUSTODY RECORD

LAB #:

CLIENT NAME:	PROJECT NAME:	PROJECT #:	PAGE_____OF_____
MAILING ADDRESS:	REPORT DUE DATE:	TURN AROUND TIME: Standard Rush	# OF SAMPLES:
	ANALYSIS REQUESTED		MATRIX / TYPE:
PROJECT MANAGER:			CUSTODY SEAL INTACT? Yes No N/A
PHONE: EMAIL:			SYSTEM #:
FAX: RESULTS SENT: Email Fax EDD Mail			EDD TYPE:
INVOICE TO: PO#:			QC: Standard Level II

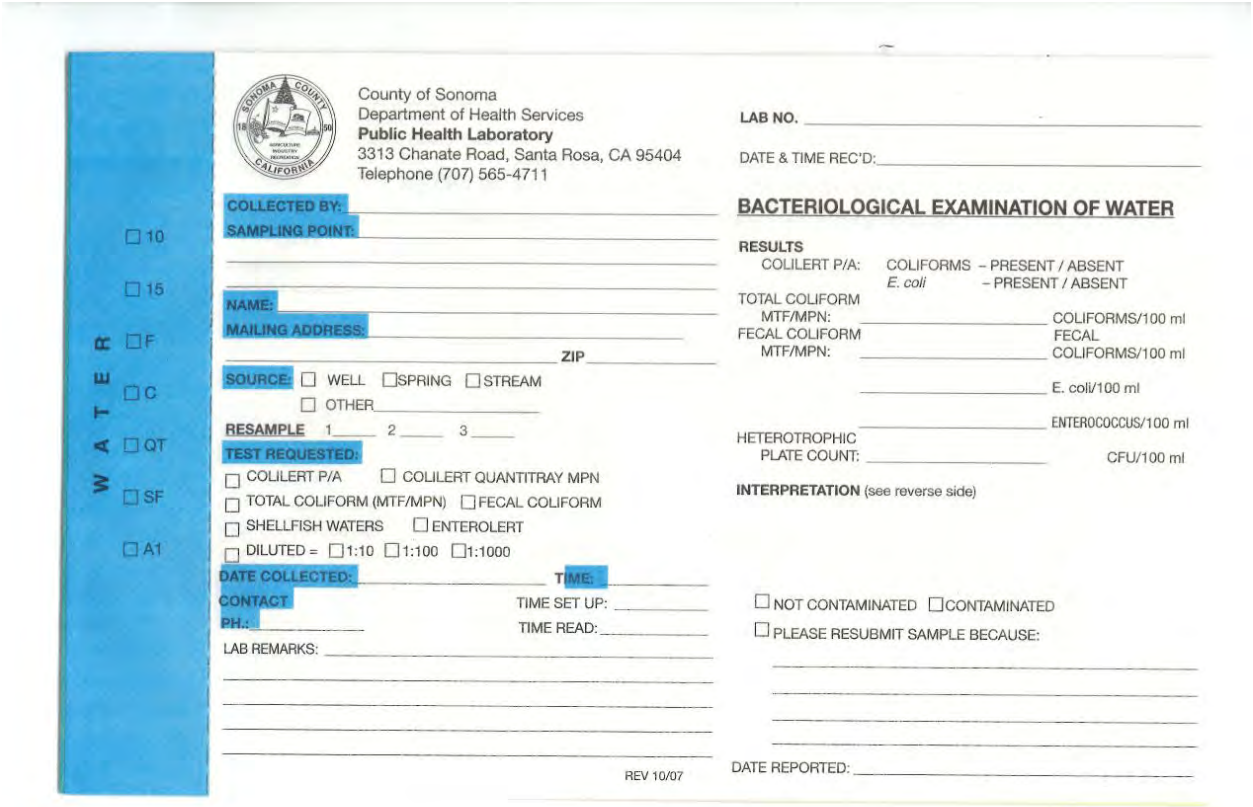
SAMPLE DATE	SAMPLE TIME	WATER	COMP	SOLID	SAMPLE LOCATION / IDENTIFICATION	NUMBER OF BOTTLES										LAB ID	CHLORINE RESIDUAL OR COMMENTS

PRESERVED WITH: HNO_3 H_2SO_4 NaOH ZnAce/NaOH HCL NaThio OTHER_____

SAMPLED BY (PRINT):	SAMPLE DATE/TIME:	RELINQUISHED BY:	DATE/TIME:
RECEIVED BY:	DATE/TIME:	RELINQUISHED BY:	DATE/TIME:
RECEIVED BY (LAB):	DATE/TIME:	PROCESSED AND VERIFIED BY:	DATE/TIME:
LOGGED IN BY:	DATE/TIME:	CARRIER: _____ COOLER TEMPERATURE: _____°c	

INSTRUCTIONS, TERMS AND CONDITIONS ON BACK.

SAMPLE TYPE: 1 = ROUTINE, 2 = REPEAT, 3 = REPLACEMENT, 4 = SPECIAL 5 = RAW



WATER
☐ 10
☐ 15
☐ F
☐ C
☐ QT
☐ SF
☐ A1

County of Sonoma
Department of Health Services
Public Health Laboratory
3313 Chanate Road, Santa Rosa, CA 95404
Telephone (707) 565-4711

LAB NO. _____

DATE & TIME REC'D: _____

BACTERIOLOGICAL EXAMINATION OF WATER

COLLECTED BY: _____
SAMPLING POINT: _____
_____
_____
NAME: _____
MAILING ADDRESS: _____
_____ ZIP _____
SOURCE: ☐ WELL ☐ SPRING ☐ STREAM
☐ OTHER _____
RESAMPLE 1____ 2____ 3____
TEST REQUESTED:
☐ COLILERT P/A ☐ COLILERT QUANTITRAY MPN
☐ TOTAL COLIFORM (MTF/MPN) ☐ FECAL COLIFORM
☐ SHELLFISH WATERS ☐ ENTEROLERT
☐ DILUTED = ☐ 1:10 ☐ 1:100 ☐ 1:1000
DATE COLLECTED: _____ TIME: _____
CONTACT
PH.: _____
TIME SET UP: _____
TIME READ: _____
LAB REMARKS: _____
_____
_____
_____
_____

REV 10/07

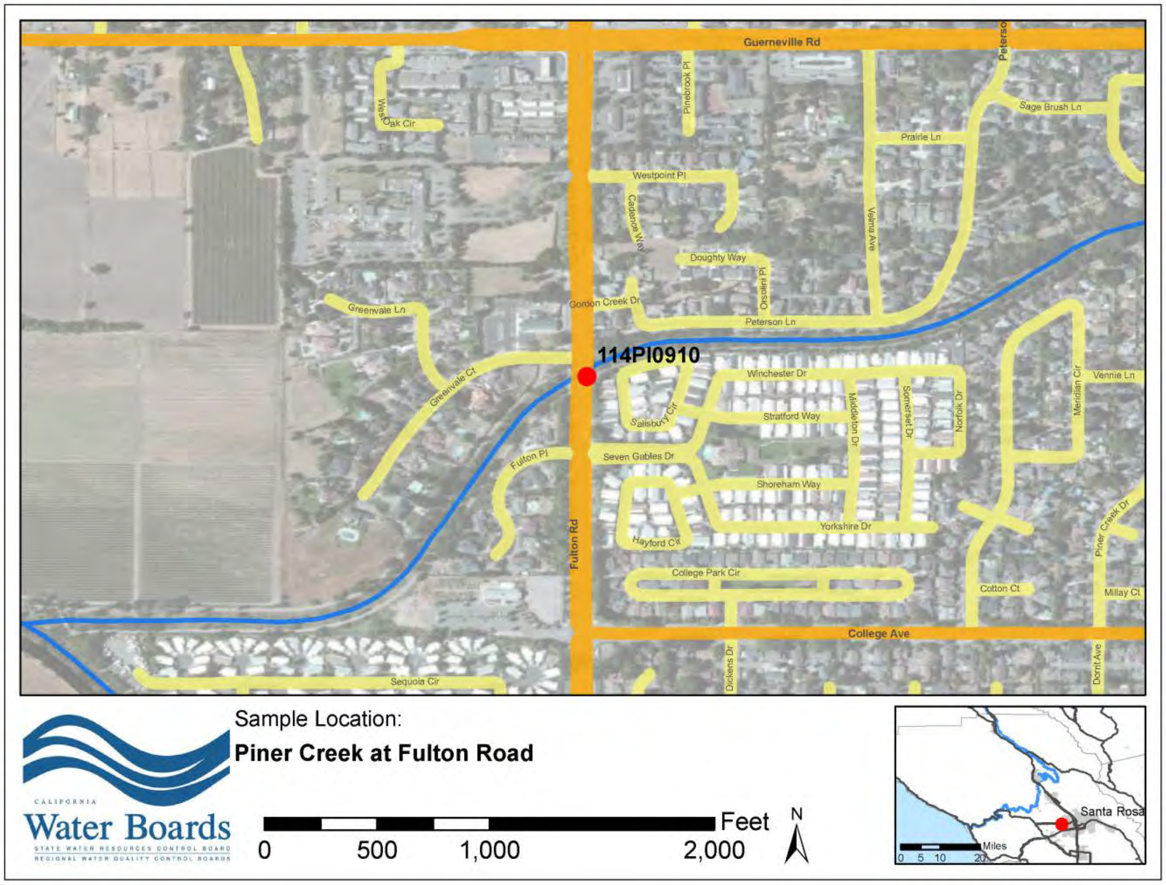
RESULTS
COLILERT P/A: COLIFORMS – PRESENT / ABSENT
E. coli – PRESENT / ABSENT
TOTAL COLIFORM
MTF/MPN: _____ COLIFORMS/100 ml
FECAL COLIFORM
MTF/MPN: _____ FECAL COLIFORMS/100 ml
_____ E. coli/100 ml
_____ ENTEROCOCCUS/100 ml
HETEROTROPHIC
PLATE COUNT: _____ CFU/100 ml

INTERPRETATION (see reverse side)

☐ NOT CONTAMINATED ☐ CONTAMINATED
☐ PLEASE RESUBMIT SAMPLE BECAUSE:
_____
_____
_____
_____

DATE REPORTED: _____

Appendix 4: Sampling Location Maps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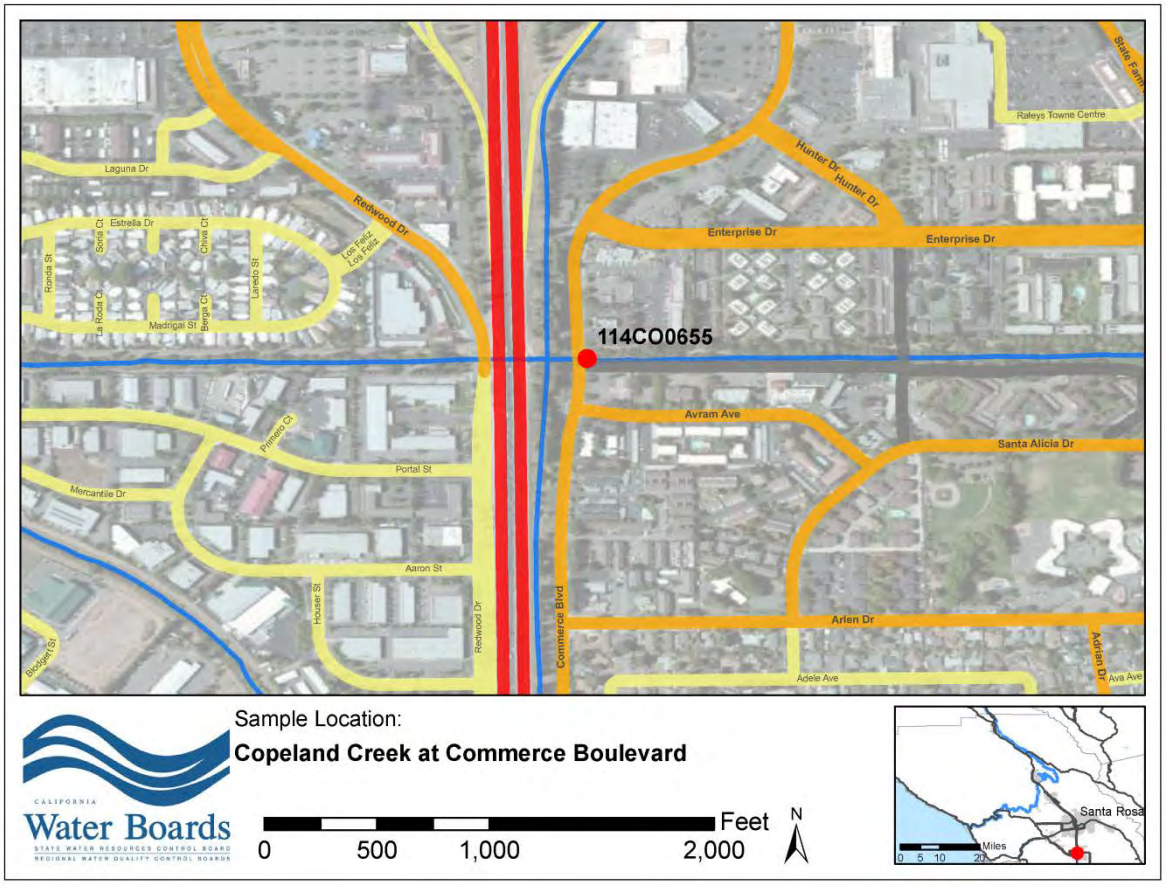
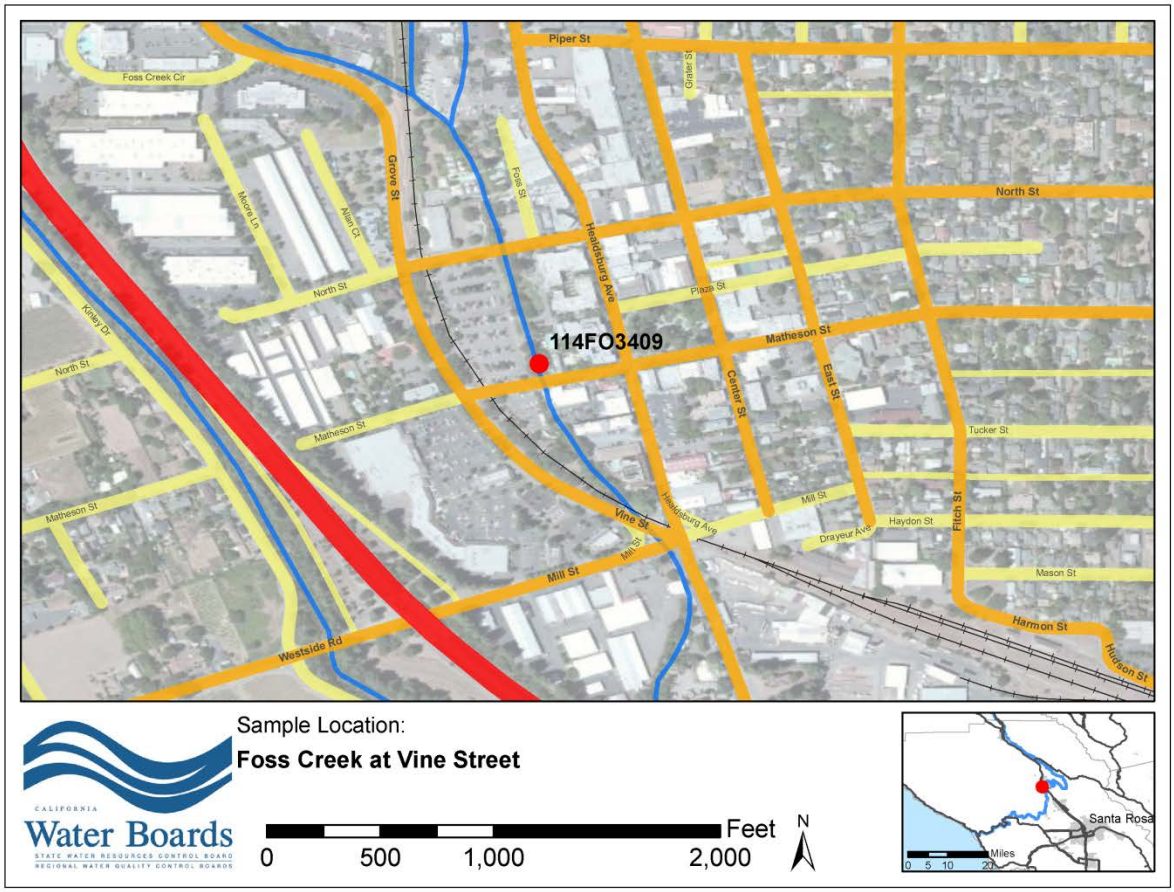
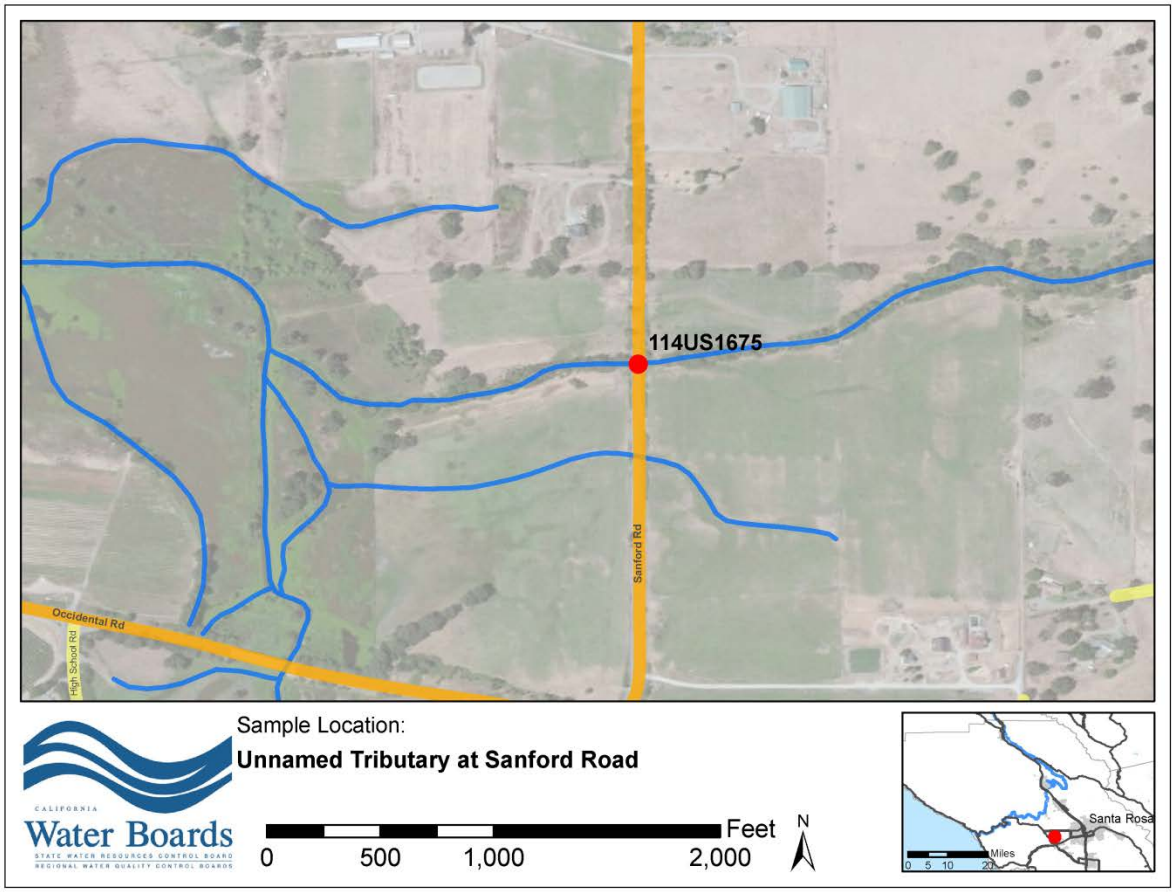
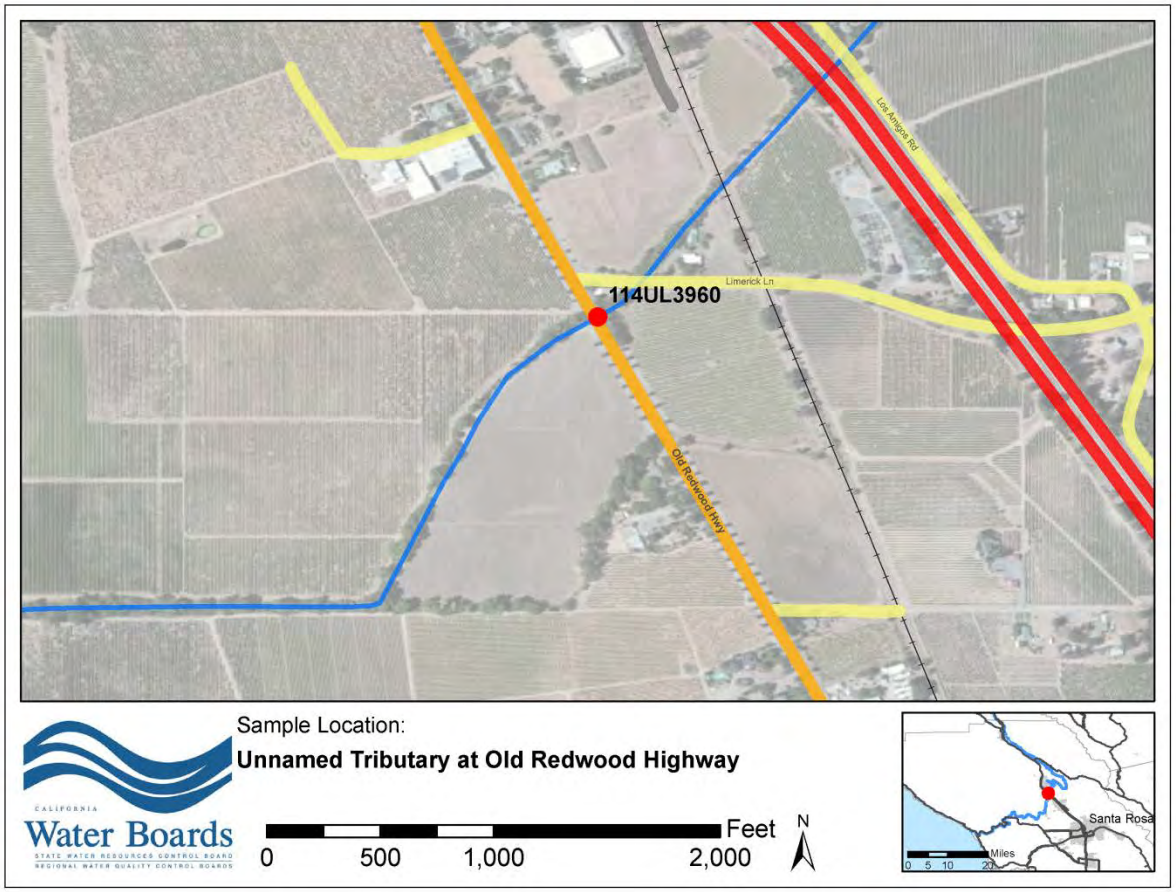
Fulton Rd
College Ave
114PI0910
Peterson Ln
Winchester Dr
Stratford Way
Seven Gables Dr
Shoreham Way
Yorkshire Dr
College Park Cir
Greenvale Ln
Greenvale Ct
Fulton Pl
Sequoia Cir
Sample Location:
Piner Creek at Fulton Road
Water Boards
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles


Laguna Dr
Redwood Dr
Estrella Dr
Sonia Ct
Olivet Ct
Los Feliz
Los Feliz
Ronda St
La Roda Ct
Madrigal St
Berga Ct
Laredo St
114CO0655
Enterprise Dr
Enterprise Dr
Hunter Dr
Hunter Dr
Raleys Towne Centre
State Farm
Avram Ave
Santa Alicia Dr
Primero Ct
Portal St
Mercantile Dr
Aaron St
Houser St
Redwood Dr
Commerce Blvd
Arlen Dr
Adele Ave
Adrian Dr
Ava Ave
Bodgan St
Sample Location:
Copeland Creek at Commerce Boulevard
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20


Piper St
Foss Creek Cir
Grove St
Foss St
Healdsburg Ave
North St
Plaza St
Matheson St
114FO3409
Center St
East St
Tucker St
Mill St
Vine St
Haydon St
Fitch St
Mason St
Harmon St
Hudson St
Westside Rd
Sample Location:
Foss Creek at Vine Street
Water Boards
0 500 1,000 2,000 Feet
N
Santa Rosa
Miles
0 5 10 20


114US1675
Occidental Rd
High School Rd
Sanford Rd
Sample Location:
Unnamed Tributary at Sanford Road
Water Boards
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20


Los Amigos Rd
Limerick Ln
114UL3960
Old Redwood Hwy
Sample Location:
Unnamed Tributary at Old Redwood Highway
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
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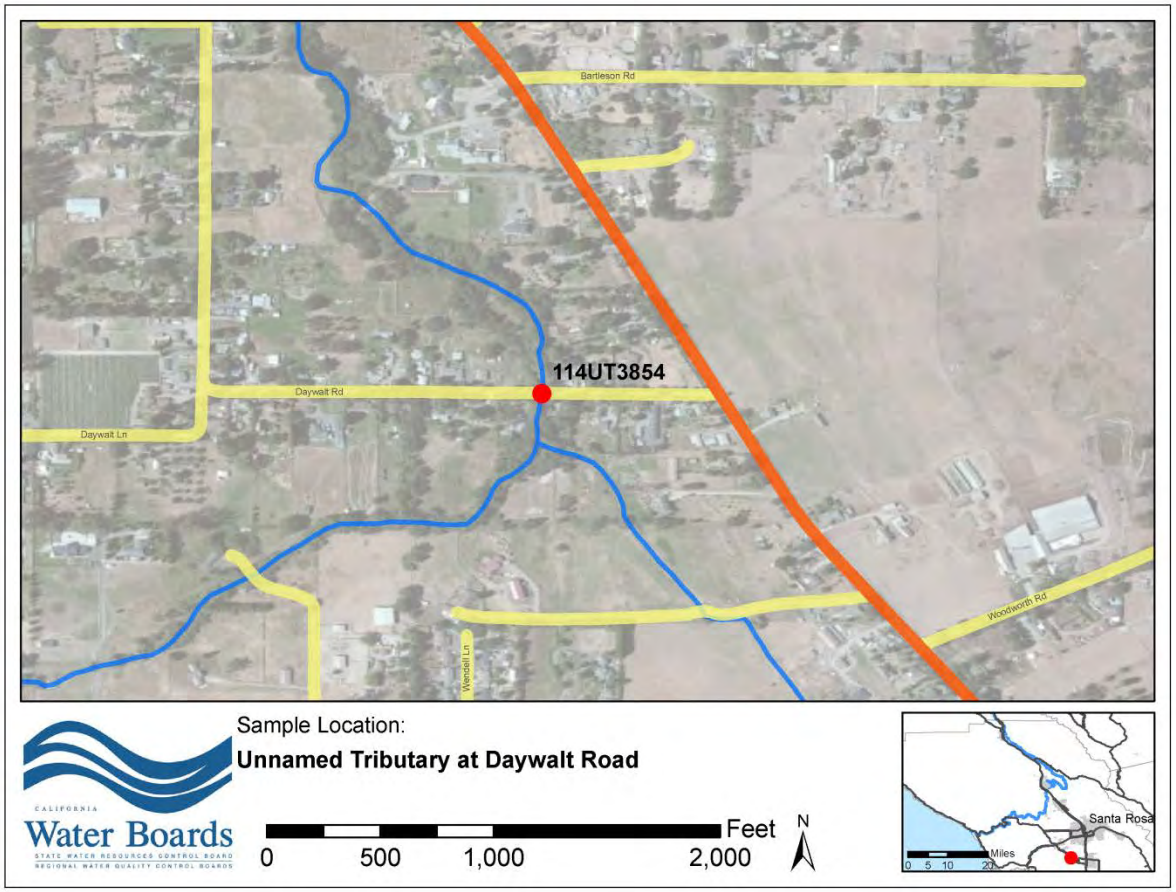
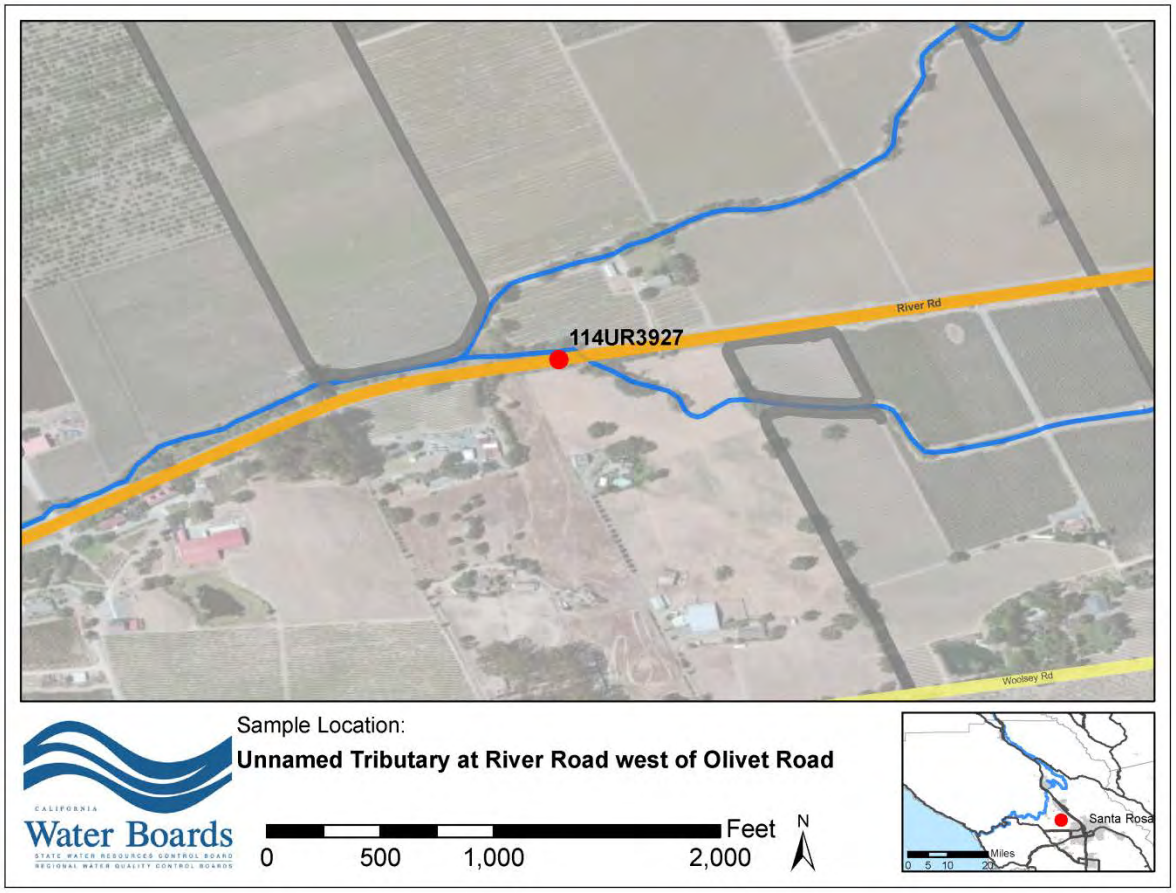
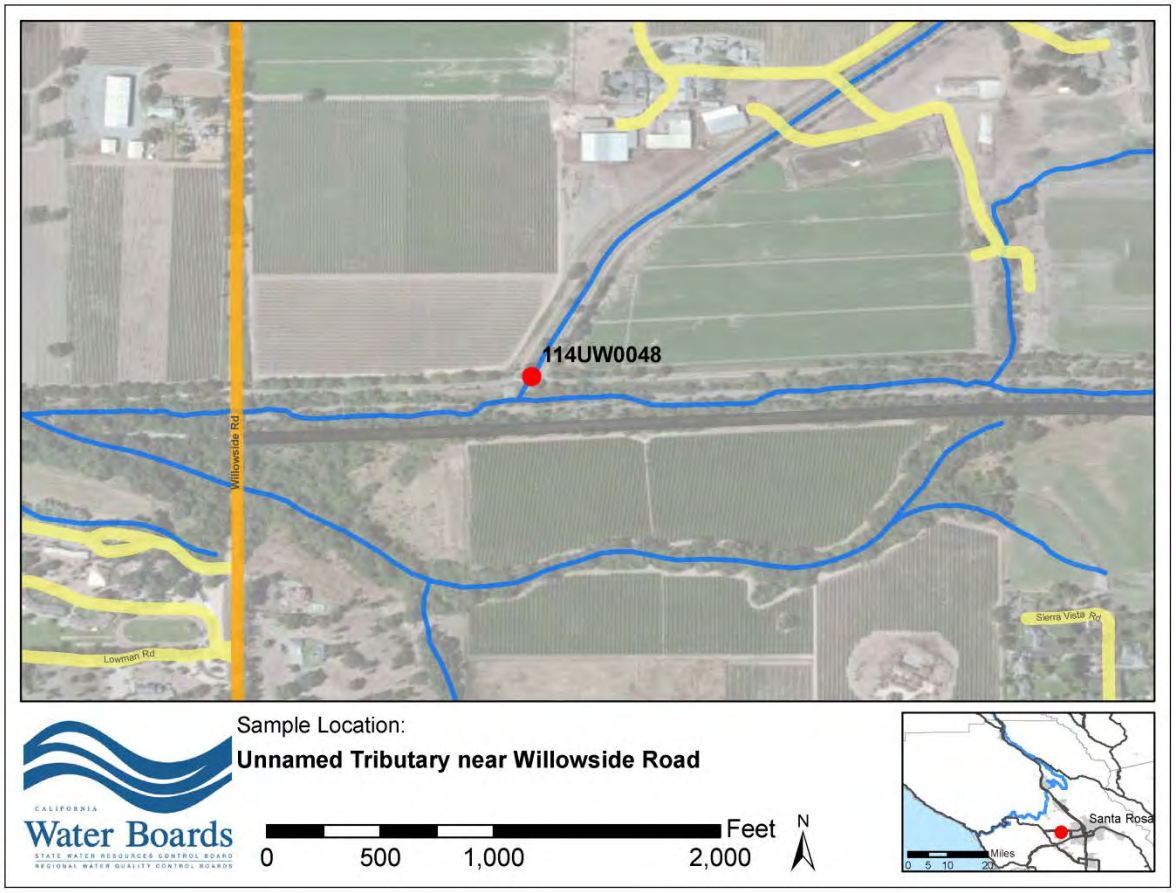
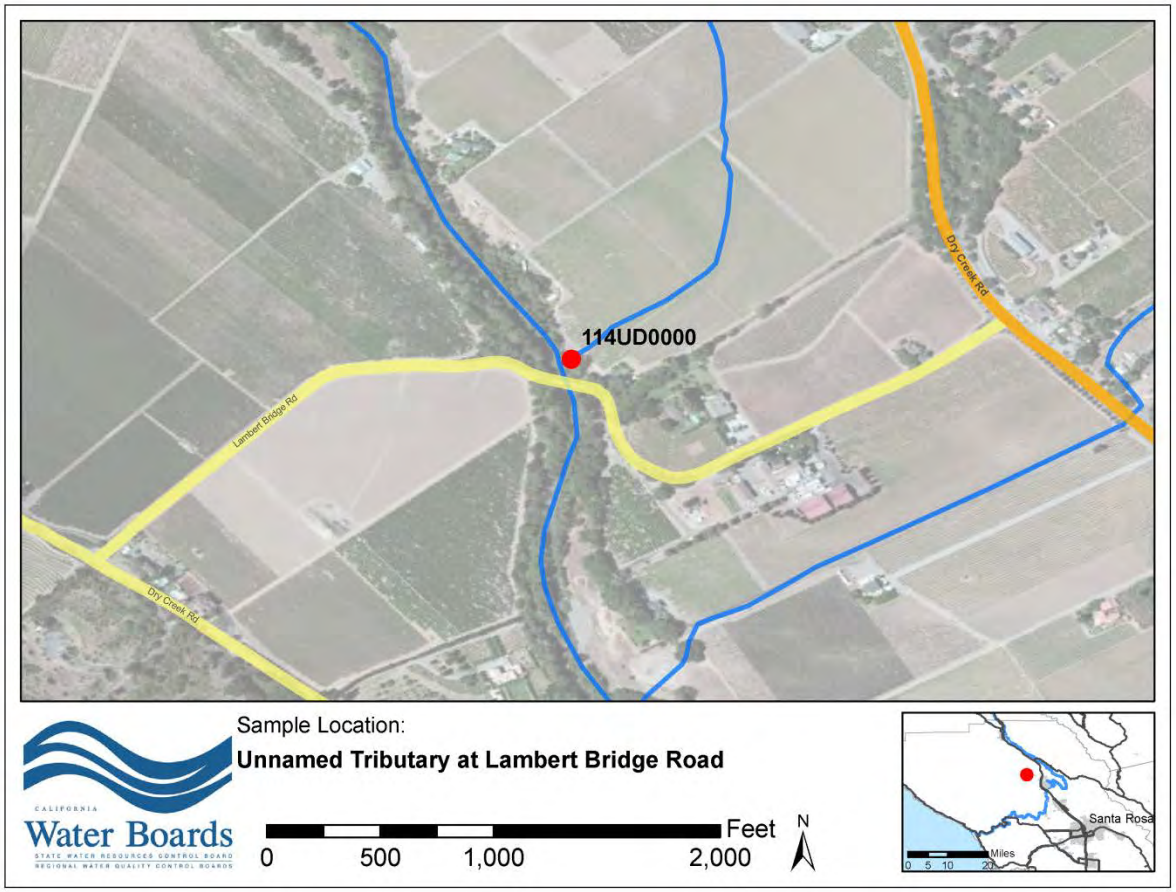
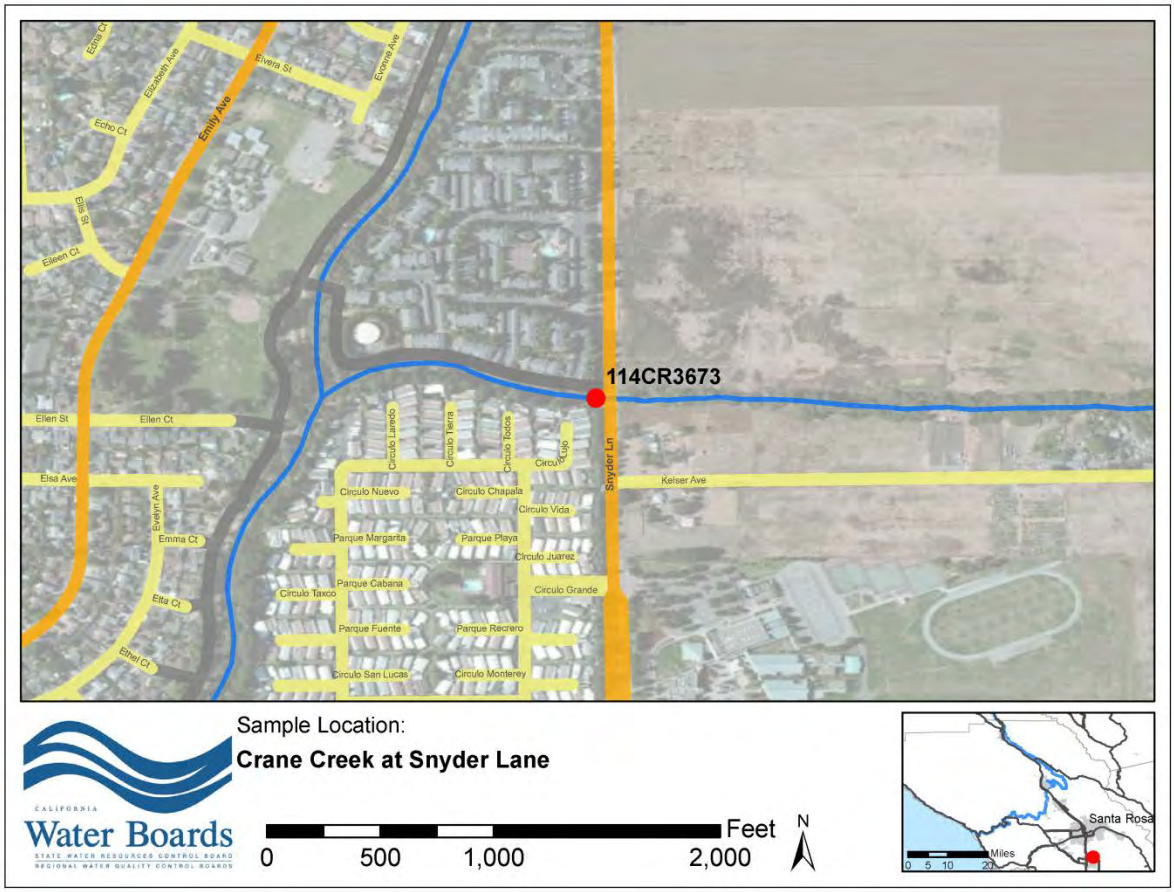
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

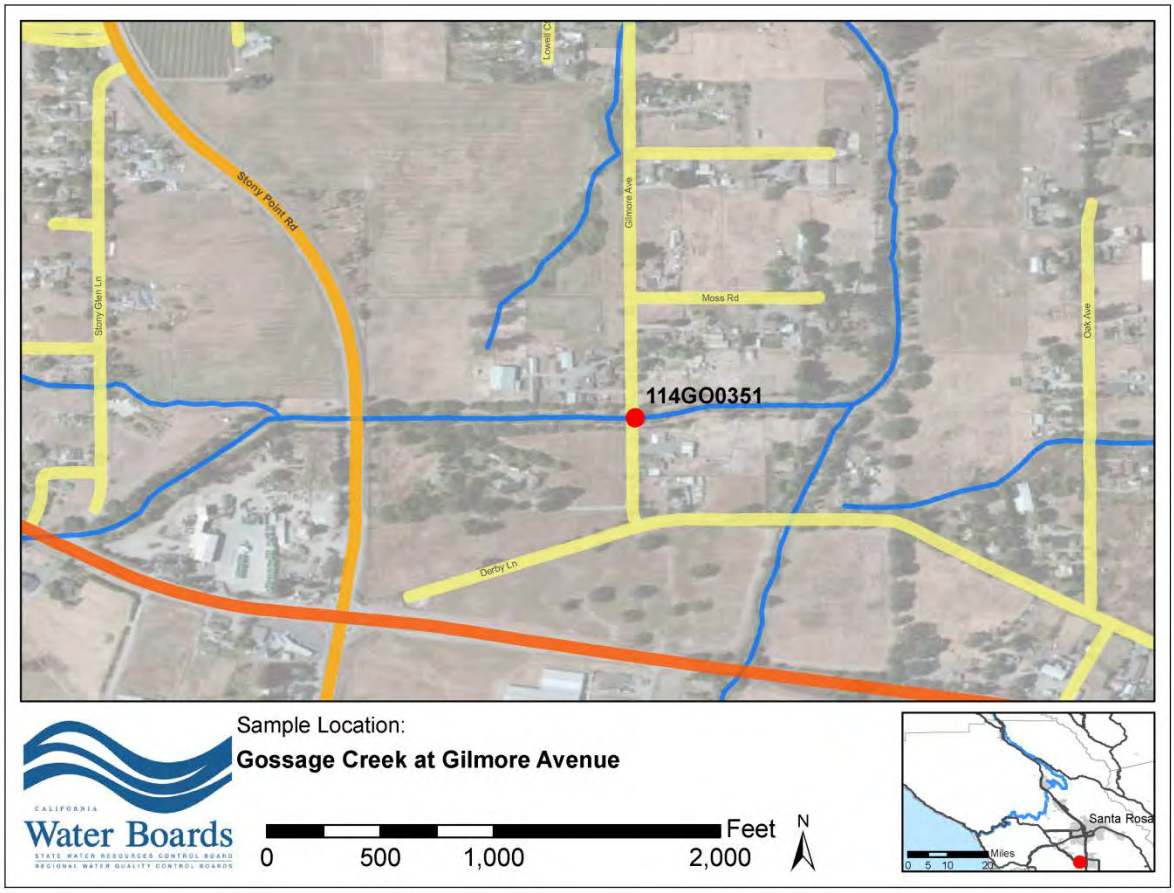

Bartleson Rd
114UT3854
Daywalt Rd
Daywalt Ln
Wendell Ln
Woodworth Rd
Sample Location:
Unnamed Tributary at Daywalt Road
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

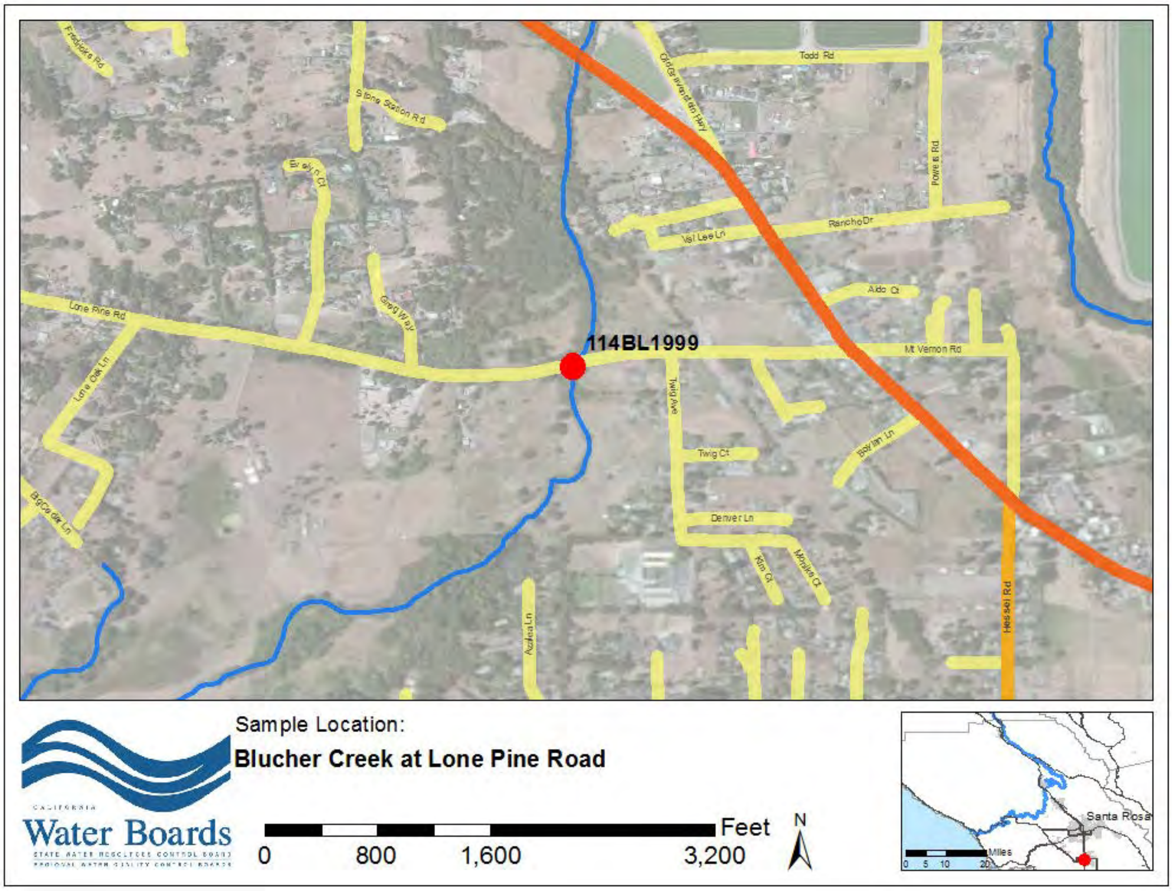

114UR3927
River Rd
Woolsey Rd
Sample Location:
Unnamed Tributary at River Road west of Olivet Road
Water Boards
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

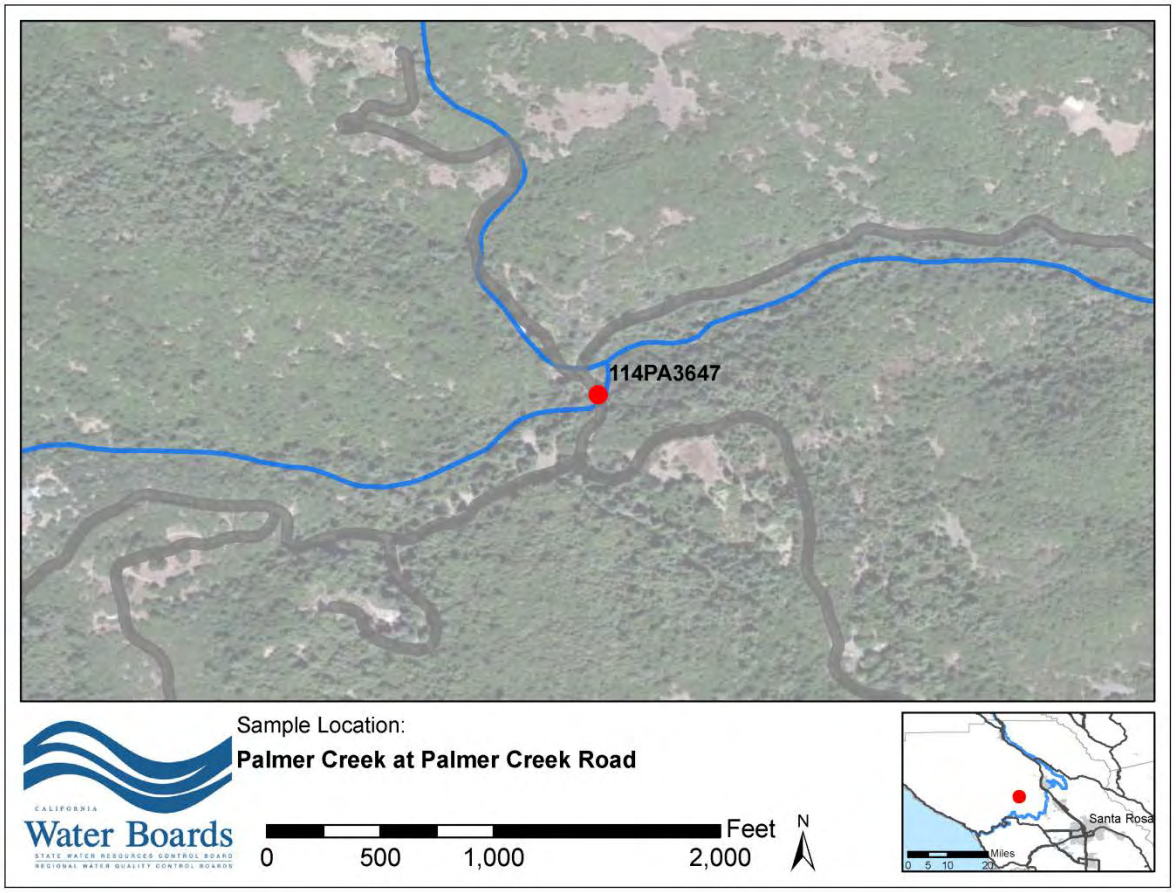

114UW0048
Willowside Rd
Lowman Rd
Sierra Vista Rd
Sample Location:
Unnamed Tributary near Willowside Road
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

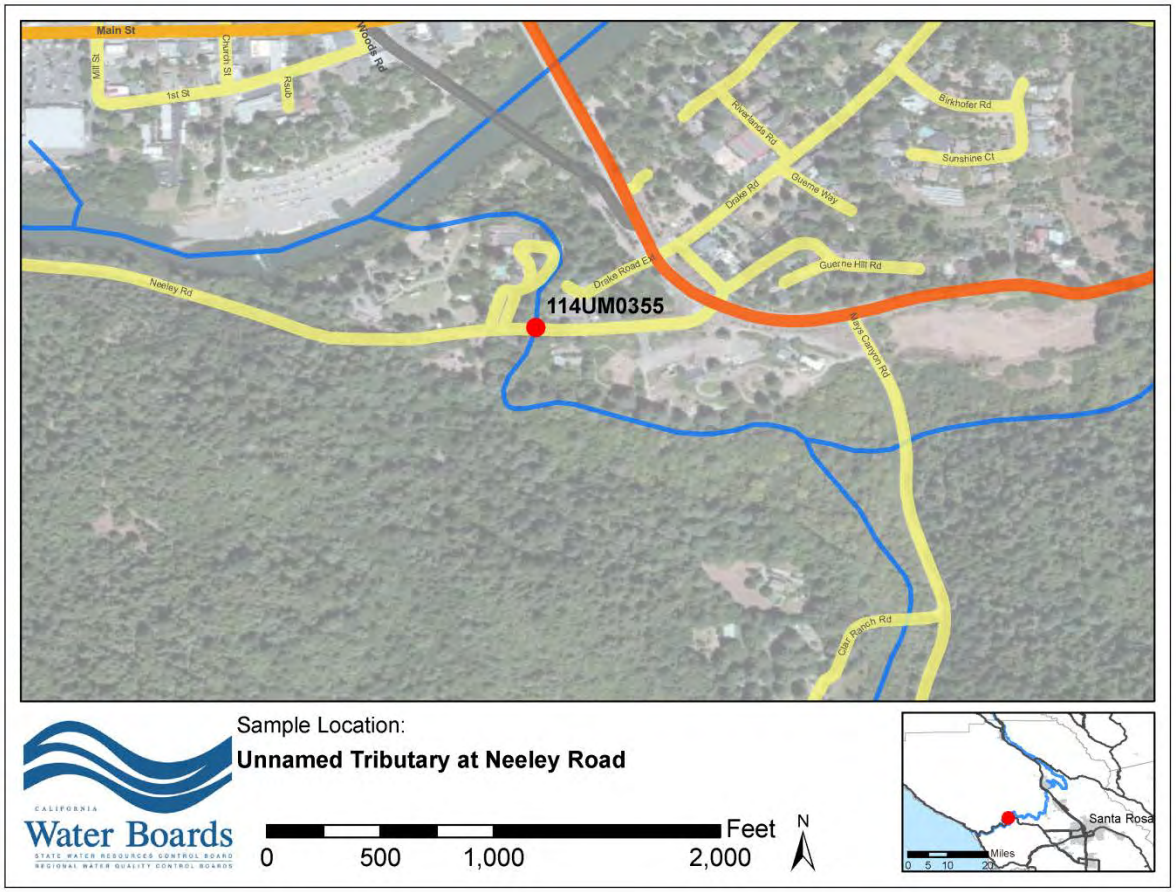

114UD0000
Lambert Bridge Rd
Dry Creek Rd
Dry Creek Rd
Sample Location:
Unnamed Tributary at Lambert Bridge Road
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

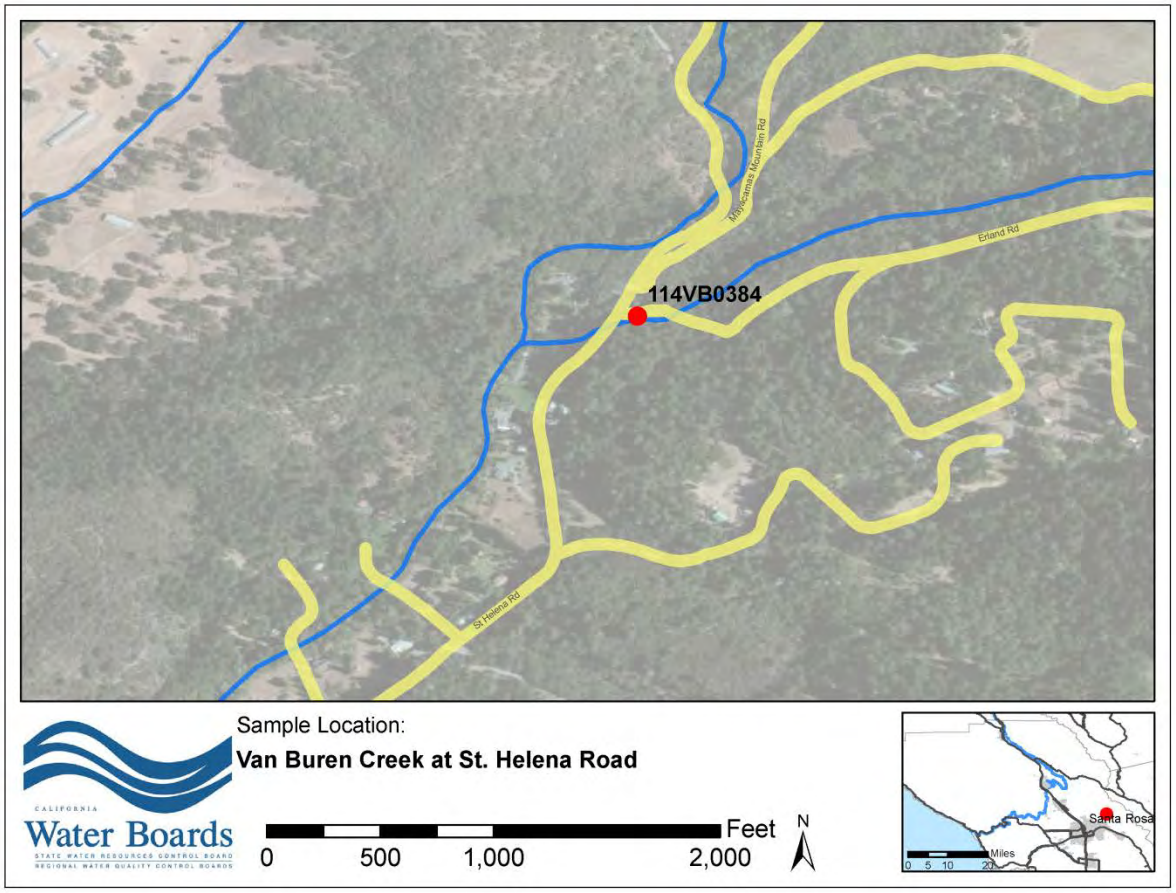

114CR3673
Sample Location:
Crane Creek at Snyder Lane
Water Boards
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

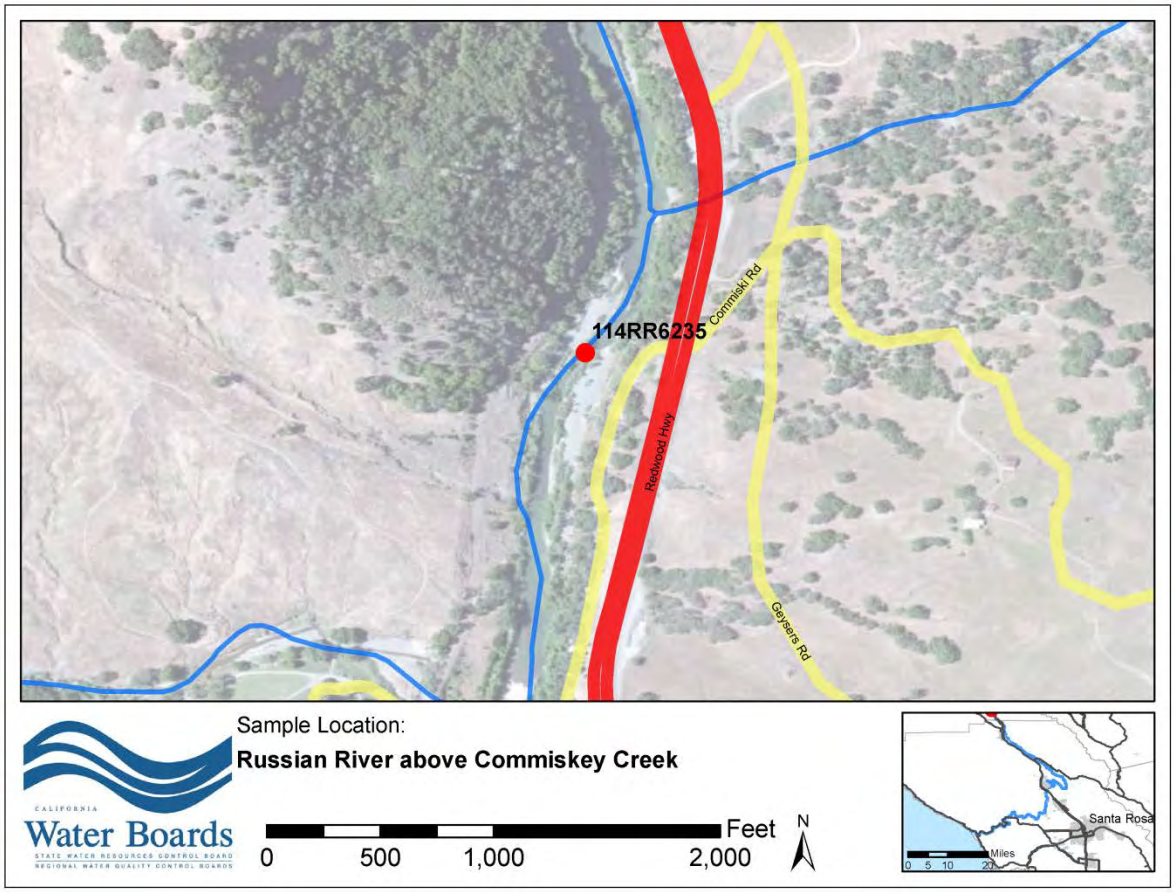

Stony Point Rd
Stony Glen Ln
Gilmore Ave
Moss Rd
Oak Ave
Derby Ln
114GO0351
Sample Location:
Gossage Creek at Gilmore Avenue
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

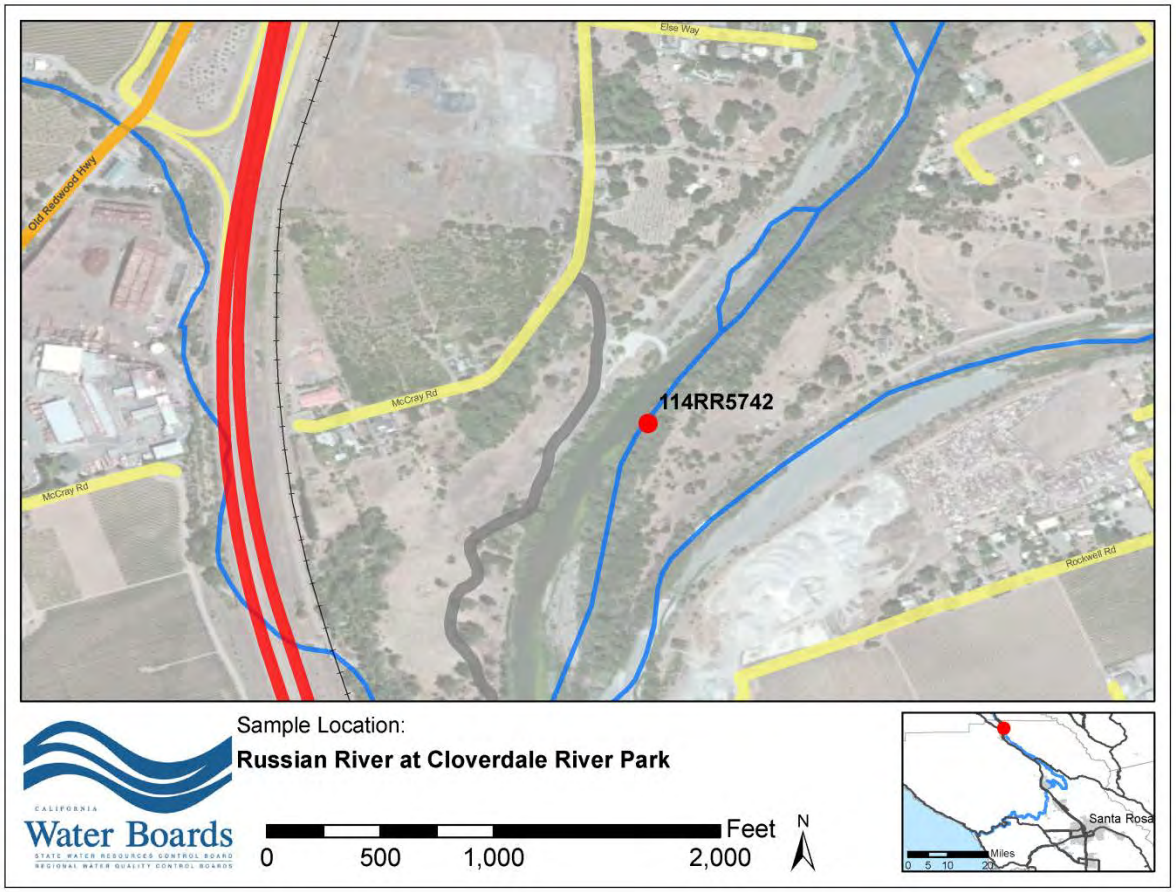

114BL1999
Todd Rd
Stone Station Rd
Lone Pine Rd
Rancho Dr
Val Lee Ln
Aldo Ct
Mt Vernon Rd
Twig Ave
Twig Ct
Denver Ln
Hessel Rd
Azalea Ln
Sample Location:
Blucher Creek at Lone Pine Road
Feet
0
800
1,600
3,200
N
Santa Rosa
Miles

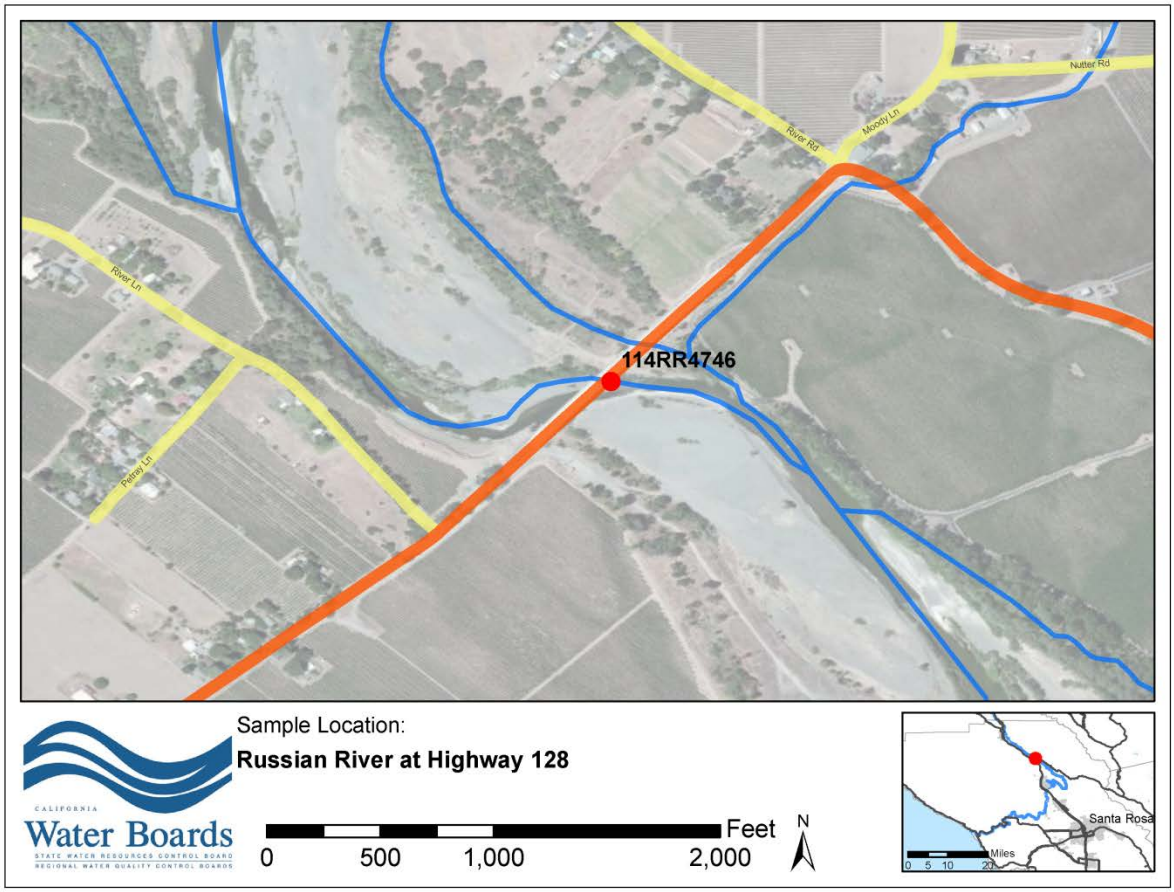

114PA3647
Sample Location:
Palmer Creek at Palmer Creek Road
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

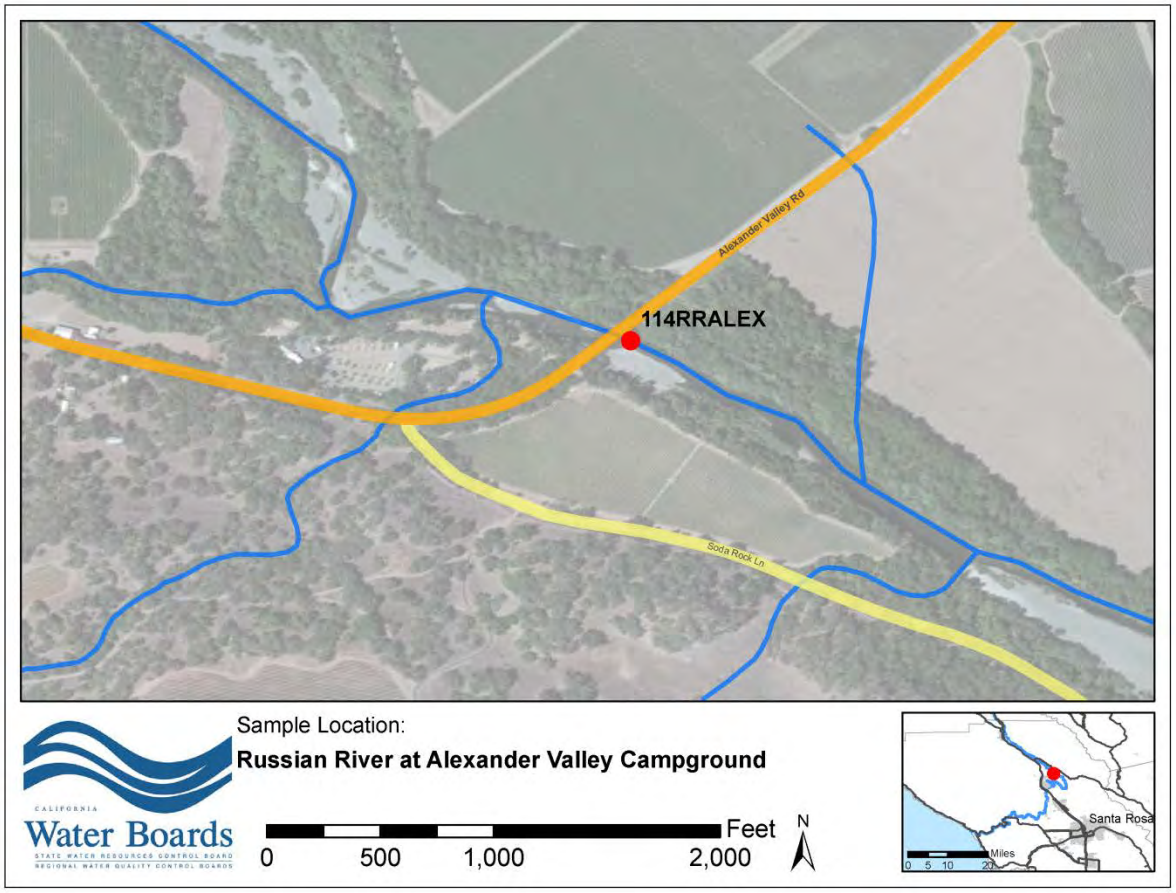

Main St
Mill St
Church St
1st St
Woods Rd
Riverlands Rd
Birkhofer Rd
Sunshine Ct
Drake Rd
Guerne Way
Drake Road E
Guerne Hill Rd
Neeley Rd
114UM0355
Mark Canyon Rd
Carr Ranch Rd
Sample Location:
Unnamed Tributary at Neeley Road
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0 500 1,000 2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

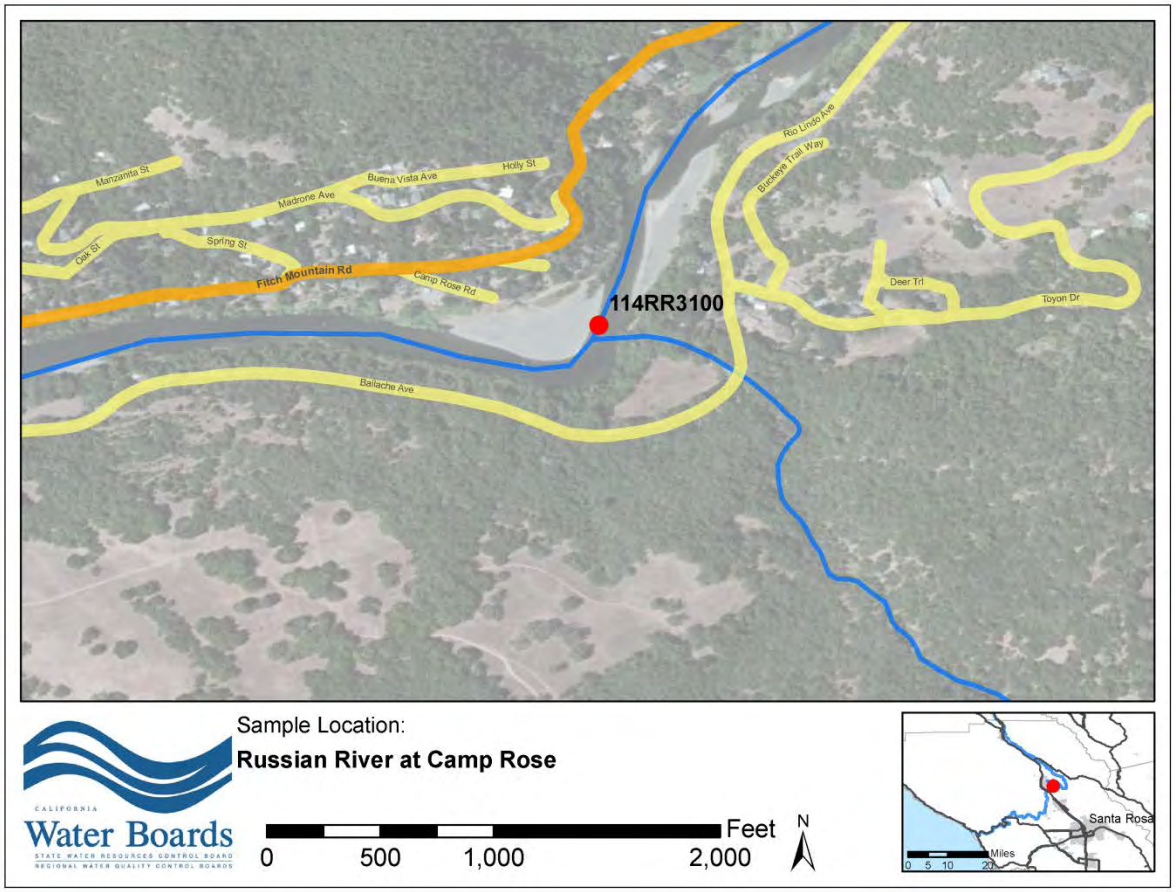

Mayacamas Mountain Rd
Erland Rd
114VB0384
St Helena Rd
Sample Location:
Van Buren Creek at St. Helena Road
California
Water Boards
State Water Resources Control Board
Regional Water Quality Control Boards
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

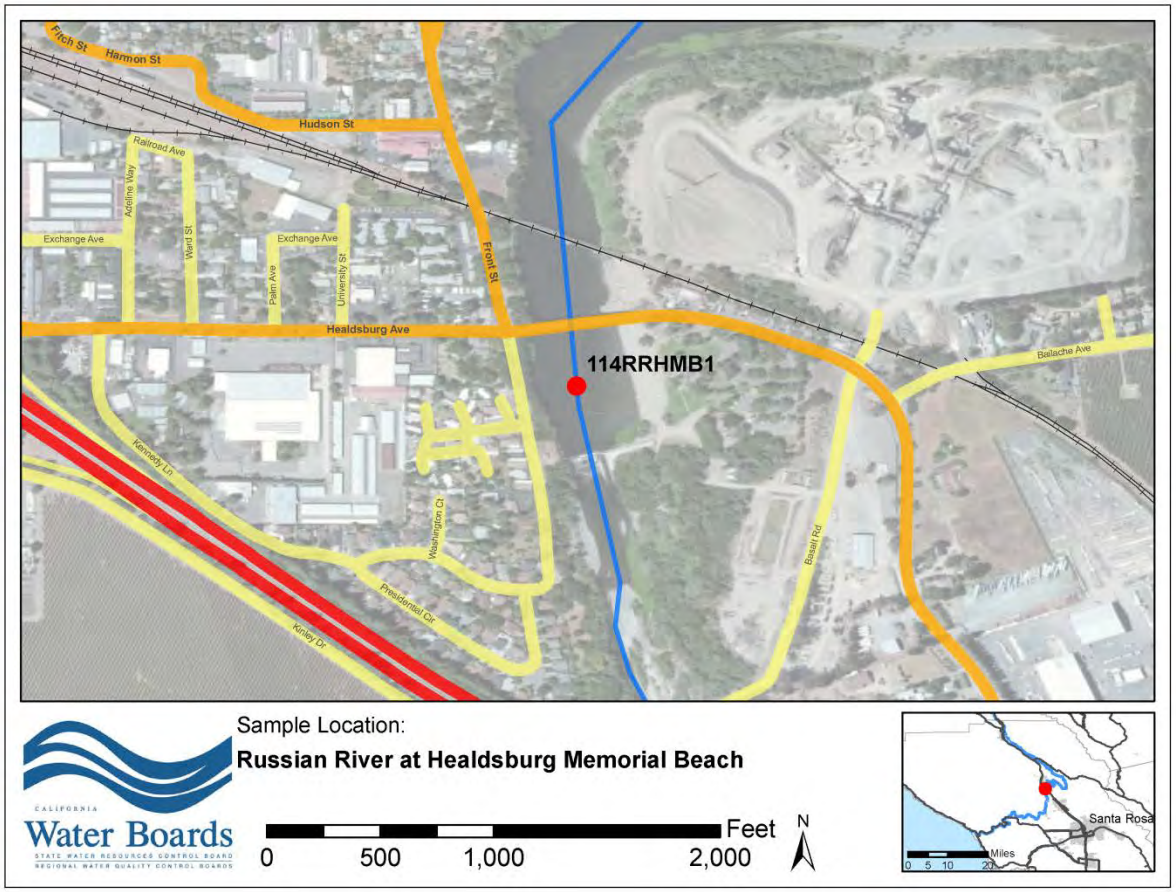

114RR6235
Commiski Rd
Redwood Hwy
Geysers Rd
Sample Location:
Russian River above Commiskey Creek
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

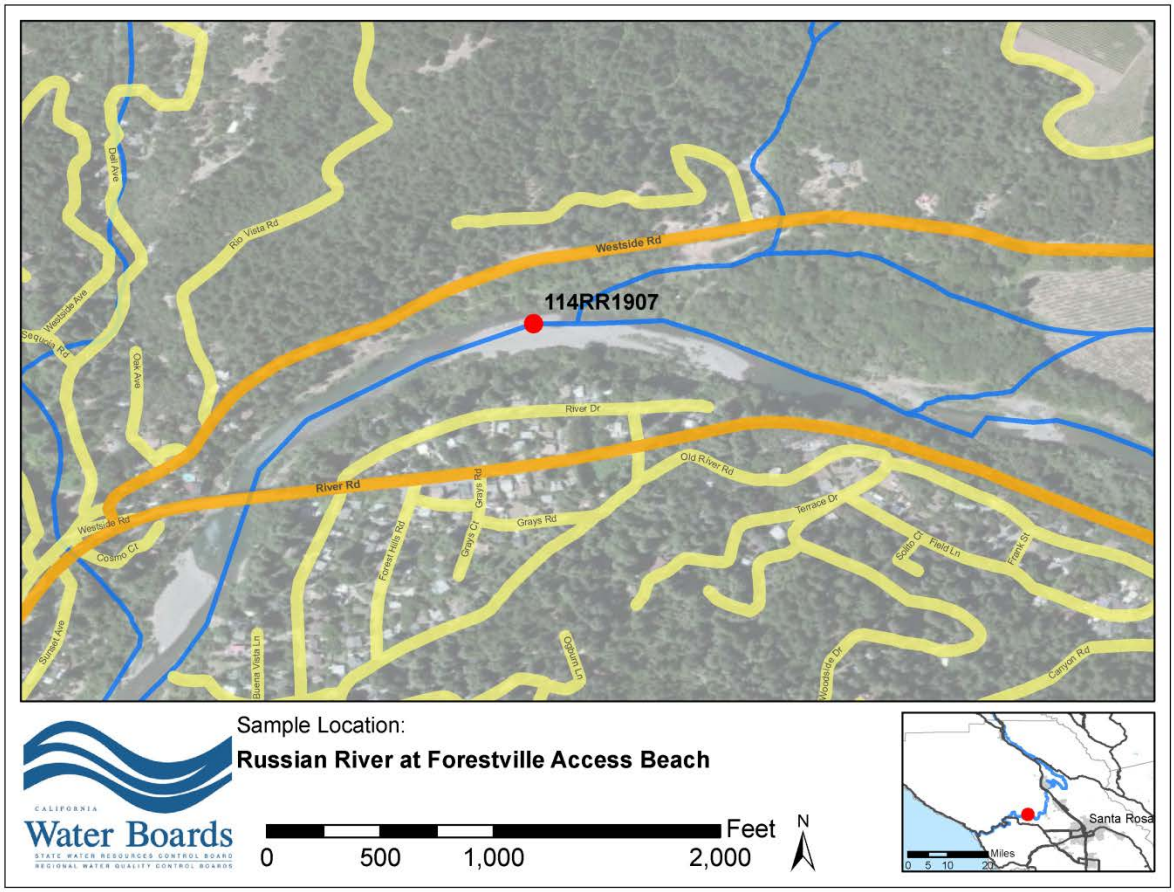

Else Way
Old Redwood Hwy
McCray Rd
McCray Rd
114RR5742
Rockwell Rd
Sample Location:
Russian River at Cloverdale River Park
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

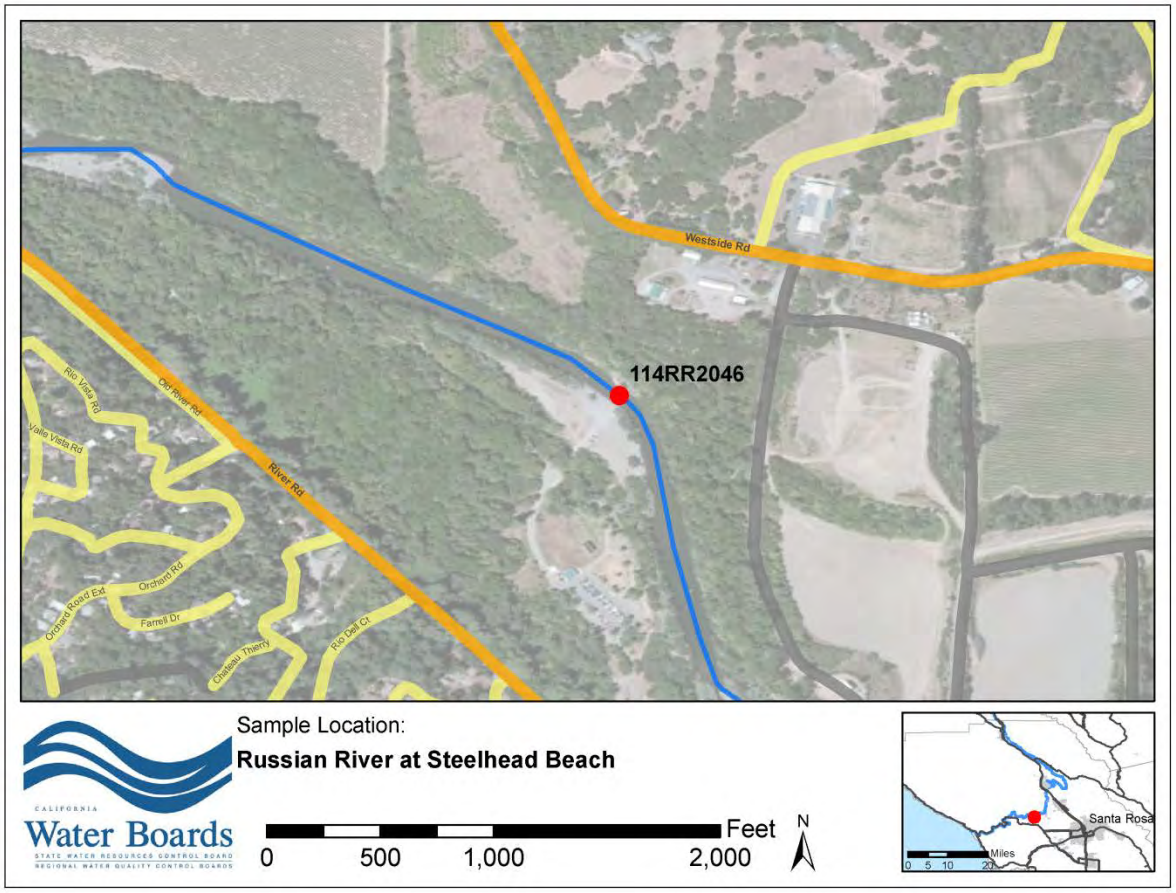

Nutter Rd
River Rd
Moody Ln
River Ln
Petray Ln
114RR4746
Sample Location:
Russian River at Highway 128
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

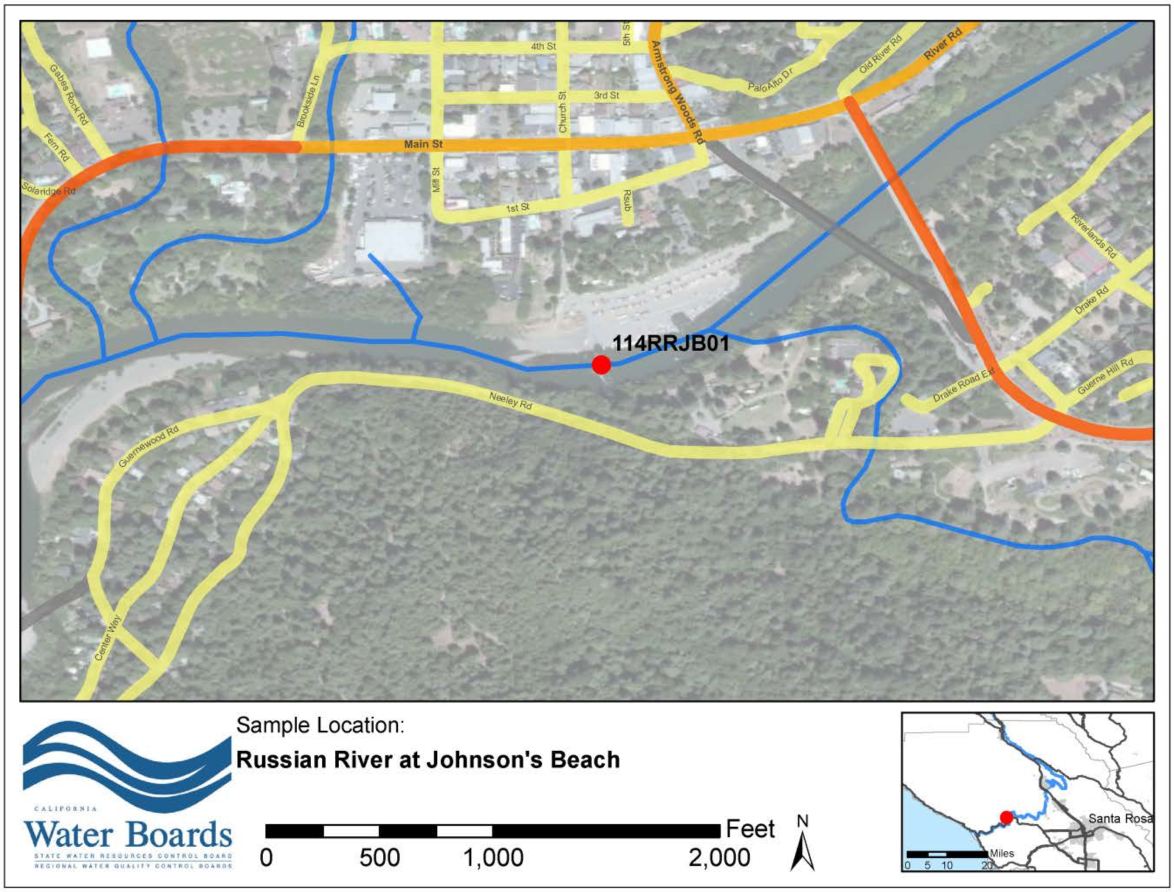

Alexander Valley Rd
114RRALEX
Soda Rock Ln
Sample Location:
Russian River at Alexander Valley Campground
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

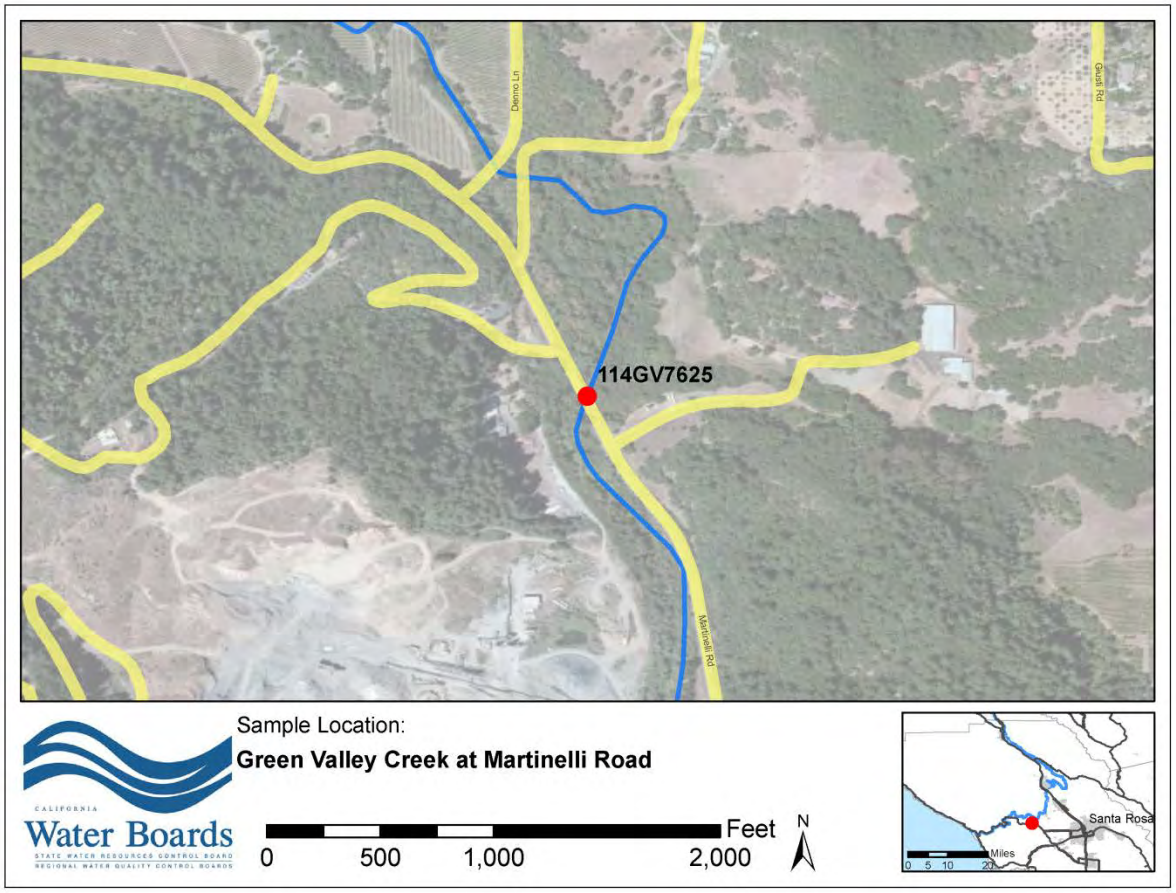

Manzanita St
Madrone Ave
Buena Vista Ave
Holly St
Oak St
Spring St
Fitch Mountain Rd
Camp Rose Rd
Rio Lindo Ave
Buckeye Trail Way
Deer Trl
Toyon Dr
Bailache Ave
114RR3100
Sample Location:
Russian River at Camp Rose
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0 500 1,000 2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

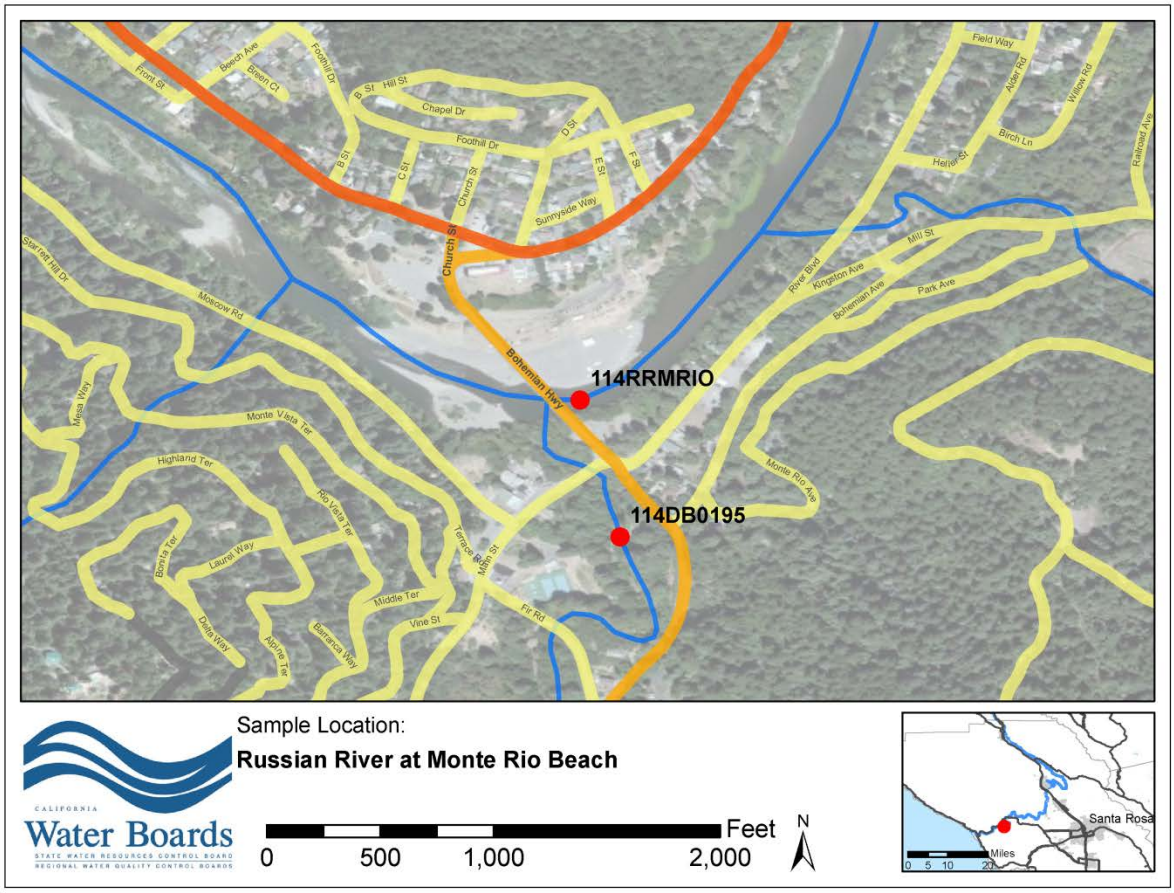

Fitch St
Harmon St
Hudson St
Railroad Ave
Adeline Way
Exchange Ave
Ward St
Exchange Ave
Palm Ave
University St
Front St
Healdsburg Ave
114RRHMB1
Bellache Ave
Kennedy Ln
Washington Ct
Bosafi Rd
Presidential Dr
Kinley Dr
Sample Location:
Russian River at Healdsburg Memorial Beach
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

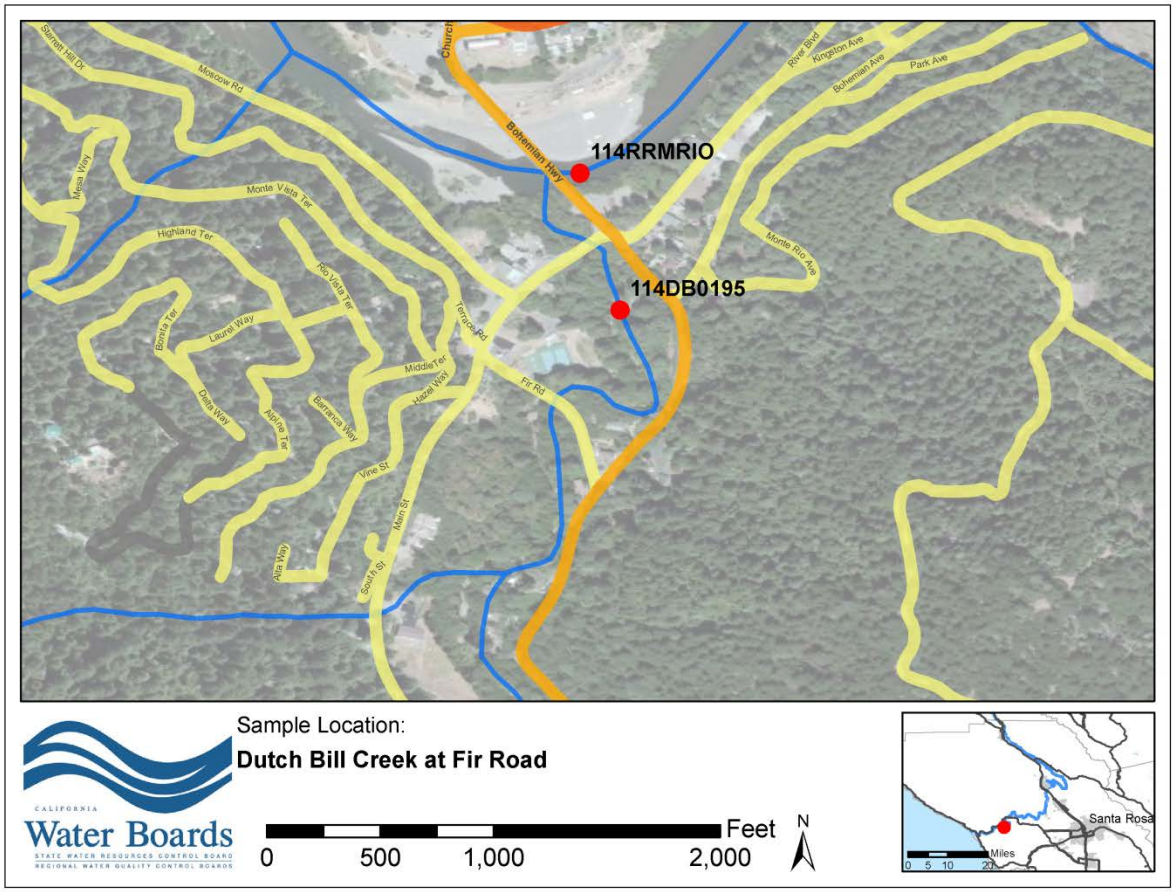

114RR1907
Westside Rd
River Rd
River Dr
Old River Rd
Grays Rd
Terrace Dr
Rio Vista Rd
Del Ave
Westside Ave
Sequoia Rd
Oak Ave
Cosmo Ct
Forest Hills Rd
Grays Ct
Scalio Ct
Field Ln
Frank St
Sunset Ave
Bluewater Ln
Opdyke Ln
Woodside Dr
Canyon Rd
Sample Location:
Russian River at Forestville Access Beach
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

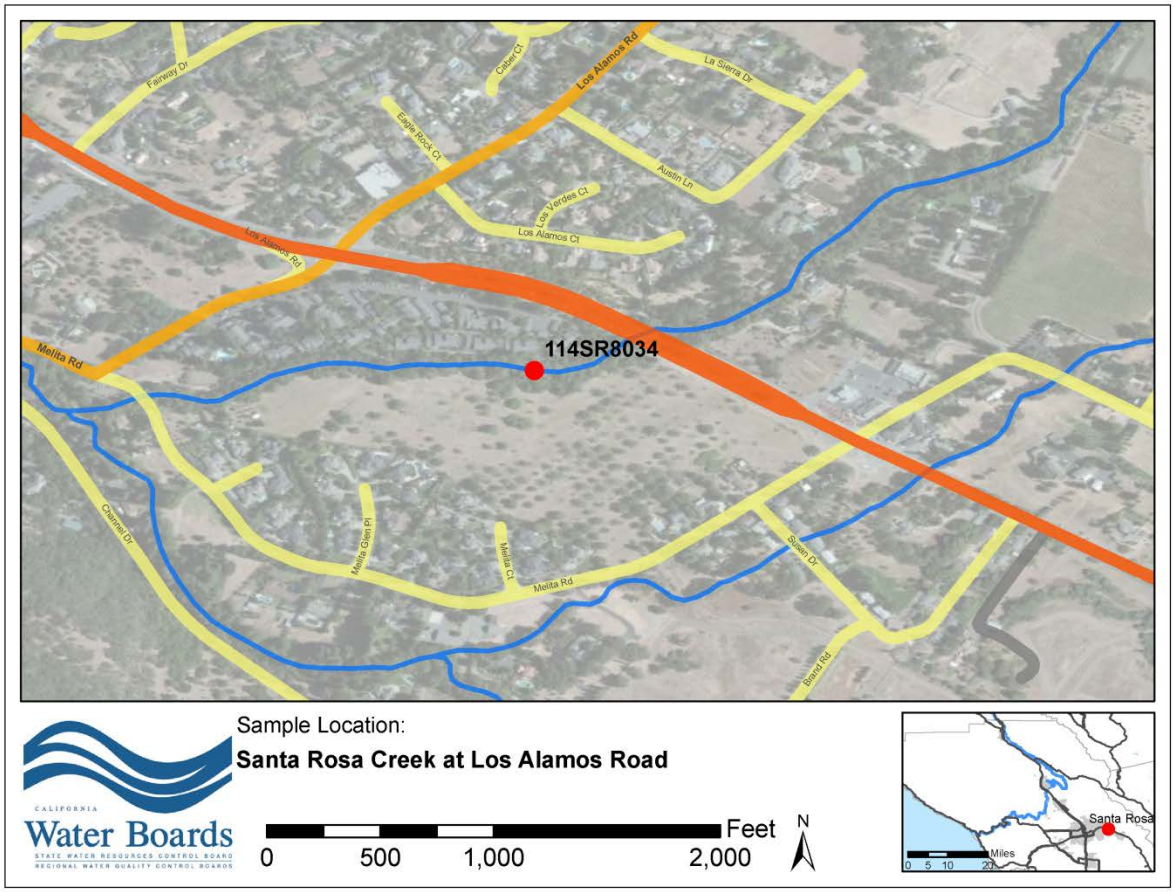

Westside Rd
114RR2046
Rio Vista Rd
Del River Rd
Valle Vista Rd
River Rd
Orchard Rd
Orchard Road Ext
Farrell Dr
Chateau Thierry
Rio Dell Ct
Sample Location:
Russian River at Steelhead Beach
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

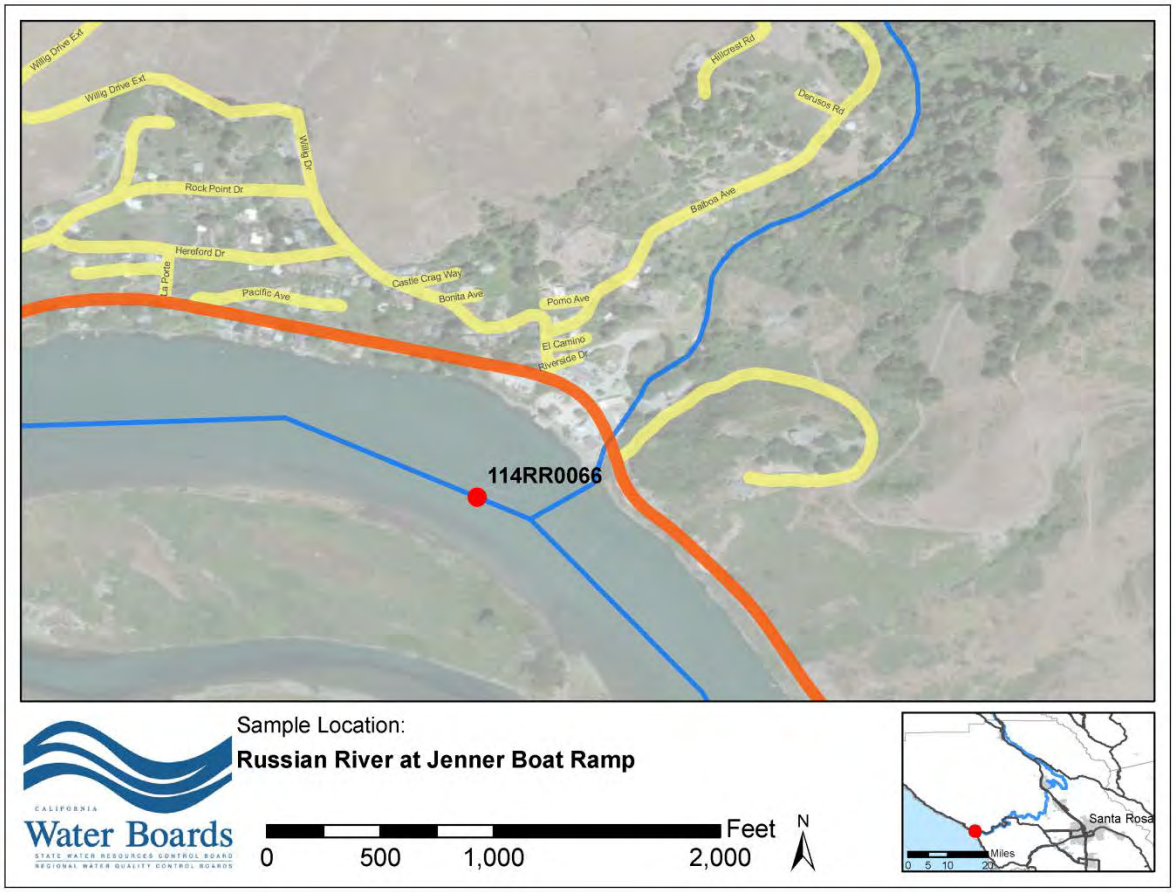

4th St
3rd St
Main St
1st St
Church St
Mill St
Brookside Ln
Armstrong Woods Rd
Old River Rd
River Rd
Palo Alto Dr
Riverlands Rd
Drake Rd
Drake Road Ext
Guerne Hill Rd
Neeley Rd
Guernewood Rd
Center Way
Solaridge Rd
Fern Rd
Gables Rock Rd
114RRJB01
Sample Location:
Russian River at Johnson's Beach
Water Boards
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0
5
10
20

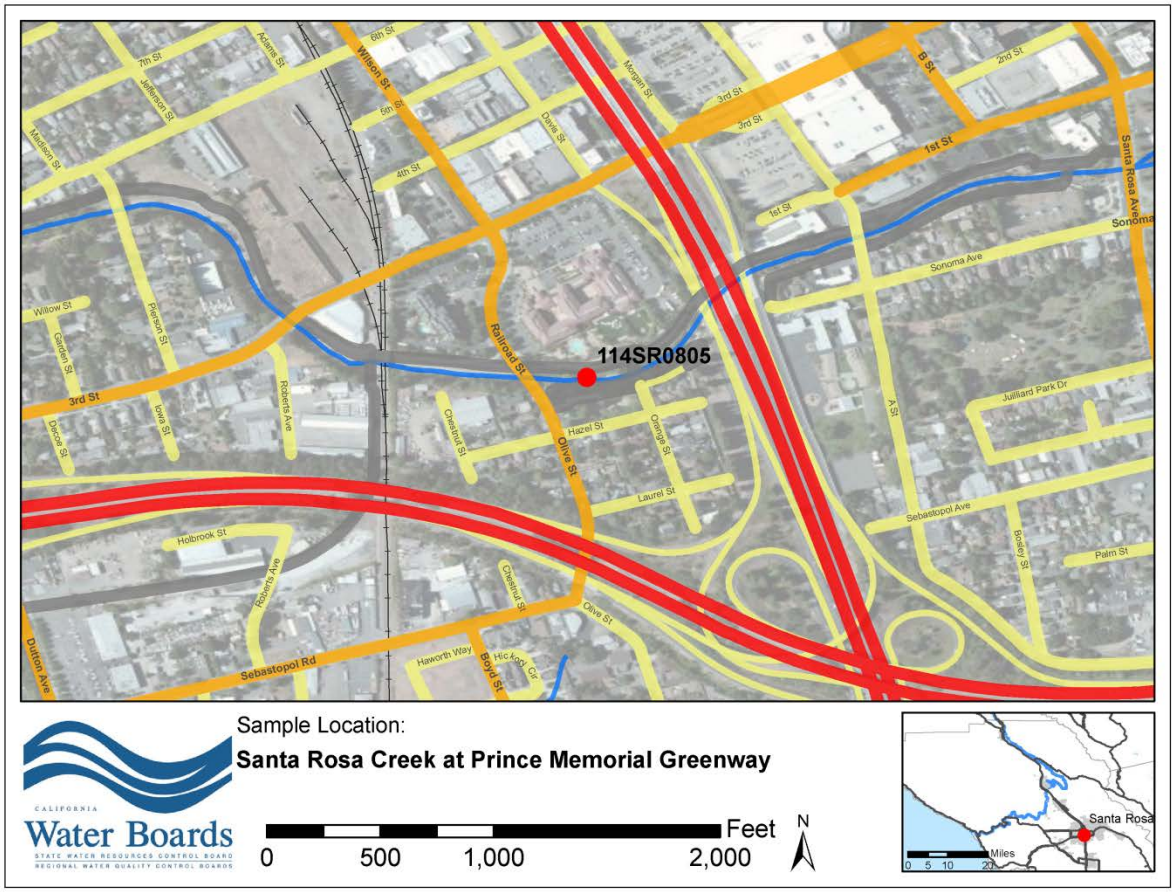

Denno Ln
Green Valley Rd
114GV7625
Martinelli Rd
Sample Location:
Green Valley Creek at Martinelli Road
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

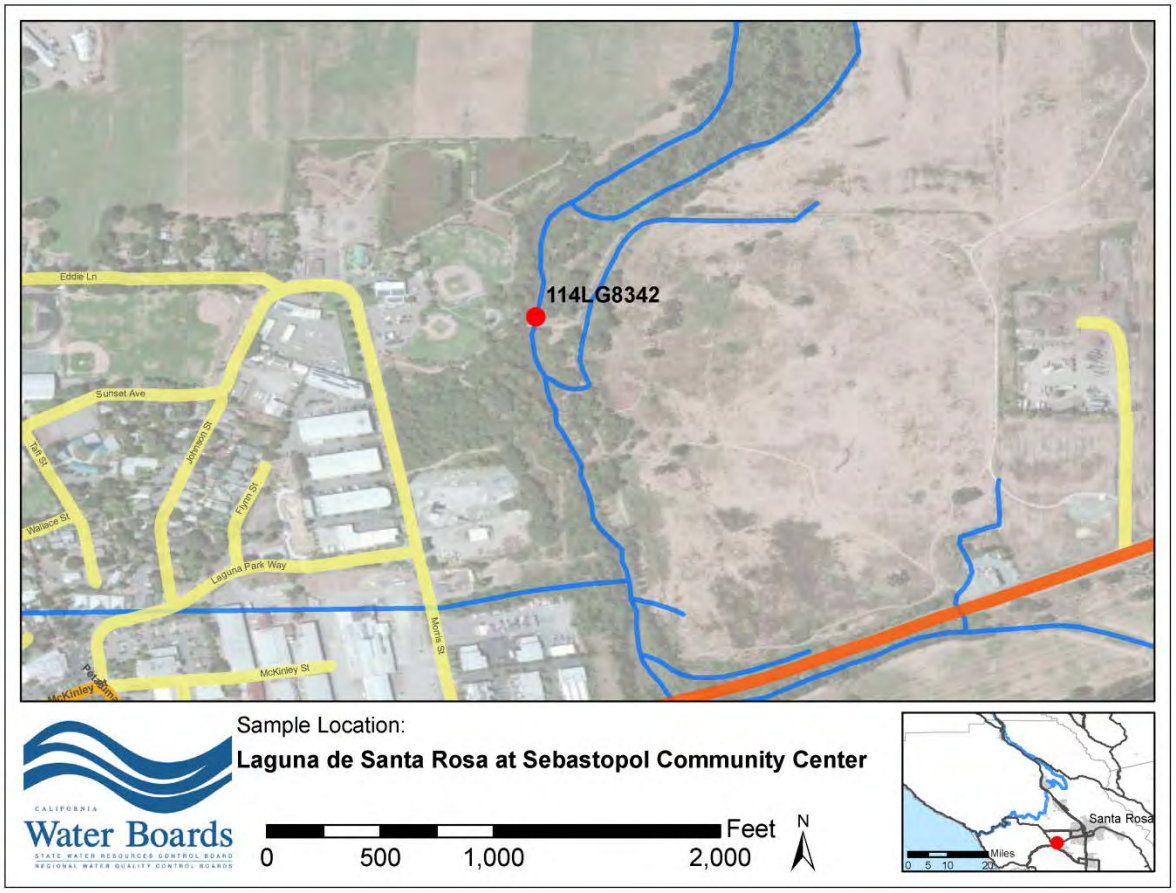

114RRMRIO
114DB0195
Bohemian Hwy
Church St
Moscow Rd
Monte Vista Ter
Highland Ter
Middle Ter
Vine St
Fir Rd
Terrace Rd
Main St
Monte Rio Ave
Park Ave
Bohemian Ave
Kingston Ave
River Blvd
Mill St
Sunnyside Way
Foothill Dr
Chapel Dr
Hill St
Field Way
Birch Ln
Helen St
Railroad Ave
Sample Location:
Russian River at Monte Rio Beach
California Water Boards
State Water Resources Control Board
Regional Water Quality Control Boards
0 500 1,000 2,000 Feet
N
Santa Rosa
Miles
0 5 10 20


114RRMRIO
114DB0195
Bohemian Hwy
Monte Vista Ter
Highland Ter
Laurel Way
Middle Ter
Fir Rd
Terrace Rd
Vine St
Main St
South St
Alpine Ter
Delta Way
Bonita Ter
Hazel Way
Park Ave
Bohemian Ave
Kingston Ave
River Blvd
Monte Rio Ave
Moscow Rd
Mesa Way
Sample Location:
Dutch Bill Creek at Fir Road
Water Boards
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20


Fairway Dr
Caber Ct
Los Alamos Rd
La Sierra Dr
Eagle Rock Ct
Austin Ln
Los Verdes Ct
Los Alamos Ct
Los Alamos Rd
Melita Rd
114SR8034
Channel Dr
Melita Glen Pl
Melita Ct
Melita Rd
Susan Dr
Brainard Rd
Sample Location:
Santa Rosa Creek at Los Alamos Road
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0 500 1,000 2,000
Feet
N
Santa Rosa
Miles
0 5 10 20


Willig Drive Ext
Willig Drive Ext
Willig Dr
Rock Point Dr
Hereford Dr
La Porte
Pacific Ave
Castle Crag Way
Bonita Ave
Pomo Ave
El Camino Riverside Dr
Hillcrest Rd
Deruses Rd
Balboa Ave
114RR0066
Sample Location:
Russian River at Jenner Boat Ramp
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0
500
1,000
2,000
Feet
N
Santa Rosa
Miles
0 5 10 20


114SR0805
Railroad St
Olive St
Wilson St
3rd St
Sebastopol Rd
Sonoma Ave
1st St
Hazel St
Laurel St
Holbrook St
Sample Location:
Santa Rosa Creek at Prince Memorial Greenway
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0 500 1,000 2,000
Feet
N
Santa Rosa
Miles
0 5 10 20


114LG8342
Eddie Ln
Sunset Ave
Johnson St
Tyler St
Wallace St
Ferrell St
Laguna Park Way
Morris St
McKinley St
Sample Location:
Laguna de Santa Rosa at Sebastopol Community Center
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS
0 500 1,000 2,000
Feet
N
Santa Rosa
Miles
0 5 10 20

Appendix 5: Standard Operating Procedure for PhyloChip Analysis

Standard Operating Procedure for Phylochip Sample Filtering:

1. Each sample will be collected using a vacuum pump. Set up filtering apparatus. Check each connection point to ensure components are snug and pressure loss is minimized.
2. Clean each filter setup (glass top and filter stage) using 70% ethanol or isopropanol. Wipe dry with paper towels.
3. Gently place a sterile 47mm 0.2µm filter on each filter stage using alcohol-cleaned flamed forceps. Place glass top onto filtering stage and use metal clamps to hold components together.
4. Start vacuum pump, allow to run ~5 sec before adding samples.
5. Homogenize water sample by shaking gently.
6. Pour ~300mL of water from the sample onto the filter.
7. If filtration stops before the entire contents have been filtered, decant off the remaining sample into a graduated cylinder and record the volume of sample that remained unfiltered on the chain-of custody form.
8. Once sample has been filtered, gently remove the glass tops, and using alcohol-cleaned flamed forceps, carefully roll-up the filter with the filtered contents inside..
9. Carefully place the filter into a single DNA extraction bead tube (with 0.5 mm beads that are more commonly used for bacteria). Label with sample name and date.
10. Immediately transfer on ice the bead tubes to Sonoma County Public Health Labs for freezing at -80C. Frozen samples will be transferred to LBNL on dry ice after all samples have been collected.
11. If additional samples will be filtered, rinse filter stage and glass top with de-ionized water and wipe down with 70% alcohol solution. Filter next sample as described above.
12. Once filtration of all samples is complete disassemble sampling apparatus. Rinse filter setups and glassware with de-ionized water and 70% alcohol. Store tubing, vacuum pump, and connectors. Autoclave all glass ware between uses.

Standard Operating Procedure for Phylochip Sample Filter Analysis:

G3 PhyloChip Manual: 12-07-2010 Edition:

Target Preparation.

PCR amplification of 1.5 kb product for target hybridization

To prepare DNA, use either a one-temperature amplification with a degenerate primer if you do not have very much template, or use a non-degenerate primer at 3 - 8 temperatures on a gradient thermocycler (from 48.0 to 58.0 deg. C annealing temp.).

The primers we use for non-degenerate (gradient) PCR are as follows:

Primer sequences used for microarrays and 16S rRNA clone libraries (taken from a JGI protocol)
27f.jgi (Bacteria-specific) 5'-AGAGTTTGATCCTGGCTCAG-3'

1492r.jgi (Bacteria/Archaea-specific) 5'-GGTTACCTTGTTACGACTT-3'

If amplifying Archaea, use this forward primer instead of 27f.

4fa.jgi (Archaea-specific) 5'- TCCGGTTGATCCTGCCRG-3'

The primers for the one-temp, degenerate (forward) primer PCR are:
27f.1 5'-AGRGTTTGATCMTGGCTCAG-3' and the 1492r primer.

Note: we use a gradient PCR for maximal diversity if we have enough template to do so. Also, if we are cloning and sequencing from the same PCR pool as is analyzed on a chip, then we use 50 uL reactions on an 8-temp. gradient (48° - 58° C, 25 cycles). We would do an 8-temp. gradient set at 25 uL per reaction per temperature if we knew we would get lots of PCR product at each temperature. All temperature products are combined (usually through isopropanol ppt.) and used as a pool for microarray analysis (and clone library formation, if that is being done).

When amplfying Bacteria and Archaea, keep the PCR products separate, so you can quantify them separately. Ideally, use 500 ng of Bacterial PCR product and 100 ng Archaeal PCR product for microarrays. If there is not enough product for any one sample, consider cutting back on all of them so that an equal amount of product is loaded onto each chip. These values are for gel quantitation. If you use a spec. or Nanodrop, be sure to know the equivalent amounts needed for these values on a gel. It is common for a Nanodrop measurement to be twice as high as a gel measurement.

1. TAKARKA TAQ PROTOCOL (Used for most studies). May use Excel spreadsheets (see Attachments). Changing the number of samples on any worksheet generally causes the volumes to be updated to reflect your current set of samples.

TAKARA PCR for chips
PCR and/or cloning

Reagent	type	batch/date open	Stock Conc.	Final Conc.	units	vol (uL)
Water	Milli-Q, autoclaved					34.2
Buffer	Takara buffer w/MgCl2		10	1.0	X	5.0
BSA *	Roche		20	0.8	mg/mL	2.0
dNTP	Takara (2.5mM each)		10	0.8	mM	4.0
27f.jgi	primer		50	0.3	pmol/uL (uM)	0.3
1492r.jgi	primer		50	0.3	pmol/uL (uM)	0.3
Polymerase	Takara Ex Taq		5	(1.5U) 0.02 **	U/uL	0.2*
Template	10 – 30 ng***					4.0
					Total	50.0

* Use BSA as needed for your template. If not needed to get good amplification, then it can be omitted.
** This can be increased to 0.03 final concentration and 0.3 uL volume added per reaction, but there may be increased background also.
*** Add 2-4µL environmental DNA (enough to give 10 – 30 ng of template), to the reaction mixture. Remember a positive and negative control sample. The positive control is E. coli (at least 100pg and 1pg).

Thermocycling program:

Protocol:	
95C 3'	
95C 30s 53C 30s 72C 1'	25 – 35 cycles as needed, 48 – 58 deg C annealing temp (gradient) if sufficient template, archaea too if sufficient template
72C 7'	
4C hold	

2. Quantitation of PCR amplicons

a. Invitrogen "E gels"

Quantify the PCR products by loading 5uL of product with 10uL water for a 2% E-gel (Invitrogen). If doing a temperature gradient, then combine the products (isopropanol ppt. or using YM-100 Microcon filter units as described below) and run 1 uL on a gel.

Optional: Add 2uL of loading dye to one lane as an indicator of how far the DNA is traveling. Note that the dye will affect the adjacent lane and may impair visualization.

Load designated amount of Invitrogen Life Technologies low mass ladder (should be 10 uL).

Product is 1.5 kb. Run for 20-25 minutes at 60V for a single comb gel. If a double comb gel is used, run for 18 minutes at 60 volts.

- Remember to pre run the gel for less than 2 minutes (60-70V) prior to loading.
- No running buffer is required in this reaction.
- If there are remaining empty wells, remember to fill with an equivalent volume of water.
-

Analyze gels using the FluorS-MultiImager (BioRad, Hercules, CA).

b. Biorad Geldoc Operating Instructions

Load the gel onto the middle of the glass surface.

On the attached computer, select "Quantity One" program, then pick "Geldoc."

Step 1: To view the gel, press "Trans UV."

Step 2: Select position. Gel will appear on screen: focus and zoom out if gel is not in a good orientation. Ensure there are no saturated pixels for quantifying. Saturated pixels will appear as red spots; to eliminate these, you can close the iris further.

Step 3: Once the saturated bands are eliminated, choose '"analyze" to create an enhanced gel image.

Steps such as "transforming" enable a clearer image of product bands. Zoom into desired bands and standards using the zoom tool.

Step 4: Save the raw image data.

Step 5: Take picture of gel: File> Print> Video Print

c. DNA mass quantitation

Open the Volume Contour tool in the volumes quick guide. The tool is activated when you see a + symbol: put the + on the desired band: move it around until it outlines the band with a green line. Continue outlining all bands, including mass ladders and unknowns. Once all bands are outlined, zoom back out.

Using the volume rectangle tool, pick an area representative of the background near the unknown bands. Highlight the rectangle to be yellow: double click for volume properties, fill in form to say background. The background sample should be an area larger than the bands, to enable a better average to be calculated.

For standard bands: highlight each band yellow: double click: fill in form: label as standard and fill in band mass concentrations (key is taped onto computer screen).
For unknown bands: highlight each band yellow, select unknown.
After all inputs are made, save the file.

Using the Volume Regression Curve tool, calculate the correlation coefficient. Anything above 95% is acceptable. It is okay to remove any bands on the dye line that don't fall around the curve (the background is different and these bands don't usually correlate well to the others).

The volume analysis report tool lists the information generated. Reformat the report so that index, name, and concentration only are reported. Print the report.
The report shows the concentration of the product loaded on the gel: since 5 µl was added to the gel, divide by 5 to get the ng/µl concentration.

3. Concentrating Samples

If there are more than 40 uL of PCR product to be prepared for chip analysis (such as when combining PCR products from a gradient PCR run), then the sample will need to be concentrated to 40 uL or less.

Step 1: Label a Microcon YM-100 (blue) regenerated cellulose 100,000 MWCO concentrator insert (Amicon, Cat. # 42413) or equivalent, and label the catch tube "flow."

Step 2: Add 40 uL water to the filter and spin at least half the vol. through before adding any sample (spin at 500 x g for 3 min or longer). <u>This step is very important for good recovery of product.</u>

Step 3: Add up to 500 uL of PCR product to the concentrator insert.

Step 4: Spin the concentrators at 500 x g for 1 – 5 minutes, check the vial for volume by measuring with a pipette, continue until there is less than 40 uL retained in the insert. Toward the end of the concentration, you may need to spin for only 30 sec. at a time to keep from drying the filter.

Step 5: Invert the insert into a new, labeled tube and spin it at 1,000 x g for 2 – 3 minutes (or pulse spin) to recover the retentate.

Step 6: Measure the retentate using a pipette.

Step 7: Run 1 uL of retentate and 10- 20 uL of "flow" on a 2% E-gel. All DNA should be in the retentate. Otherwise, there was a leak.

At this point, calculate the amount of DNA recovered in the retentate for both the band of interest (on the gel) and the total amount of DNA in the gel lane. Both numbers will be used in the calculations for DNA fragmentation.

4. PCR Product Fragmentation – customized from Affymetrix protocol. May use Excel spreadsheet ChipCalcs (see Attachments). If making your own spike mix, see Appendix I.

Dnase I calcs — Combine Bacterial and Archaeal

Sample	vol of conc Bacterial PCR	total Bacterial PCR ng/uL	vol of conc Archaeal PCR	total Archaeal PCR ng/uL	total PCR (ug)	Spike mix added (ug)	Total DNA (ug)	units needed @ 0.02U/ug	Dnase (1/20 dilution of 1U/uL stock)	2x DNAse
Sample Set_1.1	20.0	28	5.3	29	0.7	0.202	0.91	0.018	0.36	0.73
Sample Set_1.2	9.1	78	6.5	34	0.9	0.202	1.13	0.023	0.45	0.91
Sample Set_1.3	9.3	68	8.8	33	0.9	0.202	1.12	0.022	0.45	0.89
Sample Set_2	10.5	55	0.0	0	0.6	0.202	0.78	0.016	0.31	0.62

Fragmentation Rxn (50uL) calcs

Sample	Water	10x DNAse I Buffer (uL)	(9-09-09) Spike Mix (uL)	Bacterial Product (uL)	Archea Product (uL)	1/20 Dnase I (uL)	Total
Sample Set_1.1	-0.09	3.0	1.08	20.0	5.3	0.73	30.00
Sample Set_1.2	9.45	3.0	1.08	9.1	6.5	0.91	30.00
Sample Set_1.3	7.00	3.0	1.08	9.3	8.8	0.89	30.00
Sample Set_2	14.80	3.0	1.08	10.5	0.0	0.62	30.00
		13.2	4.752				

(Dnase) Incubate: 25C - 25min 98C - 10min hold @ 4C

4.1 per tube

1/20 Dnase	
1	Dnase (Invitrogen) 10x DNAse I
2	Buffer
17	water
20uL	

- Optional: Dilute DNase 1 to 0.05U/uL in 1 X One-Phor-All Buffer (a 1/20 dilution). This must be used immediately. This can even be diluted further to increase accuracy when pipetting very small amounts. Adjust the volume used accordingly.
- Add DNase I last, prior to putting in the thermocycler, and add it either to the side of the tube (hanging drop) or to the lid of the tube. Mix gently by tapping the tube (DNAse is physically fragile) and spin the sample.
- Remember to keep 200ng for gel analysis as a control (optional).

2. Incubate the reaction at 25ºC for 20 minutes, typically in a thermocycler.

3. Inactivate DNase I at 98ºC for 10 minutes.

4. Store at -20ºC or add directly to the terminal labeling reaction.

Recommended for each new batch of enzyme:

* Affymetrix claims that the DNase I activity will vary from lot to lot. A titration assay is recommended for each new lot of enzyme to determine the dosage of the DNase I (meaning the unit of DNase I per ug of cDNA) to be used in the fragmentation reaction.

Run fragmented and non-fragmented samples on a 4% E-gel. Use the low mass DNA ladder (2ul)+ 1ul dye and 17ul H20. Also use 25bp ladder (1.5ul) + 1ul dye + 17.5ul H_20. Majority of DNA fragments after fragmentation should be around 50-200bps.
Run at 60V for 50 minutes.

If the fragment range is less than 50 – 200 bp, then extend the time of the incubation at 25 deg C. If the fragments are too small, then decrease the time of incubation.

If PCR was performed to create uracil-incorporated PCR product, use the following table:

Fragmentation Rxn (30uL) calcs

Tube	Sample	Water	Bacterial PCR (uL)	Archea PCR (uL)	10X Frag Buffer	2:1 Spike Mix (uL)	UDG	APE
1	Sample_1	19.86	1.98	0.83	3.00	1.99	0.94	1.41
2	Sample_2	19.83	1.86	0.97	3.00	1.99	0.94	1.41
3	Sample_3	19.70	2.27	0.69	3.00	1.99	0.94	1.41
4	Sample_4	19.73	2.17	0.76	3.00	1.99	0.94	1.41
5	Sample_5	19.67	2.09	0.91	3.00	1.99	0.94	1.41
					16.50	10.95	5.15	7.74
						7.33	per tube	

Incubate:
37C - 60min
93C - 2min
hold @ 4C

5. Terminal labeling- same as Affymetrix protocol

Requires:

- Affymetrix GeneChip WT Double Stranded DNA Terminal Labeling Kit, P/N: 900812

1) Make sure reagents are fully thawed and then mix according to the chip calculation worksheet.
2) Incubate at 37°C for 60 min, followed by a 10 min, 70°C step. Add 2uL of .5M EDTA to each reaction.

Biotynalation Rxn

Reagent	x1 (uL)	MM (uL)
5X Rxn buffer	8	36
GeneChip Labeling Reagent	0.664	2.988
50X TDF	1.336	6.012
Fragmented spike	0	0
water	0.00	0
Fragmentation product	30	--
Total uL	40	45
	per tube=	10

Incubate:
37C - 60min, 70C - 10min, 4C - 2min
Add 2uL 0.5M EDTA
to stop Rxn

NOTE: reaction scaled down to 40uL
to add larger amount of DMSO during hybe

To assess the efficiency of the labeling procedure, a gel shift assay can be used. See Appendix X.

6. Pre-hybe chips

Requires:

- Affymetrix GeneChip Hybridization, Wash, and Stain Kit, P/N: 900720

Fill each chip with 130 uL Pre-Hybe solution from the kit. Incubate chips in hybe oven for 20 – 30 min. at 48°C and 60 RPM.

7. Target Hybridization

Requires:

- Control Oligo B2, P/N: 900301

Hybridization Mix	x1 (uL)	MM (uL)
Fragmented, labeled product	42.0	--
3nM Control Oligo B2	2.2	9.9
2X MES Hybe Buffer	65.0	292.5
DMSO	20.4	91.8
H2O	0.4	1.8
total uL	88.0	396
	per tube=	88

Incubate:
99C - 5min
48C - 5min
hold @ 48C

1) Make sure DMSO and Control Oligo are fully thawed, and mix reagents according to the chip calculation worksheet.
2) Incubate samples at 99°C for 5 minutes to denature DNA, followed by a 5 minute incubation at 48°C. Hold reactions at 48°C.
   Take the first 4 - 6 chips from the hybe oven. Vent chips and remove pre-hybe solution. Remove the corresponding samples from the thermocycler and load entire contents of each hybe mix onto their chips as

follows: Use two pipette tips when filling the probe array cartridge. Use one (unfiltered) to vent air from the hybridization chamber.

3) Cover vent septa with stickers to prevent evaporation, and place microarrays back in the hybe oven. Rotate chip to loosen any bubbles; they should move freely around the chamber. Repeat this process until all chips have been loaded.
4) Save PCR tubes on the bench top until tomorrow.
5) Load probe arrays in a balanced configuration around the rotisserie axis. Hybridize chips at 48°C/60RPM for 16 hours.

The next day, remove the hybe solution and save in case the sample needs to be run again. Store this frozen, protected from light. Fill the chip with Wash Buffer A (non-stringent wash buffer) if there is a delay in using the fluidics station.

8. Washing, Staining and Scanning –same as Affymetrix protocol.

Requires:

- Affymetrix GeneChip Hybridization, Wash, and Stain Kit, P/N: 900720

Before Beginning:

- Turn on the scanner at least 10 minutes before scanning so that it can warm up.
- Aliquot a few milliliters of Wash A into a centrifuge tube.

Chip Prep

1) Remove chips from hybe oven and remove stickers covering vents.
2) Transfer hybe mix to the appropriate PCR tubes. Hybe mix can be stored at -20°C in case a chip must be repeated.
3) Vent chips and add 150uL of Wash A buffer to each array.
4) Bring chips to the computer terminal/fluidics station and place them inside the drawer.

Priming the Fluidics Station (This can run in the background as you register chips)

1) Place bottles of Wash A, Wash B and DI water in appropriate positions. Note that much less Wash B is required for a wash cycle than Wash A. 250mL of wash B is more than sufficient.
2) On the start menu open Affymetrix launcher.
3) Select Fluidics control.
4) Select “Maintenance Protocols Only”
5) Select Prime_450 from the drop down menu.
6) To the right, click the Check/Uncheck All Stations and Modules box so that all modules are highlighted. If you are running less than 8 chips deselect the modules that will not be in use.
7) Click the “Copy to Selected Modules Button”
8) Click on each Station ID tab at the top of the screen. Prime_450 should be in the protocol window of each module you selected. Run the relevant modules.

Creating a Project (skip this section if you already have a project on the system for the chips you want to run)

1) Return to the Affymetrix Launcher and select AGCC Portal.
2) To start a new project, first make a subdirectory for the system to save your data to. This will make finding your data later much easier. Go to the Create subfolder field and enter a name for your directory. Press the create button.
3) Highlight the folder you just created in the directory tree to the left.
4) Enter a name for your project in the Create Project field and press the create button.
5) All Cel data for your project will be stored in this default directory.

Sample Registration

1) In AGCC Portal go to the Samples tab and select Quick Register.
2) Select the number of chips to be run in the drop down list.
3) Select your project, and select LBL-Phy3b520660 for the Probe Array Type.
4) For each of your chips scan in the barcode and type in the chips sample name into the appropriate fields.
5) Scroll to the bottom of the page and press the Next button.
6) Place chips not to be immediately stained back in the fridge, wrapped in foil.

Washing and Staining PhyloChips

1) Bring chips to be stained up to room temperature by placing them face down in a drawer.
2) In the Fluidics Control window go to the top menu bar and press edit → email messages. Enter your email address in the "To" field. The system will send you error messages if there are fluidics or scan errors.
3) Press the Filters button at the top of the page. Select today's date for both date fields and press ok. This will allow you to see the arrays you just registered in drop down menus.
4) On the Master tab select LBL-Phy3b520660.Universal as the array type.
5) Select "List Compatible Protocols Only" and make sure GeneChip HWS Kit is selected.
6) Select protocol FS450_0002 from the drop down menu.
7) To the right side, select the modules you will use.
8) Press the "Copy to Selected Modules" Button.
9) Go to each station tab and select the chips to be run on each module.
10) Press the run button for each module to be used.
11) After staining the fluidics station will instruct you to eject the chip and look for bubbles. If you see bubbles don't insert the array back into the fluidics station as instructed. Simply take the chip, vent it, remove the holding buffer contained in the chip and reload it by hand with 150uL of fresh holding buffer.
12) Insert empty vials so that the system can do a rinse of the lines and prime for the next set of chips.
13) If another set of chips will be stained, take them out of the fridge and place them in the drawer to warm them up.
14) Start scanning completed chips as described in the next section. You should have enough time to get them set up in the autoloader while the system primes.
15) Repeat from step 9, as necessary.

Affymetrix recommends a weekly bleach protocol and a monthly decontamination protocol to maintain cleanliness of the fluidics station, especially if staining with antibodies. See the Affymetrix manuals for the weekly and monthly cleaning protocols.

Scanning PhyloChips

1) Go to the launcher and select Scan Control
2) Place stickers over the vents on each PhyloChip to prevent possible dripping of holding buffer onto the optics of the scanner.
3) Load the autoloader beginning at the red-bracketed position one, and load sequentially. The slots in the autoloader are numbered.
4) In Scan Control, press start. When prompted confirm that the chips are at room temperature.
5) The autoloader will do a count of the number of chips in the carousel, then it will scan the chips one by one. If it cannot read a barcode that chip will have to be scanned manually.
6) Allow about 5 minutes per chip.
7) Empty the carousel. Set aside any chips that were unable to be scanned (due to misread barcodes). They will have to be scanned manually.
8) Go to Edit → options. Set the system to manual mode.
9) Load the first chip into position 1 of the carousel and press start.
10) From the drop down list, select the name of the chip that was loaded into the carousel and continue.
11) Repeat as necessary for the remaining chips.

Viewing PhyloChip Scans

1) Go to the launcher and select AGCC Viewer
2) Newest chips will appear at the bottom of the review window list. Scroll to your first chip and double click the sample name to open the file.

3) A cel file image will appear in the viewer. To the left are icon buttons that will take you to each corner of the cel image.
4) Verify that the control oligo probes are aligned to the grid at each corner. If realignment is necessary left click the grid at the corner and drag it such that most of the control oligos in view generally occupy the center of their grid space. Realignment is rarely necessary, but if required, make sure that you are making adjustments at full zoom.
5) Zoom out so that you can scroll along the image's edges and verify there are no blotches on the image, and that the oligo is present around the chip's perimeter in a checkerboard pattern.
6) Hover over individual cells if you want to view their intensity (it will appear along with the x,y coordinates of the cursor at the bottom of the program window). Oligo B2 intensity should be between 1500 and 3000.
7) When your done reviewing your image, review date and time will automatically be entered in the review window.
8) When you're done, select your image files in the review window and press the "Remove Selected" button. This will remove you files from the review window. Cel files can be added back to the review window if needed by going to File→Open File. Data is stored by default at: D:/Program Files/Affymetrix/GeneChip/Affy_Data/Data/(Your project folder)

Additional Info: Uracil incorporation for amplifying 16S rRNA gene

Clontech
TITANIUM Taq PCR Kit (includes buffer), P/N: 639209 (500 rxns)

Promega
dUTP, P/N: U1191
Set of dATP, dCTP, dGTP, dTTP, P/N: U1240
→ Make a stock dNTP mix with T:U of 2:1 (2.5 mM each dATP, dCTP, dGTP; 1.67 mM dTTP; 0.83 mM dUTP).

Primers 27f.jgi: 5'-AGAGTTTGATCCTGGCTCAG-3', 1492r.jgi: 5'-GGTTACCTTGTTACGACTT-3'

4fa.jgi: 5'- TCCGGTTGATCCTGCCRG-3' (use instead of 27f when amplifying Archaea)

PCR set up sheet for use with Clontech Titanium Taq					
Date: 9/2/2009 Name:					
Final Volume: (uL)	Number of samples (incl. controls) *				97.9
25 Component	Starting Conc.		Vol. per rxn	Final conc.	Vol in mastermix
10X Buffer	10	X	2.5	1X	244.75
Forward primer 27F.jgi	3	uM (=3pmol/uL)	2.5	300nM (=0.3pmol/uL)	244.75
Reverse primer 1492R.jgi	3	uM (=3pmol/uL)	2.5	300nM (=0.3pmol/uL)	244.75
BSA	20	mg/mL	1.25	1 ug/uL	122.38
dNTP mix **	10	mM each	2	800 uM	195.80
Clonetech taq	50	X	0.5	1X	48.95
Template - for bacteria	10 - 30	ng	1		
Total Reagents			11.25		
Water			12.75		1248.23
Total Vol.			25		
Vol. per well or tube			24		

* 8 tempertures per sample; 1 negative control per set
** Starting dNTP conc. = 2.5 mM ea. dATP, dCTP. dGTP; 1.67 mM dTTP; 0.83 mM dUTP

Isis
48-58C
25 cycles

Samples		tube # (start - finish)	
1	Sample name	1	8
2	Sample name	9	16
3	Sample name	17	24
4	Sample name	25	32
5	Sample name	33	40
6	Sample name	41	48
7	Sample name	49	56
8	Sample name	57	64
9	Sample name	65	72
10	Sample name	73	80
11	Sample name	81	88
12	neg	89	

Note: we use a gradient PCR for maximal diversity if we have enough template to do so. Also, if we are cloning and sequencing from the same PCR pool as is analyzed on a chip, then we use 50 uL reactions on an 8-temp. gradient (48° - 58° C, 25 cycles). We would do an 8-temp. gradient set at 25 uL per reaction per temperature if we knew we would get lots of PCR product at each temperature. All temperature products are combined (usually through isopropanol ppt.) and used as a pool for microarray analysis (and clone library formation, if that is being done).

When amplfying Bacteria and Archaea, keep the PCR products separate, so you can quantify them separately. Ideally, use 500 ng of Bacterial PCR product and 100 ng Archaeal PCR product for microarrays. If there is not enough product for any one sample, consider cutting back on all of them so that an equal amount of product is loaded onto each chip. These values are for gel quantitation. If you use a spec. or Nanodrop, be sure to know the equivalent amounts needed for these values on a gel. It is common for a Nanodrop measurement to be twice as high as a gel measurement.

Appendix 6: Standard Operating Procedure for Stable Isotope Analysis of Nitrogen and Oxygen.

Stable Isotope Facility
Department of Plant Sciences
University of California – Davis

Standard Operating Procedure UCD-SIF QA-04:

Isotopic analysis of nitrate for stable isotopes of N and O by Continuous-Flow Isotope-Ratio Mass Spectrometry (CF-IRMS)

Author: _____

Signature: _____

TABLE OF CONTENTS

A. Protocol Summary

Stable isotope ratios of nitrogen and oxygen are measured by continuous flow isotope ratio mass spectrometry (20-20 mass spectrometer, Sercon, Crewe, UK) interfaced to a trace gas concentration system. Gas samples are purged from vials through a double-needle sampler into a helium carrier stream (20 mL/min). N_2O is quantitatively trapped and concentrated in 2 liquid nitrogen cryo-traps operated in series such that the N_2O is held in the first trap until the non-condensing portion of the sample gas has been replaced by helium carrier, then passed to the second, smaller trap. Finally the second trap is warmed to ambient temperature, and the N_2O is carried by helium to the IRMS via a Poroplot Q GC column (25m x 0.53 mm, 25°C, 1.8 mL/min). This column separates N_2O from residual CO_2. A reference N_2O peak is used to calculate provisional isotope ratios of the sample N_2O peak.

Final delta ^{15}N and ^{18}O values are calculated by adjusting the provisional values such that correct delta ^{15}N and ^{18}O values for working standards are obtained. The calibration of the N_2O is problematic since there are no suitable international standards. Thus, we calibrated ^{15}N and ^{18}O by reacting the N_2O with glassy carbon at 1400°C to convert N_2O to N_2 + CO. The resulting N_2 was calibrated against the Oztech N_2 standard, and the CO was calibrated against an Oztech CO_2 standard (after converting CO_2 to CO in a similar manner).

B. Safety

1. The sample preparation and preconcentration systems contain toxic and corrosive chemicals. Use proper protective clothing (gloves, eye protection) during sample preparation and disposal, as well as system maintenance.

C. Sample Preparation

1. Clients are instructed to supply filtered, frozen water samples (30-60mL), shipped in insulated packaging. Polypropylene (PP) sample containers are preferred.

2. Samples are not to contain antibiologicals of any type.

3. Sample lists accompanying the water samples shall identify samples as natural abundance or enriched, provide nitrate concentration data, source information, and unique sample identifiers.

D. Quality Control

1. Communication. If uncertain, communicate with the client regarding sample nitrate concentration, $^{15}N/^{18}O$ enrichment, or sample preparation. Ensure that full sample details and sample lists are provided both in electronic and hard copy, and verify correspondence between client paperwork and shipment upon receipt.

2. Inspection. Inspect sample containers for leakage and trays for any sample migration.

3. Laboratory Reference. The laboratory standards are potassium nitrates (calibrated against NIST 8568 & 8569). Linearity and scale compression standards are analyzed at the beginning of each batch. Drift and check standards are analyzed with every 15 samples.

E. Instrumentation

All major components of the Trace Gas IRMS system described below must be fully functional and quality tested before client samples are analyzed.

1. Preconcentration (TG) unit.
    a. Autosampler
        i. Prior to loading the autosampler, the needle should be wiped clean with water, then greased with a thin layer of Krytox™ lubricant. Inspect both needle holes for blockage; clean or replace the needle if necessary.
    b. CO_2/water traps
        i. Inspect the condition of the CO_2 and water trap. Replace with fresh magnesium perchlorate and carbosorb, if necessary.
    c. Carrier gas and GC flow rate
        i. Inspect flow meters. Flows should be 15mL/min and 8mL/min respectively for carrier flow and valve 8.
        ii. Once a week, check the GC column flow with the handheld flow meter at the "GC 2" outlet. Flow should be 1.5-2mL/min.
        iii. If flows are dramatically changed, inspect autosampler needle, chemical traps, and helium supply for potential issues. Report any unresolved issues to your immediate supervisor.
    d. Liquid nitrogen (LN_2)
        i. Verify the LN_2 dewar contains enough liquid to last throughout the batch.
        ii. Make sure the autofill unit is on and program the timer for automated shutoff.

2. Sercon GEO/20-20 IRMS.
    a. System Check.
        i. Peak shape tuning for 45/44 and 46/44 shall be performed prior to each batch.
        ii. Instrumental conditions will be measured and matched to in the System Check file.

F. Data Analysis and Results Reporting

1. Open and review chromatograms in Callisto Reprocessor. Check that sample peaks are properly integrated and that standards are correctly identified.
2. Calculations. Use Excel file: "NO3-Denitrifier" to fully calibrate the data.
3. This includes:
    a. Blank correction
        i. Apply blank correction
    b. Linearity ("size") correction
        i. Choose a correction that best fits the data (linear, logarithmic, etc.)
    c. Scale expansion/compression
        i. Apply two-point scaling factor from selected scaling standards, or three-point scaling factor if samples contain enriched nitrate.
    d. Drift correction
        i. Apply drift correction using the three drift standards.
    e. Shift to known
        i. Perform a final shift to known.
4. Send both the raw and corrected data files and accompanying data report to your supervisor for approval. Await approval or instruction for changes.
5. Deliver the data report to client.

Appendix 7: Standard Operating Procedure for Genetic Profiling of Bacteroides

Part I. DNA Extraction
Part II. A) PCR Sample Preparation
B) 7300 System Software Run Setup

Part I. DNA Extraction

Items needed:

- General PPE
- Pipettes, p1000, p100, p20 w/ respective sterile tips
- 2.0 ml and 1.5 ml sterile (autoclaved) microcentrifuge tubes
- Qiagen MinElute Gel Extraction kit
- Water bath at 56°C
- 100% EtOH
- Sterilizing solutions- 20% Bleach, ddH_2O, and 100% EtOH
- Forceps
- PBS pH 7.4

1. Open microcentrifuge tube and unfold filter using sterile forceps and then refold the filter so that the inside, which contains bacteria, will now be on the outside and place into a 2ml microcentrifuge tube.
   **make sure to sterilize forceps between each sample*
2. Add 250µl of PBS to sample along with 20µl of Proteinase K
3. Repeat steps 1 and 2 for all samples
4. Add 500 µl of Buffer AL to the sample and vortex for 15s.
5. Incubate at 56°C for 10 min and quick spin.
6. Add 500µl of 100% EtOH and vortex/quick spin
7. Add 700µl of mixture from step 6 to the QIamp Spin Column, which should be within a clean microcen. tube.
8. Spin at 8000 rpm for 1 min.
9. Place spin column in new microcen. tube and add the remaining solution from step 6 and repeat step 8
10. Add 500µl of buffer AW1 and centrifuge at 8000 rpm for 1 min. Place Spin Column in a clean 2ml tube and discard filtrate collection tube
11. Add 500µl of buffer AW2 and spin at full speed for 4 min
12. Place the Spin Column in a clean 1.5 ml tube (not provided in kit) and discard collection tube. Add 50µl of buffer AE and spin for 1 min at 8000rpm.
13. To the same spin column, add another 50µl of AE buffer, making sure to use the same 1.5ml collection tube as in step 12.
14. Store the eluate in the -20°C fridge

Part II. A) PCR Sample Preparation

Items needed:

- General PPE
- Pipettes, p100, p20, p2 w/ their corresponding sterile tips
- Real-Time Thermal Cycler
- Power SYBR green PCR Master Mix
- Molecular Grade Water
- 1.5 ml sterile microcentrifuge (autoclaved) tubes

- 96 well PCR plate (non-fast)
- Optic PCR plate film
- Ice bucket w/ ice

1. Thaw all materials including PCR Master Mix, H_2O, extracted DNA, positive control (196B for HuBac and 186 for BoBac and AvBac) and primers.
2. When an individual item is thawed, vortex/quick spin, and immediately place in ice.
   **Note, It is imperative that the Taq is kept cold at all times*
3. Calculate master mix depending on total samples to be run including PC and NC plus one: n+PC+NC+1, where n=number of DNA samples.
4. Refer to the Matrix presented below when calculating reagents and add to sterile microcentrifuge tube in the order as listed.
   **Note, Take appropriate steps to ensure reagent contamination does not occur*

Primer Series, ie. HuBac	Amount per 20µl Rxn	Multiple needed,ie for 10 samples 10+PC+NC+1=**13**
H_2O	11.25	111.25
PCR Master Mix	12.5	12.5
F_{primer}	0.125	1.25
R_{primer}	0.125	1.25

5. Once master mix is made, vortex/quick spin
6. Pipette 24µl of the master mix into an appropriately labeled PCR tube
7. Pipette 1µl of template DNA(or water for blank) into the assigned PCR plate well containing the master mix
8. Trombone the solution within the well to mix
9. Cover PCR plate with optic film and seal
10. Place in thermal cycler and run appropriate program (see Part II B)

Part II. B) 7300 System Software Run Setup

1. Open 7300 Software on Desktop
2. Create a new document
   a. Within new document wizard, only change plate name
3. Select appropriate detectors for the plate (i.e. HuBac if using HuBac primers)
4. Highlight areas of plate that correspond to the locations of the sample wells being used for current run
5. Add dissociation state, change default volume from 50µl to 25µl, and run samples with default settings* after saving run setup.

* Default PCR Conditions:

Step 1 50^0C for 2 min
Step 2 95^0C for 10 min
Step 3 95^0C for 15 sec
Step 4 60^0C for 1 min

Appendix 8: LBNL Laboratory QAP

OPERATING AND QUALITY MANAGEMENT PLAN

LBNL/PUB-3111, Rev. 10

EFFECTIVE DATE: October 28, 2008

Ernest Orlando Lawrence
Berkeley National Laboratory

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Review and Approval

The Department of Energy and Lawrence Berkeley National Laboratory Office of Institutional Assurance approve the Operating and Quality Management Plan.

Signature maintained on file

James Krupnick

Director, Institutional Assurance

Lawrence Berkeley National Laboratory

Signature maintained on file

Aundra Richards

Manager, Berkeley Site Office

Department of Energy

Table of Contents

Record of Revisions

Rev. No.	Date	Description
3	DRAFT (11/11/92)	Rewrite of the LBL *Institutional Quality Assurance Program Plan, Rev.* 2, dated December 21, 1988, to incorporate requirements of DOE Order 5700.6C.
3	2/3/93	Comments incorporated. Issued to Laboratory for use.
4	6/15/94	Updated in the following areas: -suspect/counterfeit parts program -maintenance management -DOE Order 5480.25, *Accelerator Safety* -total quality management -general editorial
5	1/15/96	Rewrite of OAP, Rev. 4
6	1/15/98	Revise to integrate with ISMS
7	4/18/00	Updated in the following areas: - 10 CFR 830.120 and 414.1, *Quality Assurance* - conduct of operations - maintenance management
8	12/06	Rewrite of the LBNL *Operating and Quality Management Plan*, dated April 18, 2000, to incorporate requirements of DOE Order 414.1C and ISO 9001-2000. Also updated to reflect current LBNL organizations and operations. Appendix D demonstrates how OQMP addresses DOE O 414.1C requirements.
9	06/08	Clarified section 2.7, Document Control policy.
10	10/08	Clarified and expanded upon section 2.6.5, Software Quality Assurance

Statement of Laboratory Policy

It is the policy of the Ernest Orlando Lawrence Berkeley National Laboratory (LBNL) to carry out all our activities in a reliable, safe, and quality manner. The Operating and Quality Management Plan (OQMP) provides the framework for a results-oriented management system that focuses on performing work safely and meeting mission and customer expectations efficiently through continuous process improvement. It is line management's responsibility to set and execute annual performance objectives. In addition, every LBNL employee is individually responsible for the quality and safety of his or her work.

It is our policy to implement the OQMP in a way that enables compliance with DOE quality assurance requirements and other customer's agreements, that ensures our continued scientific research and programmatic success, and that is resource-efficient. The OQMP is integral towards keeping the Laboratory on course in achieving its mission and eliminating non-conformances and unacceptable risks. Our program emphasizes three principles:

- The most essential resources at LBNL are the creative scientists, engineers, and support personnel.
- People who perform the work have the greatest effect on outcome and process quality.
- Problem prevention is more cost-effective than problem correction.

Accordingly, our program establishes a management system that (1) recognizes that managing a laboratory that supports research is different from managing the research itself and (2) provides a process for continuous improvement in our performance in both aspects of Laboratory management.

Director
Ernest Orlando Lawrence
Berkeley National Laboratory

Objectives and Applicability

The LBNL Operating and Quality Management Plan (OQMP) is a set of operating principles, requirements, and practices used to support LBNL organizations in achieving reliable, safe, and quality performance in their work activities. The OQMP is designed to fulfill three main objectives:

- Describe the elements necessary to integrate quality assurance, management systems, and process controls into Laboratory operations.
- Provide the framework for LBNL administrators, managers, supervisors, and staff to plan, manage, perform, and assess their work.
- Comply with the contractual and regulatory requirements specified in DOE-University of California (UC) Contract No. DE-AC02-05CH11231, DOE O 414.1C, *Quality Assurance*, 10 CFR 830, Subpart A, *Quality Assurance Requirements*, DOE O 5480.19, Paragraph 4, *Conduct of Operations*, DOE P 450.4, *Safety Management System Policy*, and DOE O 226.1, *Implementation of DOE Oversight Policy*.

The OQMP applies to all LBNL organizations. All LBNL operating units should be engaged, at some level, with organizing their resources, managing and ensuring the safety and quality of their processes and activities, and evaluating the results of their performance. However, the level of rigor in applying the OQMP principles, requirements, and practices is based on a *graded approach*, with consideration given to the organization's mission, its programmatic or operational significance, and its environmental, safety, and health consequences to personnel, environment, and the general public. Appendix A contains one methodology that can be used to grade processes, activities, and facilities to determine the applicable level of rigor. Alternate methodologies, such as the use of DOE guidance documents or EH&S hazard reviews, may be used if the rationale is appropriately documented and approved.

Depending on contractual or regulatory requirements, certain organizations may require additional program- or facility-specific plans to ensure that relevant policies, administrative and work procedures, and technical information are provided to affected individuals. LBNL radiological facilities apply the OQMP through the Lab's Radiation Protection Program to meet the quality assurance requirements of 10 CFR 830, Subpart A. The use of national or international consensus standards is encouraged for organizations that have unique or specific work activities that require consistent results and/or conformity to specifications.

Offices of Institutional and Contract Assurance

The Director of the Office of Institutional Assurance (OIA) is the senior LBNL manager who has the responsibility and authority to develop, implement, assess, and improve the LBNL Operating and Quality Management Plan. Under the Director's charge, staff from the Office of Contract Assurance (OCA) has the day-to-day operational responsibility to ensure that compliance, scientific excellence, best management practices, and continuous improvement are achieved at LBNL. In partnership with LBNL line management, the OCA regularly monitors project performance, develops and tracks performance metrics and leading indicators, identifies and

records deficiencies, and organizes independent project and performance reviews. The OCA has critical oversight, feedback and process improvement roles with respect to performance deficiencies and maintains centralized tracking of corrective actions and lessons-learned for regular reporting to relevant line managers, the Laboratory Directorate, UC Office of the President (UCOP), the UC LBNL Contract Assurance Council, and the DOE/ Berkeley Site Office.

The Director of OIA ensures that the OQMP is in conformance with the requirements of DOE O 414.1C, *Quality Assurance*, and with applicable elements of ANSI/ISO/ASQ Q 9001-2000, the international consensus standard for quality assurance. Appendix D delineates OQMP conformance with quality assurance requirements and standards.

Section 1
Organization

An appropriate management structure, a proficient staff, and a systematic approach in planning work are key elements in sustaining a safe and high level of performance. This section describes the steps for implementing these concepts in LBNL organizations.

1.1 Organizational Structure

The Laboratory is organized hierarchically by divisions, departments, groups, and offices. A description of the organization must be maintained for each of these levels. This information is the basis for identifying the functional responsibilities, levels of authority, and interfaces both within and among organizations. Organizational information must be clearly communicated to all affected Lab personnel and guests.

The description of the organization should include the following information:

- The organization name
- The core function(s) or mission of the organization
- The roles, responsibilities, and authorities of manager(s) and staff, including clear and concise safety responsibilities

The recommended and official vehicle to describe Laboratory organizations is through the LBNL internet web site (http://www.lbl.gov/Workplace/organization.html). Each division is responsible for maintaining the currency of its organization chart in both printed and electronic versions.

Roles, responsibilities, and authorities of managers and staff must be clearly defined in position descriptions and/or job expectations. Environment, safety, and health responsibilities and duties must be part of the description and expectation. Such information should be reviewed and updated at least annually. Safety roles and responsibilities for managers and staff are detailed in the LBNL Health and Safety Manual (LBNL PUB-3000).

1.2 Planning

Planning is a vital step in implementing a quality work process. Planning is a systematic approach used to identify, in advance, the parameters and actions necessary to execute or arrange an activity, function, or project. Good planning generally results in higher efficiency, effectiveness, safety, quality in products and services, and customer satisfaction. It is an ongoing process that begins as early as practical to allow sufficient time to address issues such as the following:

- Funding
- Organizational interfaces and authorities for those managing, performing, and assessing work

- Resource allocation
- Requirements for written procedures and drawings
- Identification of work standards and requirements
- Identification of safety, environmental and security requirements and controls
- Staff training needs

Evidence of organizational planning is required. Examples of planning include:

- Strategic plans
- Operation and planning meetings (e.g., staff meetings, project meetings, program reviews)
- Research and program proposals that describe the work objectives and the proposed actions/steps
- Work plans or work authorizations that address work objectives, resource requirements, work hazards, and the implementation of safety controls
- Work or project schedule
- Operational policies and procedures
- Division integrated safety management plans that describe each division's environment, safety and health management system
- Performance measures and results

1.3 Staff Proficiency

Staff proficiency involves hiring and retaining staff who have the appropriate skills, experience, and qualifications to carry out their work assignments successfully and safely. To ensure consistent hiring practices, the Human Resources Department provides the institutional policies and procedures for personnel qualification, selection, and training. [See the LBNL *Regulations and Procedures Manual (RPM)*, Chapter 2, *Personnel*.] Supervisors and managers must follow these requirements in hiring new staff.

Supervisors and managers must also ensure that the following activities related to staff proficiency are accomplished and documented for each individual in their organization:

- **Position requirements** must be established at the time of recruitment and selection. The requirements define the minimum education, experience, and skills necessary to fill the position. Requirements for certification and licenses are also identified at this time. Candidates' qualifications must also be verified during the hiring process (see RPM 2.01).
- **Training needs** for each position must be determined and documented based on the scope, hazards, and complexity of the job and on any institutional and regulatory training requirements (see RPM 2.04).

- **Job orientation, ES&H training, required reading, and on-the-job training** must be completed as early as possible after the job assignment. Some training is required prior to the actual performance of work. On-the-job training must be administratively controlled to ensure that such training is not allowed to adversely affect work quality or operational safety (see RPM 2.01, 2.04).
- **Guest/visitor training or orientation** may be required based on the scope, length, hazards, and complexity of the job assignment. Training for guests and visitors must be documented (see RPM 1.06).
- **Periodic training and retraining** must be provided to ensure continued job proficiency and to improve overall performance and safety (see RPM 2.04).
- **Performance evaluations** must be conducted at least annually for every position to ensure that job proficiency is being maintained and improved. This process is described in the Performance Evaluations Guidance issued annually by Human Resources. (See RPM 2.03.)
- **Where appropriate, professional development plans** are developed to encourage staff to improve their knowledge, abilities, and skills. These plans require management approval. This process is described in the Performance Evaluations Guidance issued annually by Human Resources. (See RPM 2.03, 2.04D.)

Section 2
Management Systems and Process Controls

2.1 Managing Principles

Management develops and implements the management systems and controls to direct the execution of work at the Laboratory. To address the interaction between people, materials, equipment, and actions unique to a national laboratory, the systems and controls must include the following underlying principles of good management:

- **Conduct of Operations:** Management, in particular the Laboratory Director, sets the tone for conduct and behavior in the work environment. The foundation for appropriate conduct and behavior is integrity, ethics, and competency. This draws people to follow rules and regulations, produce quality services and products, embrace Laboratory stewardship, and interact with co-workers appropriately.
- **Information and Communication:** Pertinent information is identified and communicated in a form and timeframe that enables personnel to carry out their duties and responsibilities. At the onset of employment, all personnel must receive clear information of management expectations and the operating principles described herein. During employment, means of communication should be two-way and on-going, allowing both management and staff to initiate discussions on work issues. Information systems should readily provide reports and data on Laboratory performance and results so that line managers, supervisors, and staff can act upon the information as necessary. Key Laboratory functions, such as operations, finance, and compliance, are especially dependent on having timely and accurate reports and data.
- **Risk Assessment:** At all organizational levels and functions, risks to achieving work objectives and risks to workers, the public, and the environment must be continuously assessed and controlled. Organizational units must assess work activities to minimize adverse impacts while maximizing reliability and performance of work. Risk assessments by Laboratory units are in addition to the assessments conducted by independent third parties or the LBNL Office of Institutional Assurance.
- **Controlled Work:** Work is executed by following prescribed policies and procedures. Work within the framework of policies and procedures helps to ensure that Laboratory Management's directives are carried out. When the policies and procedures are not followed, the work is not authorized, and actions must be taken to address the nonconformance and potential risks arising from the non-authorized work. For work that has higher risk significance, authorization requires greater formality than only policies and procedures. Formal work authorizations can take the form of written approvals, authorizations, and verifications; documented reconciliation processes, and formal reviews of operating conditions, security of assets, and delineation of duties.
- **Monitoring:** Management systems and process controls need to be monitored – a process that assesses the efficiency and effectiveness of the systems and controls over time. This is accomplished through on-going monitoring by managers, supervisors, and staff in the course of performing their duties. The scope and frequency of monitoring is dependent on the assessment of risks and the effectiveness of the monitoring procedures. Monitoring by Laboratory units is in addition to the reviews conducted by independent third parties or the LBNL Office of Institutional Assurance.

2.2 Safety Management

2.2.1 Hazards in the Work Process

Safety must be integrated into the work process. For all core functions and other significant activities, line managers must implement an integrated safety management process as outlined in Appendix B to ensure that safety-related work issues have been addressed comprehensively. At a minimum, line management must have auditable evidence of the identification and control of hazards in their responsible workplace. Managers must follow the requirements in Chapter 6 of LBNL PUB-3000, *Health and Safety Manual*, to identify hazards and implement appropriate controls. LBNL's Environment, Health and Safety Division (EH&S) and division ES&H personnel provide the support and guidance to line managers for identifying and mitigating the hazards in their workplaces.

All line managers must perform the following safety functions:

- Define the scope of work
- Analyze the hazards
- Develop and implement controls
- Perform work within the controls
- Provide feedback and continuous improvement

Documentation of the above functions can be in work plans, division ES&H reports, or authorization/contract agreements.

2.2.2 Formal Work Authorization

Depending on the programmatic or operational significance and environment, safety, and health consequences, some work processes may require formal work authorization from the management of LBNL organizations. Formal authorization is a review and approval process by management to ensure that appropriate procedures, controls, and resources are in place before the work begins. Formal authorization results in a written document that describes:

- The scope of work
- Required procedures and controls
- Authorized materials and equipment to be used
- Authorized staff to conduct the work

The document must be signed off by the appropriate manager(s) and/or staff to signify approval of such work.

When formal authorization is not warranted based on a graded approach (see Appendix A), line managers must still review and approve work under their supervision. Line authorization need not be formalized into an authorization document. Work plans, position descriptions, and job expectations are acceptable vehicles for line authorization.

Regardless of the type of authorization, all managers and staff must consider the following work principles in the review and approval of their work:

- Line management accountability
- Clear roles and responsibilities

- Competence commensurate with responsibilities
- Balanced priorities
- Identification of work quality and safety standards
- Conditions and requirements for performing work
- Work and hazard controls tailored to the work being performed

2.2.3 Stopping Unsafe Work

All LBNL employees, contractors, and participating guests are responsible for stopping work activities considered to be an imminent danger. Stopping unsafe work applies to all activities conducted at the Laboratory and to all off-site facilities operated by Laboratory personnel (PUB-3000, section 1.5).

An "imminent danger" is defined as any condition or practice that could reasonably be expected to cause death or serious injury, or environmental harm. Whenever an employee, contractor, or participating guest encounters conditions or practices that appear to constitute an imminent danger, such individuals have the authority and responsibility to:

- Alert the affected employee(s) or contractor(s) engaged in the unsafe work creating an imminent-danger condition and request that the work be stopped.
- Call LBNL incident notification telephone number (x6999) and report the incident. EH&S staff will investigate.
- Notify the immediate supervisor and/or responsible division/department manager (if known).

EH&S staff will ensure that the supervisor is notified and will assist the supervisor in preparing a report to the EH&S Division Director, describing the unsafe activity and identifying corrective actions and responsibilities.

2.3 Environmental Management

Executive Order 13148, *Greening the Government Through Leadership in Environmental Management*, and DOE Order 450.1, *Environmental Protection Program*, have both mandated the development of Environmental Management Systems (EMS) to implement sound environmental stewardship practices that:

- Protect the air, water, land, and other environmental resources potentially impacted by facility operations and
- Meet or exceed applicable environmental laws and regulations.

LBNL has established a documented EMS that: 1) Complies with applicable environmental and public health laws and regulations; 2) prevents pollution and conserves natural resources; and 3) continually improves the Laboratory's environmental performance. Detailed information on the LBNL EMS, including environmental aspects, current objectives and targets, and environmental assessment reports can be found at: http://www.lbl.gov/ehs/esg/emsplan/emsplan.htm.

LBNL managers, employees, and guests who have activities that may impact the environment must assume their individual responsibility for environmental stewardship. At a minimum, they must:

- Strictly comply with all LBNL environmental policies and procedures as promulgated by the Environmental Services Group and Waste Management Group of the EH&S Division.

- Support the Lab's effort to meet environmental objectives and targets as identified in the LBNL EMS plan.
- Prevent pollution, conserve natural resources, and practice sustainability at all opportunities.

2.4 Safeguards and Security Management

LBNL achieves a balance between protecting its critical assets and maintaining an open working environment that fosters collaborative science. The Lab's Integrated Safeguards and Security Management Plan (ISSM) identifies and implements protection programs capable of assuring graded safeguards to theft, sabotage, and other malicious acts. The ISSM plan includes programs for cyber security, export control, physical and intellectual property control, and counterintelligence.

LBNL managers, employees, and guests are directed to comply with all prescribed safeguard and security policies and procedures and adhere to the following ISSM principles:

- Line management is responsible for integrating appropriate security controls into his/her work and for ensuring active communications of security expectations up and down the management line and with the workforce.
- Clear security roles and responsibilities are defined and communicated in position descriptions, performance reviews, and other forms of feedback.
- Without compromising the security, an open environment is promoted to support the Laboratory mission.
- Security controls are tailored to LBNL organizations through the development of integrated security plans, as appropriate, to meet the mission of the organization.

2.5 Other LBNL Management Systems

The use of documented management systems is the preferred method of operation for all LBNL organizations, but in particular, units in Operations and other support organizations should use accepted management practices and systems common to their work discipline or field. Selected management systems should be cost effective, process efficient, and improve customer satisfaction.

LBNL managers and employees who utilize the services of Operations and other support organizations are required to follow the policies and procedures of the applicable management system.

2.6 Process Control

Process control is intended to reduce variation in the work process, thereby improving performance, safety, and quality. Line managers must review their core functions and other significant activities to ensure that appropriate controls are in place. Examples of process controls include:

- Check points in the process where management review and approval are required
- Use of safety standards and requirements necessary and sufficient to mitigate the hazards of the work process
- Assurance that only qualified and trained personnel are assigned to perform the work
- Assurance that only the appropriate equipment and material are used, maintained, and safeguarded

- Assurance that up-to-date written procedures to direct the work are being used
- Acceptance criteria for final review of end product or service

2.6.1 Core Functions

LBNL organizations must identify and describe the key processes used to meet the organization's scientific or operational objectives. The description of core functions is part of the organization description (see Section 1.1).

2.6.2 Descriptions, Procedures, Instructions, and Drawings

Core functions must have descriptions, procedures, instructions, and/or drawings to direct and inform personnel how to perform the functions in an efficient and safe manner. In addition to the core functions, other LBNL work activities may require similar written procedures, based on the activity's complexity, ES&H hazard, programmatic or operational significance, and consequences to other organizations.

Procedures for core functions and other significant work processes must be written formally to ensure clarity and proper review and approval. The procedures should contain the following:

- Approval signatures and effective date
- A unique title or other identifier
- Purpose and scope
- Definitions (for special acronyms or terms)
- Procedural work steps with associated responsibilities and controls
- References (sources of requirements)

Modification of approved procedures for core functions and other significant work processes requires use of a formal change control process if the changes impact the quality and/or safety of the activity. Change control must include approval signatures, effective date, and revision number for the changed procedure.

Activities with low or moderate significance or consequences (as determined by the supervisor or manager) may have less formal procedures or instructions. Notes, desk manuals, memos, operator aids, logbooks, notebooks, postings, and drawings are acceptable methods for this level of written communication. Modification of these types of procedures requires, at a minimum, written concurrence by the immediate supervisor if the changes have an impact on the quality and/or safety of the activity.

Oral instruction, when it is the only communication method used, is not considered sufficient for directing and/or communicating with personnel on core functions or other significant work processes.

2.6.3 Consensus Standards

The use of consensus standards where practicable and consistent with contractual or regulatory requirements is the preferred method on which to base a controlled work process or program. Consensus standards, for the purpose of the OQMP, include national and international standards, DOE technical standards, and standards and codes from nationally recognized professional societies. These standards, as applicable, may supplement or replace written procedures, instructions, and drawings.

The LBNL Work Smart Standards (WSS) Set provides a listing of standards that the Laboratory utilizes in its contract with DOE. Consensus standards, such as ANSI, ASME, ACGIH, and NFPA, are listed

along with local, state, and federal laws in the WSS Set. For the most current Work Smart Standards Set, go to: http://labs.ucop.edu/internet/comix/contract/LBNL/wss_lbnl.pdf.

ANSI/ISO/ASQ Q 9001-2000 is the international consensus standard for quality management. LBNL is committed to the use of all applicable elements of the standard (see Appendix D for conformance with the standard).

2.6.4 Suspect/Counterfeit Items

Line managers must be cognizant of the presence of suspect/counterfeit items (S/CIs) in their work processes. A suspect item is one in which there is an indication by visual inspection, testing, or other information that it may not conform to established government or industry-accepted specifications or national consensus standards. A counterfeit item is a suspect item that is a copy or substitute without legal right or authority to do so, or one whose material, performance, or characteristics are knowingly misrepresented by the vendor, supplier, distributor, or manufacturer. The use of suspect/ counterfeit items can lead to unexpected failures and undue risk of mission impacts, environmental impacts, and personal injury, contamination, or death.

To mitigate the use of suspect/counterfeit items in Laboratory work processes, line managers must implement the Laboratory Policy and Procedure for Controlling S/CIs, as described in Appendix C. The controls include:

- Guidance on identifying S/CIs
- Procurement procedures to prevent the purchase of S/CIs
- Detection and disposition of S/CIs from Laboratory facilities and installed equipment
- Reporting requirements for discovered S/CIs

2.6.5 Safety Software Quality Assurance

The LBNL approach to Safety Software Quality Assurance is based on the principle that software which is part of a system where degradation of the confidentiality, integrity, or availability of the software can have a foreseeable, significant impact on human safety, taking into account compensating controls, must be appropriately controlled and tested.

Software which forms part of a safety chain in high-risk facilities, but for which adequate non-software controls exist to prevent a degradation of the software from impacting human safety may adopt a subset of these controls at management discretion, but is not covered by this policy directly.

The SSQA approach sets four core requirements:

1. The process owner must consider the system as a whole in considering the risks, taking into account software and non-software components.
2. The Software must be documented to a level where users, developers, and those providing oversight can understand its functions.
3. Tests must be created and executed which clearly show that the software is performing as intended across a range of operating conditions. These tests must be repeated at any time that the environment or the software changes in a way that could create differences in behavior.
4. Changes to the software must be approved, documented, tested, and archived to provide for rigorous, continuous oversight

Safety software includes safety system software, safety and hazard analysis software and design software, and safety management and administrative control software, and performs a safety function as part of a system, structure or component (SSC), and is cited in either a DOE-approved safety analysis document (SAD) or a Lab Directorate-approved hazard analysis document (HAD).

- Safety and Hazard Analysis Software and Design Software is used to classify, design or analyze nuclear/radiological facilities.
- Safety Management and Administrative Controls Software performs a hazard control function in support of nuclear facility or radiological safety management programs or Technical Safety Requirements or other software that performs a control function necessary to provide adequate protection from nuclear facility or radiological hazards.

2.6.5.1 Applicability

[A] System Software

System Software includes operating systems such as Windows, Linux, and system utilities such as compilers, assemblers, translators, interpreters, query languages, word processing programs, spreadsheet programs, database managers, and graphing programs.

System Software **is exempt** from these requirements.

[B] Library (Data) Files

Library files [e.g., nuclide library files used for Nondestructive Assay (NDA)] containing vendor-supplied data shall be controlled by the qualified supplier. Change control is required for the initial library files when they are introduced into the LBNL program for installation, and when they are updated with qualified supplier changes.

Data files that do not affect accuracy or precision, or the quality and correctness of the information, **are exempt** from these requirements.

[C] Applications Developed Within Commercial-Off-The-Shelf (COTS) or System Software

Specific applications developed for use that fall under the applicability of this Plan, based on COTS or System Software (e.g., Microsoft (MS) Excel spreadsheets, MS-Access databases, detailed formulas, or macros) ***are not exempt*** from these requirements.

Calculations that are 100% validated by an alternate calculation method **are exempt** from these requirements.

[D] Acquired Software

Software that's initial design, programming, and testing is performed by a third party. Supplier Software can be categorized as COTS, System Software, or Firmware and could be supplied by either a Qualified or Non-qualified Supplier.

[D-1] COTS Software

COTS is short for Commercial Off-The-Shelf, an adjective that describes software products that are general purpose, ready-made and available for sale to the general public. COTS products are mass-produced, and are designed to be implemented easily into existing systems without the need for customization by the vendor. COTS excludes Sections A and B above.

COTS software **is not exempt** from these requirements.

[D-2] Firmware

Firmware is vendor-supplied software that is included as an integral part of an instrument (e.g., programmed in a read-only memory [ROM] chip) and cannot be modified by another party.

Firmware that cannot be removed or updated **is exempt** from the requirements of this plan. Firmware that is updated by the vendor without respect to particular LBNL requirements is treated as COTS software above.

[D-3] Firmware, modified

Firmware, modified, is vendor-supplied software that is included as an integral part of an instrument (e.g., programmable logic controller) and can be modified by another party. Firmware that is removable or updated will be treated as software if the specifications are created by LBNL.

Firmware that can be removed or updated **is not exempt** from these requirements.

[D-4] Vendor-Developed Software

Vendor-Developed software is software that is developed for LBNL to perform a specific end-user function.

Vendor-Developed software **is not exempt** from these requirements.

[E] LBNL-Developed Software

LBNL-Developed Software is software developed for use by LBNL to perform a specific end-user function.

LBNL-Developed Software **is not exempt** from these requirements.

2.6.5.2 Software Evaluation

New and modified safety software used in facilities will be evaluated to determine the applicable criteria of Software Quality Assurance.

Table 2, *Software Evaluation Checklist*, will be used for this evaluation.

2.6.5.3 Software Lifecycle

Changes to software will be uniquely identified and cataloged. An inventory of related systems will be maintained at the system-owner or functional-owner level. An inventory of safety software systems (broadly) will be maintained by the Office of Contract Assurance (OCA).

[A] Labeling System

A labeling system for configuration items shall be implemented by the responsible organization that:

- Uniquely identifies core configuration items.
- Identifies changes to configuration items by revision or version identifier.
- Provides the ability to uniquely identify each approved configuration of the revised software that is available for use.

[B] Change Control

Changes to software shall be systematically proposed, evaluated, documented and approved to ensure that the impact and rationale for making the change is carefully assessed prior to updating the software baseline. Changes to previously accepted software will be subject to the same level of control as the original software.

Information concerning approved changes will be transmitted to affected organizations if software functionality is impacted. A system of change management will be developed and documented. Software verification activities will be performed for the changes, as necessary, to ensure that the change is appropriate reflected in the software documentation and to ensure traceability is maintained. The degree of software validation will be commensurate with the nature and scope of change.

[C] Software Inventory

A software inventory of all applicable software/systems will be maintained that identifies the software name, version classification, , operating environment and the person and responsible organization for the software (i.e. the organization that owns and/or uses the software).

The Software Inventory List (SIL) is maintained as an institutional document by the Office of Contract Assurance, and will be updated by the responsible organization (i.e. the organization that owns and/or uses the software).

The SIL will be evaluated periodically by all responsible organizations, at least once annually, to ensure the data is accurate, current and complete.

[E] Software Problem Reporting

Problems with software, the potential impact of the problem and the corrective action(s) taken are documented by the responsible organization (e.g. may be the organization that owns and/or uses the software or the assigned software developer(s)).

[F] Software Removal and Retirement

The removal and retirement of software applications will be documented by the responsible organization (e.g. may be the organization that owns and/or uses the software or the assigned software developer(s)). Software covered by this policy must be archived in accordance with archive and records schedules for the safety-area they supported.

2.6.5.4 Software Documentation

[A] Verification and Validation Plan (V&VP)

V&V processes are used to determine if developed software products conform to their requirements, and whether the products from each development phase fulfill the requirements or conditions imposed by the previous phase (verification) and whether the final systems or components comply with specified requirements (validation). This includes analysis, evaluation, review, inspection, assessment, and testing of the software products and the processes that produced the products. Also, the software testing, validation, and verification processes apply when integrating purchased or customer-supplied software products into the developed product. The verification plan should document the verification tasks and the validation plan should document the validation tasks.

Each plan defines the verification and validation tasks and required inputs and outputs needed to maintain the appropriate software integrity level. It also provides a means of verifying the implementation of the requirements of the Requirements Document in the design as expressed in the Design Document and in the testing as expressed in the project's test documentation.

If desired, the verification plan and validation plan may be packaged together in a single document (i.e. V&V P).

[B] Change Control Document

Change control documentation define the methods and facilities used to maintain, store, secure and document controlled versions and related artifacts of the software through all software life cycle phases.

[C] Requirements Document

The software requirements will be identified, documented and reviewed. The Requirements Document will specify requirements for a particular software product, program, or set of programs that perform certain functions in a specific environment. The Requirements Document may be written by the supplier (internal or external), the customer, or by both. The Requirements Document should address the basic issues of functionality, external interfaces,

performance, attributes, and design constraints imposed on implementation. Each requirement will be specified in sufficient detail to permit the accomplishment of design and validation activities. Software Requirements will be traceable throughout the software development cycle, and a V&V Plan will be prepared after the requirements have been documented and approved.

[D] Design Document

The software design will be based on the software requirements and will be documented and reviewed. The Design Document specifies the overall structure of the software (control and data flow) and the reduction of the overall structure into physical solutions (e.g. algorithms, control logic, data structures). The Design Document should describe the components and subcomponents of the software design, including databases and internal interfaces. The Design Document may be prepared first as the architecture design but should be subsequently expanded to address additional detail. The design may necessitate the modification of the Requirements Document and the V&V Plans.

[E] Test Plan

Test Plans identifies the scope, approach, resources and schedule of the testing activities. They also identify the items and features that will be tested, the tests that will be performed, the people responsible for each test and any risks associated with the Plan.

Test Plans define the test approach and identifies the features that are covered by the design and its associated tests. This plan identifies the specific test cases and test procedures, if nay, required to accomplish the testing and specifies the acceptance criteria.

[F] Test Results

Test results are documented and reviewed. Test results describe the results of designated testing activities. Test results identify the summary of the results, any variances that occurred during the test, a summary of activities, the person(s) who performed the tests, the name of the reviewers of the test results

[G] User Documentation

User documentation guides users in installing, operating, managing and maintaining software products. This does not include modifying source code. User documents should describe the data control inputs, input sequences, options, program limitations, error messages and corrective actions to correct those errors, and other essential information for the software product.

2.6.5.5 Software Reviews

Software Reviews are conducted at various stages of software development and may include managerial reviews, acquirer-supplier reviews, technical reviews, inspections, walk-throughs, and audits. Software reviews will be documented as well as further actions, implementation of those actions and verification of those actions, if applicable. The following are examples of possible reviews:

[A] A Requirements Review will be performed to assure the adequacy of the requirements, as appropriate.

[B] A Design Review will be performed to determine the acceptability of the detailed software designs as depicted in the detailed Design Description in satisfying the requirements of the Requirements Document, as appropriate.

[C] The V&VP Review will be performed to evaluate the adequacy and completeness of the verification and validation methods defined in the verification and validation plans.

[D] Other reviews and audits may include the user documentation review. This review is held to evaluate the adequacy (e.g., completeness, clarity, correctness, and usability) of the user documentation.

All reviews performed will be documented by the responsible organization.

2.6.5.6 Software Testing

Testing will be performed to ensure that the design as implemented in code to assure adherence to the requirements and to assure that the software produces the correct results for the test cases.

To evaluate technical adequacy, the software test case results can be compared to results from alternative test methods such as:

a. Hand calculations
b. Comparison with other validated software of similar purpose
c. Calculations using comparable proven problems
d. Empirical data and information from confirmed published data and correlations
e. Manual inspections or qualitative checks not involving numerical manipulations (visual inspection of database reformatting or data plotting)

Tests may be performed by persons who did not implement the design who are technically competent.

2.6.5.7 Supplier Control

Applicable quality assurance requirements will be specified and the required vendor-supplier software documentation, plans, and procedures will be identified in software procurement documentation.

Procured software will be tested in accordance with documented and approved test plans using approved test case specification to ensure that the acquired software will perform satisfactorily in its operating environment. Test plans, and the results of the tests will be identified, documented and retained by the responsible organization.

The responsible organization will perform an acceptance test prior to use of procured software to verify the functional capability of the software and the acceptability of the supplier's supporting documentation (e.g. user documentation, technical specifications and results of supplier testing). In cases of multiple components from a single vendor, a sampling process approved by the functional owner may be utilized. In some cases, then a representative sample may be one component.

2.6.5.8 Records

Software quality assurance documentation will be retained by the responsible organization in accordance with the records requirements outlined in the RPM.

2.6.5.9 Training

Training requirements needed to develop or use the software application, if required, will be identified by the responsible organization

2.6.5.10 Graded Approach

Software Quality Assurance is performed using a graded approach, considering aspects such as software applications that are important to safety that may be included or associated with SSCs.

This graded approach takes into consideration that LBNL has multiple radiological and accelerator facilities that have been determined to need a documented and DOE-approved SAD or a Lab Directorate-approved HAD. Radiological and accelerator facilities where a documented SAD is required are considered to be medium to high hazard facilities, and LBNL applies the complete process detailed here to software which meets the definitions in Section 1.

Table 1: Software Quality Assurance Graded Approach

Software Type	Software Evaluation	Change Control Document	V&VP	Requirements Document	Design Document	Source Code Review	Test Plan/ Results	Installation & Checkout Test Plan/Results	User Document
Applications developed within COTS or System Software	X	X	-	X	-	-	-	X	-
COTS	X	-	-	-	-	-	-	X	X
Firmware, modified	X	X	-	X	X	-	X	X	X
Acquired	X	X	VD	VD	VD	VD	VD	X	X
Vendor-developed	X	VD	VD	VD	VD	VD	VD	VD	VD
LBNL-developed	X	X	X	X	X	X	X	X	X

- = Requirement Not Required
X = Requirement to be Satisfied
VD = Vendor-Developed

Table 2 – Software Evaluation Checklist

1. Initiator: (Print Name)	**2. Date:**	**3. Bldg/Facility:**
4. Software Name:	**5. Software Version:**	**6. Process Affected:**
7. Purpose/function of software (including whether software is used for data collection, design, analysis or scientific reasons):		
8. If any of the answers to **c** through **g** are "Yes", then the Software Quality Assurance requirements are applicable.		
a. Is this System Software as defined below? (System Software includes operating systems such as Windows, Linux, and system utilities such as compilers, assemblers, translators, interpreters, query languages, word processing programs, spreadsheet programs, database managers, and graphing programs.) If YES, this software is exempt from the requirements of this Plan. Designate Blocks **b** through **f** as "N/A", and complete Block 9. If NO, continue to Block 8**b**.		☐ Yes ☐ No
b. Can the software output adversely impact hazard control functions in support of nuclear facility or radiological safety management program or Technical Safety Requirements or other control functions necessary to provide adequate protection from nuclear facility or radiological hazards; or the classification, design or analysis of nuclear or radiological facilities? If YES, continue to Block 8c. If NO, this software is exempt from the requirements of this Plan. Designate Blocks **c** through **f** as "N/A", and complete Block 9.		☐ Yes ☐ No ☐ N/A
c. Is this an application developed within COTS or System Software as defined below? (Applications developed within COTS or System Software are those applications that may include formulas or calculations that are easily reproduced by hand or with a calculator. This would include databases that transfer data or interface with other software.) If YES, Section 2.6.5 of the OQMP is applicable. Designate Blocks d through **f** as "N/A", and complete Block 9.. If NO, continue to Block 8**d**.		☐ Yes ☐ No ☐ N/A
d. Is this COTS Software as defined below? (Commercial off the shelf software is software that is downloaded onto a system or PC, Instrument, or tool that interacts with system software defined in 8a.) If YES, Section 2.6.5 of the OQMP is applicable. Designate Blocks **e** through **f** as "N/A", and complete Block 9. If NO, continue to Block 8**e**.		☐ Yes ☐ No ☐ N/A
e. Is this Vendor-Developed Software as defined below? (Vendor-Developed Software includes software that is owned and/or maintained by a Vendor) If YES, Section 2.6.5 of the OQMP is applicable. Designate Block **f** as "N/A", and complete Block 9. If NO, continue to Block 8**f.**		☐ Yes ☐ No ☐ N/A
f. Is this LBNL-Developed Software as defined below? (LBNL-Developed Software is software developed and/or maintained by LBNL) If YES, Section 2.6.5 of the OQMP is applicable. Complete Block 9.		☐ Yes ☐ No ☐ N/A
9. Evaluation performed by:	**Approved by:**	
Initiator (Print Name/Sign	Date	Responsible Person / Date

2.6.6 Function-Specific Controls

If a Laboratory organization's core functions include any of the activities listed in the tables below, additional controls are required, as described in the tables.

A. Design

Activity	Application	Controls
Input	• Hardware design • Facility design	• Identify and record: – Design basis and performance criteria – Applicable codes, standards, and regulatory requirements – ES&H considerations – Security considerations • Review and approve design by the design organization and the requesting group. • Control the design documents.
Interface	• Hardware design • Facility design	Define the coordination among participating organizations.
Output	• Hardware design • Facility design	Ensure that final documents resulting from the design input are: • Approved prior to issuance • Identified uniquely and by revision status • Retained as part of the design organization's records management
Change control	• Hardware design • Facility design	Approve and record all modifications to the final design by the original design organization or a technically competent designee.
Verification	• Hardware design • Facility design	Conduct an independent review to verify that the final design is technically adequate and complies with the design specifications and applicable standards and codes.

B. Procurement

Activity	Application	Controls
General	LBNL scientific, operations, and administrative functions	Follow institutional procurement procedures as described by the University of California Procurement Policy and Standard Practices and on the LBNL web site.
Procurement planning	High-risk items or services	• Document procurement process to ensure adequate consideration for ES&H, cost and schedule, quality assurance, security, and compliance with codes and technical specifications. • Complete the Advance Acquisition Plan (AAP) for procurements costing more than $500k.
Supplier and subcontractor selection	Nonstandard and non–off-the-shelf items or services	• Evaluate and periodically monitor vendor's capability and quality assurance record in collaboration with the appropriate QA and user organizations, as requested. • Document selection.
Acceptance of items and services	Services and items under contractual agreement	• Document method of acceptance, which can include: – Receipt inspection – Verification testing – Surveillance of service provider – Certificate of conformance – Screening for suspect/counterfeit items • Segregate unaccepted items from satisfactory items.

C. Manufactured Items Inspection and Testing

Activity	Application	Controls
Inspection	Operations requiring regular inspections, as determined by line management	• Include inspections as part of written operating procedures. • Calibrate and maintain inspection equipment. • Establish inspection schedule. • Identify acceptance criteria. • Retain inspection reports and follow-up actions.
Testing	• Bench tests • Analytical laboratory • Preoperational • Maintenance • Post-modification	• Identify acceptance criteria. • Calibrate and maintain testing equipment. • Retain test results that verify process or equipment are performing as specified. • Place equipment test results on or near equipment to signify status of equipment or work process.
Follow-up on nonconforming items	Equipment or product that failed an inspection or test	• Mark, tag, label, or post failure status on or near equipment or product. • Segregate nonconforming item if feasible. • Retain retest or reinsertion that documents correction of the nonconforming item.

D. Construction Inspection and Testing

Activity	Application	Controls
Inspection to verify compliance with design drawings and specifications	Building new or remodeling existing facilities Building new or upgrading existing plant infrastructure	• Quality acceptability determined by the project's approved design drawings and specifications • Quality assurance inspectors are independent of project managers' project schedule and cost concerns
Testing	Construction materials, alone or as an assembly. New utilities and services.	• Identify acceptance criteria. • Calibrate and maintain testing equipment. • Retain test results that verify process or equipment are performing as specified. • Place equipment test results on or near equipment to signify status of equipment or work process.
Follow-up on nonconforming items	Building new or remodeling existing facilities Building new or upgrading existing plant infrastructure	• Projects don't close until all deficiencies in workmanship or materials are resolved • Inspector's signature required to close out project

E. Data Collection

Activity	Application	Controls
Design of data collection systems	Data from: • Scientific investigations • Sampling and monitoring • Environmental remediation • Waste management	Develop operating procedures that include: • Traceability to data collection or sampling activity • Validation of procedures to accepted standard or reference • Verification that all procedures are being followed • Handling and custody requirements • Statistical analysis
Data and sampling control	Data from: • Scientific Investigations • Sampling and monitoring • Environmental remediation • Waste management	• Assign unique identifiers. • Identify limitation of data or samples. • Calibrate and maintain data and sampling equipment. • Date and sign data collection and sampling document. • Retain collection and sampling documents.

F. Asset Management

Activity	Application	Controls
Traceability	Equipment and other items determined by LBNL Property Management as being capital and sensitive items and requiring property control	• Identify responsible person for each item and piece of equipment requiring accountability. • Conduct periodic physical inventory. • Trace equipment and items back to specification, procurement records, maintenance manual, and other support documents. • Identify and implement appropriate security measures.
Calibration	Measuring and test equipment (M&TE)	• Physically mark M&TE with unique identifier and recalibration due date. • Calibrate at prescribed intervals and against traceable standards. • Specify limitations on range, accuracy, and tolerance. • Retain calibration records.
Storage	Physical assets with moderate to high cost value, hazard, or operational importance	• Physically identify and control items with finite shelf life. • Verify any special equipment or protective environment required for storage. • Designate limited-access storage areas. • Prevent damage, loss, or deterioration.
Shipping, transfer, and disposal	Physical assets with moderate to high cost value, hazard, and/or operational importance	• Conform to packaging requirements. • Verify that mode of transportation is adequate. • Retain shipping, transfer, and disposal documents (i.e., ensure traceability).

2.6.7 Facility-Specific Controls

Most facility operations require controls for facilities operations and asset management.

A. Operating Practices

Activity	Application	Controls
Operating practices	Moderate- to high-hazard facilities	• Ensure that programmatic quality and safety standards and requirements are identified and communicated to affected staff. • Ensure that the primary equipment and work processes have written operating procedures, including lockout/tagout procedures. • Ensure that hazardous materials, equipment, and areas are labeled/posted. • Ensure that safety procedures, including hazardous waste procedures, are being followed. • For facilities with multiple shifts, use logbooks to issue directives, instructions, or status change information to incoming staff. • For facilities with control rooms, ensure limited access; ensure that duties of the control room operator are known and available.
Emergency procedures	All occupied facilities	• Identify the facility manager. Ensure current information for reporting an emergency, including an LBNL Emergency Response Guide, is prominently posted. • Ensure that current emergency evacuation signs are posted in all buildings two stories or higher.
Communication systems	All occupied facilities	• Regularly test emergency communication, radios, and public address systems. • Establish operating procedures for local systems. • Ensure that posting and labeling in the facility are managed.

B. Facility Management

LBNL operations take place on its 200+ acre Hill site, on portions of UCB Campus, and in leased space in Berkeley, Oakland, and Walnut Creek. Most of the Laboratory's scientific, administrative, and support activities are housed on the Hill, where LBNL currently occupies approximately 1.7 million gross square feet in over 100 buildings and 50 trailers. The Facilities organization is responsible for establishing a

corporate, holistic, and performance-based approach to real property life-cycle asset management that links real property asset planning, programming, budgeting, and evaluation to program mission projections and performance outcomes. Acquisitions, sustainment, recapitalization, and disposal must be balanced to ensure real property assets are available, utilized, and in a suitable condition to accomplish DOE missions.

2.7 Document and Records Management

2.7.1 Document Control

The institutional Document Control policy is managed by the Information Technology Division.

Document control is applied using a graded approach, which considers:

- Publication in scientific or technical journals
- Monetary investment or value
- Risk levels associated with hazards or operations.

This graded approach is the institutional documented Document Control Policy and addresses all document types. Documents may be hard copy or electronic.

Divisions will identify documents that are considered controlled by reference (e.g. AHDs) or grouping (e.g. SOPs). As necessary, Divisions should formally document the justification of exception from these requirements.

Implementation of document control requirements is outlined in RPM Section 1.0, *Document Control Implementation*.

Document Control Graded Approach Matrix

Document Types	Review/ Approval Process	Distribution Process	Change Control Process	List of Controlled Documents
Institutionally-Managed				
Institutional Documents • RPM	X	X	X	X
Science & Research-Managed				
Final distribution of Scientific or Technical publications to DOE	X	X	N/A	X
Directorate/Division-Managed				
Documents directly supporting projects that require a Quality Assurance Plan (QAP) per DOE O 413.3B for which a breakdown of document control could risk immediate impact on the safety and health of the employee, environment or public.	X	X	X	X
Documents directly supporting work for a High Hazard Facility/Activity for which a breakdown of document control could risk immediate impact on the safety and health of the employee, environment or public. • High hazard facilities are defined as facilities with DOE-approved safety analysis documents and/or LBNL-Directorate approved safety analysis documents. • High hazard activities are defined as activities that require formal work authorizations	X	X	X	X
Documents directly supporting work for a High Operational Risk Activity for which a breakdown of document control could result in immediate degradation of the ability to complete mission activities or substantial financial loss. • RPM section 1.01 establishes applicable listing of high operational risk activity documents.	X	X	X	X'
Documents directly supporting work for medium Hazard Facilities/Activities that have some impact on the safety and health of the employee, environment or public	DBM	DBM	DBM	DBM
Documents directly supporting work for medium Operational Risk Activities that: • Have some impact on science, research, operations, or safety • May result in losses of > $100K or excess costs of >$500K due to inefficiencies	DBM	DBM	DBM	DBM
Not Required				
Documents directly supporting work for low Hazard Facility/Activity that have a minor or negligible impact on safety and health of the employee, environment or public.	-	-	-	-
Documents directly supporting work for low Operational Risk Activity that • Have a minor or negligible impact on science, research, operations, or safety • May result in losses of <$25K or excess costs of <$100K due to inefficiencies	-	-	-	-

X = Required DBM = Determined by Management - = Not required

Note: Desktop instructions or other tools that assist personnel in performing a specific job task and are not used to prescribe actions to be compliant with internal or external requirements are exempt from this policy.

- **Document Control Implementation**

Documents shall be prepared, reviewed, approved, issued, used, and revised to prescribe processes, specify requirements, establish design, or provide results of scientific or technical research and associated activities. Divisions are responsible for ensuring that documents are controlled in accordance with the criteria outlined below to ensure the correct documents are being used.

- **Document Preparation, Review, Approval, and Issuance**

A. Documents are uniquely identified.

B. Documents are reviewed for technical adequacy, correctness and completeness prior to approval and issuance.

C. Documents will identify the individuals or organizations responsible for the review and approval.

D. Review criteria will consider technical adequacy, accuracy, completeness, and compliance with established requirements.

E. Reviews will be performed by individuals other than the originator, as appropriate.

F. Reviews will be performed by individuals who are technically competent in the subject area being reviewed.

G. Approvals will be documented.

- **Document Distribution and Use**

A. Documents will be made available to affected personnel.

B. Effective dates will be established and identified on approved documents.

C. The disposition of obsolete or superseded documents and forms shall be controlled to avoid their inadvertent use.

D. Controls will be established and maintained to identify the current status or revision of controlled document and forms.

- **Document Changes**

A. Changes to documents, other than editorial changes, shall be reviewed and approved.

B. Editorial or minor changes may do not require formal review.

a. The originating individual or organization is responsible for identifying, reviewing and approving editorial changes.

b. Change in an organization title accompanied by a change in responsibilities may not be considered an editorial change.

2.7.2 Records Management

Records Management ensures that records of policies, procedures, activities, and decisions are generated, maintained, and readily retrievable. Information and data that document the organization's research, operational, or administrative activities are retained as evidence of completed work and adherence to standards and procedures. Most organizations should have records filed within their offices for easy retrieval. A records or file inventory must be established and maintained by the organization's administrative unit. Semi-active records must be transmitted to the LBNL Archives and Records Office in accordance with retention and disposition requirements (see RPM 1.17).

2.7.3 Scientific and Technical Publications

Scientific and technical publications are processed through the Report Coordination Office, which assigns report numbers, obtains patent releases, coordinates the printing of required copies, and makes the required DOE/Laboratory distribution. All publications receiving a Laboratory, LBNL/PUB, or LBID number must be reviewed by a designated division reviewer other than the author. Each division is expected to maintain a current list of qualified reviewers designated by the division director and to furnish the Creative Services Office (CSO) with a copy of this list. The review must be completed, and the reviewer must sign a Publications Work Order before the document leaves the Laboratory (see RPM 5.02).

Section 3
Performance Assessment and Improvement

Assessments are performed using a graded approach, based on the risk inherent in the involved organizations, systems, and processes. At a minimum, managers must regularly assess their organizations and functions. As appropriate, independent assessments are performed to provide LBNL managers with additional insight into their operations. Results of management and independent assessments should be considered collectively in improving quality. Findings and corrective actions from all assessments, management and independent, should be tracked in the LBNL Corrective Action Tracking System (CATS), as appropriate. Findings that may benefit the Laboratory community should be considered for dissemination through the LBNL Lessons Learned Program.

3.1 Management Assessment

LBNL managers at all levels must regularly assess the performance of their organizations and functions to determine how well objectives and goals are being met. Assessments by line managers focus on identifying and resolving both singular and systematic management issues and problems that may hinder the organization in achieving its scientific and operational objectives. Managers should assess their processes for the following:

- Planning
- Organizational interfaces (internal and external to the organization)
- Integration of management systems (e.g., safety, security, quality, project)
- Organizational effectiveness, including customer satisfaction
- Use of performance metrics
- Training and qualifications
- Supervisory oversight and support

The management assessments should include an internal evaluation of such conditions as the state of employee knowledge, motivation, and morale; communication among workers; the existence of an atmosphere of creativity and improvement; and the adequacy of human and material resources. The assessments should also involve direct observation of work so that the manager is aware of the interactions at a work location. The observations can be supplemented with worker and customer interviews, safety and performance documentation reviews, and drills or exercises.

The results of management assessments must be documented and used as input to the organization's improvement process. The documentation can include agendas and minutes of staff and operations meetings, progress reports, performance evaluations, inspection reports, and self-assessment reports.

3.2 Independent Assessment

3.2.1 Independent Assessment

Independent assessments advise LBNL managers on the quality of products, services, and processes produced by or for the organization. The type and frequency of independent assessments are based on the

status, complexity, risk, and importance of the activities or processes being assessed. The assessments are performed by technically and programmatically knowledgeable personnel within LBNL who are free of direct responsibility in the areas they assess. The lead assessors must work for organizations that have sufficient authority and independence to gain access to senior Lab managers capable of directing line organizations to take actions in response to the assessment results. LBNL organizations that routinely conduct independent assessments include the Environment, Health and Safety Division; the Internal Audit Services Department; the Office of Institutional Assurance; and the Safety Review Committee. These organizations have established protocols for conducting assessments and providing feedback to the assessed organizations.

Independent assessments include:

- Evaluating work performance and process effectiveness
- Evaluating compliance to the management system requirements
- Identifying abnormal performance and potential problems
- Identifying opportunities for improvement
- Documenting and reporting results
- Verifying satisfactory resolutions of reported problems

Results of independent assessments provide an objective form of feedback to Lab management that is useful in confirming acceptable performance and identifying improvement opportunities. The results must be documented in an assessment report.

3.2.2 Peer Review

Peer reviews are a form of independent assessment. These reviews are used to assure the quality of research and operations, and they are performed by peers in that particular field who have no direct responsibility in the areas being assessed. Peer reviews are often used to review research proposals; review work in progress; review results prepared for publication in professional journals; and review and evaluate the research program for both quality and adherence to missions, goals, and objectives.

3.2.3 ES&H Self-Assessment

The LBNL ES&H self-assessment program is a four-tiered system that focuses on different aspects of Integrated Safety Management (see Section 2.3). Two forms of the ES&H self-assessments are independent and internal assessments of the LBNL divisional environment, safety, and health (ES&H) programs: the Safety Review Committee Management of ES&H (MESH) Review and the Integrated Functional Appraisal (IFA). The MESH review is conducted by LBNL Safety Review Committee members from outside the subject division, and the IFA is conducted by subject matter experts from the Environment, Health and Safety Division.

Assessment	Type of Review	Performed by
Division Self-Assessment	Workplace safety	Line management with EH&S support Independently validated by OCA

Integrated Functional Appraisal	In-depth technical	EH&S subject matter experts
Safety Review Committee MESH	Safety management	Peer researchers and staff with EH&S support
Appendix B Self-Assessment	DOE/UC Contract performance	Functional managers

Program elements and requirements of the LBNL's ES&H self-assessment are described in PUB-5344, *Environment, Safety, and Health Self-Assessment Program.*

3.3 Continuous Improvement

Continuous improvement is a combination of quality improvement and corrective actions that (1) uses feedback information to improve processes, products, and services; (2) prevents or minimizes quality or safety problems; and (3) corrects discovered problems. A quality or safety problem is a collective term that involves a deficiency in an activity, product, service, item characteristic, or process parameter; in an environment, safety, and health requirement; or in a legal and contractual requirement. Managers at all levels have the responsibility to correct deficiencies and improve, whenever possible, the processes, products, and services under their supervision.

3.3.1 Quality Improvement

Quality improvement is a disciplined management process based on the premise that all work can be planned, performed, measured, and improved. Line managers should ensure a focus on improving the quality of processes, products, and services by establishing priorities, promulgating policy, allocating resources, communicating lessons learned, and resolving significant management issues and problems that hinder the organization from achieving its objectives. Management must balance safety and mission priorities when considering improvement actions.

A quality improvement process includes:

- Planning work and allocating resources to account for quality and safety in work process
- Reviewing information and data on processes, products, and/ or services to identify conditions adverse to quality and safety
- Reviewing and analyzing assessment results, including the collective results from complementary assessments.
- Analyzing the adverse conditions and determining the causes
- Segregating the processes, products, or services if the adverse conditions may lead to significant consequences, as determined by line management
- Developing corrective actions to address adverse conditions and prevent recurrence (e.g., reducing process variability or cycle time)
- Implementing the approved actions
- Evaluating the improvements or corrections and assuring customer satisfaction
- Providing lessons learned to other organizations

The quality improvement process should be part of the normal operation of all Berkeley Lab working groups and should be documented in the normal operational records and reports (e.g., minutes from staff and operations meetings; progress and activity reports; readiness reviews; assessment and inspection reports). Conditions that have significant adverse consequences require separate disposition reports that document the actions taken to correct the problems.

3.3.2 Corrective Action

Identified findings, concerns, and deficiencies should be addressed as soon as possible. If the findings cannot be immediately resolved, corrective actions should be tracked in CATS.

A corrective action plan is often necessary for findings that require multiple corrective actions implemented over an extended time period (i.e. several weeks). A corrective action plan (CAP) must be prepared by the responsible manager to allow for additional planning and scheduling. The corrective action plan may require senior Lab management review and approval to address risk management, funding, and resource allocation issues. Once approved by the appropriate Laboratory authority, the individual corrective actions from the CAP are tracked to completion and management verification in CATS.

The Office of Contract Assurance provides regular status reports on the corrective actions to advise Laboratory management on progress and completion. The Office of Contract Assurance, EH&S Division, and others perform trending and root cause analysis to prevent recurrence of the finding, concern, or deficiency.

3.3.3 Lessons Learned

The LBNL Lessons Learned Program, managed by OCA, helps the Laboratory community learn from its experiences, both positive and negative. Through various sources, the program identifies and analyzes adverse events such as accidents and near misses, and communicates the causes and corrective actions to prevent their recurrence. Lessons Learned also communicate best practices from which others may benefit. The ultimate goal of the program is to continually improve performance across all Laboratory functions.

The program uses several different sources of information. The most common sources are lessons developed in response to adverse conditions, accidents, near misses, and best practices that occur at the Laboratory. Other sources include DOE Lessons Learned websites and Lessons Learned websites from other DOE Office of Science Laboratories.

Appendices

Appendix A
The Graded Approach Methodology at LBNL

Introduction

A graded approach is used to determine the rigor with which the elements of the Operating and Quality Management Plan (OQMP) should be applied to a given Laboratory activity. The objective of the graded approach is to ensure that work activities are managed commensurate with the risks involved. Risks include potential impact to staff and/or public health and safety, threats to the environment, institutional and programmatic consequences of noncompliance, and Laboratory mission and cost impacts.

The methodology for assessing hazards and risks in order to grade processes, activities, or facilities follows. Alternate methodologies, such as the use of DOE guidance documents or evaluation of existing hazard documentation, may be used if the rationale is appropriately documented and approved.

Methodology

- In determining risk, Laboratory managers must assess the activities associated with a facility or function and consider the primary risks inherent in these activities. Once risks are identified, managers must evaluate the potential impact of an adverse event or condition and the likelihood of occurrence. Risk is a function of the negative consequence that may result if an appropriate level of management control is not applied to prevent these negative consequences.
- Managers should evaluate the potential impact or consequence of an event or condition by considering the eight risk-potential categories described in the LBNL Risk-Based Priority Planning Grid (Table A-1). Three sets of consequence statements are provided for each category: high risk (H), moderate risk (M), and low risk (L).
- Critical to assessing risk is a measure of the probability that an event will occur. In analyzing the risk inherent in each activity, managers must estimate the likelihood that the potential risk level may be encountered. Operating experience, commonly accepted statistical probabilities, best-management information, or other relevant data can be used to estimate the likelihood of an adverse occurrence. Care should be taken to consider cost-effectiveness when developing management controls for an event. Laboratory line managers should balance the probability of an event occurring and the potential impact against the potential consequence (or cost) of achieving an effective set of controls.
- Based on this risk analysis, line management determines the rigor to use in applying the OQMP requirements to their operations. As higher risk activities require a higher level of rigor, managers use this approach to determine requirements for documentation, training, and other controls. For example, a high-risk activity may require a formal authorization, as required by chapter 6 of PUB-3000, while a similar low-risk activity may rely on standard operating procedures or guidelines (e.g., RPM, PUB-3000, or standard laboratory, shop, or business practices).

- As conditions change, as a result of the self-assessment process, or as performance problems are identified, the graded approach for each facility and function is reviewed to determine whether OQMP requirements continue to be met in an appropriate and cost-effective manner.

Table A-1. Risk Analysis Using the Berkeley Lab Priority Planning Grid (Risk-based)

For each risk category, pick the statement that best characterizes the potential consequence of a failure to apply quality assurance principles to your activity.

RISK CATEGORY		CONSEQUENCE CATEGORY		
		High	**Moderate**	**Low**
ES&H HAZARD	Public safety	Loss of life or serious injury; exposure to hazardous materials in excess of standards	Reportable non-process-related accident	Minor nonreportable events
	Researcher and staff safety	Loss of life or serious injury; exposure to hazardous materials in excess of standards	Reportable onsite work accident involving lost work time or restricted duty; exposure near acceptable limits	Minor events not resulting in hospitalization; exposures below 20% of limits
	Environmental protection	Serious damage to the environment	Release of hazardous material exceeding established limits; repairable damage	Unplanned release within established limits; minor reportable events
REGULATORY	Compliance with law, contract agreement, regulation	Major noncompliance with laws or regulations with significant possible penalties; major contractual noncompliance with potential impact on conditional payment of fee.	Major noncompliance with laws or regulations but not involving significant possible penalties; major contractual noncompliance.	Minor technical or administrative violation(s); little or no adverse regulatory results; minor contractual noncompliance.
	Best management practice		Significant deviation from good practice	Minor deviation or slow implementation
PROGRAMMATIC	LBNL mission/ programmatic impact/LBNL support services	Failure to meet critical milestone; could lead to LBNL shutdown; nondelivery of significant services; results in corrective action by DOE	Failure to meet internal DOE program commitments; high-impact service reductions	Minor degradation in performance, cost, or schedule
	Laboratory protection	Facility or equipment damage >$500k	Facility or equipment damage <$500k; increased operations cost to $250k	Equipment damage or operations cost to $50k
	Public perception	National press coverage; public demonstrations	Local press coverage; some public concern by special-interest groups	Little or no public concern

Appendix B
Integrated Safety Management (ISM)

Table B-1. Seven ISM Guiding Principles

ISM Provision	Resource/Policy References	Sample Mechanisms
Line management is responsible for the protection of the public, the workers, and the environment.	RPM, Chap. 7 PUB-3000, Chap. 1 & 6 PUB-3140 OQMP, Section 1.1	• Organization charts (roles and responsibilities) • Position descriptions
Clear and unambiguous lines of authority and responsibility for ensuring safety are established and maintained.	RPM, Chap. 7 PUB-3000, Chap. 1 & 6 PUB-3140 OQMP, Section 1.1	• Organization charts (roles and responsibilities) • Position descriptions • Authorizations via SADs, AHDs, RWAs, and division-approved protocol
Personnel possess the experience, knowledge, skills, and abilities necessary to discharge their responsibilities.	RPM, Section 2.01 PUB-3140 OQMP, Section 1.3 PUB-3000, Chap. 24	• Position descriptions • PRD performance evaluations • Authorizations via SADs, AHDs, RWAs, and division-approved protocol • ESH training
Resources are effectively allocated to address safety, programmatic, and operational considerations.	PUB-3140 OQMP, Section 1.2 PUB-5344	• Work plans • Research proposals (e.g., WFO, FTP) • NEPA/CEQA • Division self-assessment/MESH
Before work is performed, the associated hazards are evaluated and an agreed-upon set of safety standards and requirements is established.	PUB-3000 PUB-3140 OQMP, Section 2.2	• Authorizations via SADs, AHDs, RWAs, and division-approved protocol • NEPA/CEQA • EH&S functional programs
Administrative and engineering controls to prevent and mitigate hazards are tailored to the work being performed and associated hazards.	PUB-3000 PUB-3140 OQMP, Section 2.2	• Authorizations via SADs, AHDs, RWAs, and division-approved protocol • NEPA/CEQA • EH&S functional programs
The conditions and requirements to be satisfied for operations to be initiated and conducted are clearly established and agreed upon.	PUB-3000 PUB-3140 OQMP, Sections 1.2, 2.2	• Authorizations via SADs, AHDs, RWAs, and division-approved protocol • NEPA/CEQA

Table B-2. Five ISM Core Functions

ISM Function	Resource/Policy References	Sample Mechanisms
Define the scope of work.	OQMP, Sections 1.2, 2.2 PUB-3140	• Work plans • Research proposals (e.g., WFO, FTP) • NEPA/CEQA • Authorizations via SADs, AHDs, RWAs, and division-approved protocol
Identify and analyze hazards associated with the work.	PUB-3000 OQMP, Section 2.2.1 PUB-3140	• Work plans • Research proposals (e.g., WFO, FTP) • NEPA/CEQA • Authorizations via SADs, AHDs, RWAs, and division-approved protocol • Self-assessment
Develop and implement hazard control.	PUB-3000 OQMP, Sections 2.2.1, 2.2.2 PUB-3140	• Work plans • Research proposals (e.g., WFO, FTP) • NEPA/CEQA • Authorizations via SADs, AHDs, RWAs, and division-approved protocol • Self-assessment
Perform work within controls.	PUB-3000 OQMP, Section 2.2 PUB-3140	• Work plans • Research proposals (e.g., WFO, FTP) • NEPA/CEQA • Authorizations via SADs, AHDs, RWAs, and division-approved protocol
Provide feedback on adequacy of controls, and continue to improve safety management.	PUB-5344 OQMP, Section 3.3 PUB-3140	• Self-assessment • Integrated functional appraisals • IASA appraisals • CATS

Appendix C
Policy and Procedure for Controlling Suspect/Counterfeit Items

A suspect item is one in which there is an indication by visual inspection, testing, or other information that it may not conform to established government or industry-accepted specifications or national consensus standards. A counterfeit item is a suspect item that is a copy or substitute without legal right or authority to do so, or one whose material, performance, or characteristics are knowingly misrepresented by the vendor, supplier, distributor, or manufacturer. The use of suspect/counterfeit items (S/CIs) can lead to unexpected failures and undue risk of mission impacts, environmental impacts, and personal injury, contamination, or death. For these reasons, LBNL has instituted mitigating measures for the prevention, detection, and disposition of S/CIs at the Laboratory.

Identification

The range of items at the Laboratory that should be considered as possible S/CIs includes the following:

- High-strength fasteners (bolts, screws, nuts, and washers)
- Electrical/electronic components: circuit breakers, current and potential transformers, fuses, resistors, switch gear, overload and protective relays, motor control centers, heaters, motor generator sets, DC power supplies, AC inverters, transmitters, computer components, semiconductors
- Piping components: fittings, flanges, valves and valve replacement products, couplings, plugs, spacers, nozzles, pipe supports
- Pre-formed metal structures, elastomers (O-rings, seals), spare/replacement kits from suppliers other than original equipment manufacturers, weld filler material, diesel generator speed governors and pumps

DOE maintains a list of S/CIs and identification guidance on the DOE web site at http://www.sci.doe.gov.

Procurement

1. Any item known to have been counterfeited in the past (e.g., Grades 5 and 8 high-strength bolts, circuit breakers, and other S/CIs listed on the DOE S/CI web site), particularly items intended for use in safety systems or critical applications, should be procured only from qualified or dedicated suppliers. The LBNL Procurement Department can qualify suppliers and provide technical specifications and quality clauses prohibiting delivery of S/CIs in the purchase orders and contracts.
2. High-strength fasteners (graded bolts, screws, nuts, and washers) must be purchased directly through the Procurement Department. Procurement buyers will purchase from prequalified suppliers and will retain the manufacturer's certificate of conformance and/or certified material test report. Once on site, high-strength fasteners must be segregated and secured from the general stock to eliminate mixing with nongraded fasteners and to prevent general purpose use.

3. Onsite stores, shops, and end users should inspect newly received items known to have been counterfeited in the past. S/CI identification guidance is provided on the DOE S/CI web site. Periodic inspection of open stock and storage areas should continue to ensure they have been purged of S/CIs.

Installed Items

1. During routine Laboratory inspections of facilities and equipment (e.g., self-assessment, EH&S functional inspections, maintenance and construction inspections), consideration should be given to identify S/CIs (identification guidance provided on the DOE S/CI web site). Additional training for personnel to recognize S/CIs can be arranged through the Office of Contract Assurance (OCA).
2. If an installed item is suspected of being an S/CI, OCA must be contacted to coordinate any engineering evaluation, verification testing, or disposition process.
3. If it is determined that the S/CI in safety systems and critical applications (e.g., heavy equipment, critical load paths in lifting equipment, and facility structures) can adversely affect the environment or create a safety hazard, the system or application must be locked/tagged out and the S/CI removed and replaced. If there is no adverse affect or creation of a hazard, the S/CI must be identified and entered into the LBNL Corrective Action Tracking System (CATS), and either removed and replaced during routine maintenance or determined to remain in place. If the S/CI is to remain in place, the structure or equipment must be tagged or marked, and the CATS entry so annotated and closed out.
4. For non-safety systems and noncritical applications, the S/CI must be identified and entered into CATS, and either removed and replaced during routine maintenance or determined to remain in place. If the S/CI is to remain in place, the structure or equipment must be tagged or marked, and the CATS entry so annotated and closed out.

Disposition and Reporting

1. If an S/CI requires removal, OCA must coordinate and document the disposition. S/CIs must be removed from the work/use site and transferred to the LBNL Warehouse to be temporarily stored in a segregated area. Efforts will be made by the Procurement Department to identify the supplier, manufacturer, or distributor to seek restitution for the Laboratory.
2. OCA must report all discovered S/CIs to the local DOE Office of Inspector General (OIG) and the cognizant DOE operations office manager by means of the Occurrence Reporting and Processing System (ORPS).
3. After the S/CI is no longer needed as material evidence by OIG, the LBNL Warehouse will coordinate the destruction or alteration of the S/CI to render them unusable.
4. OCA will perform quarterly trending of suspect/ counterfeit item discoveries. Results will be reported in the quarterly Performance Analysis and Identification of Recurring Occurrences. As appropriate, lessons learned will be communicated via the DOE and LBNL lessons learned programs.

Appendix D
LBNL Conformance with Quality Assurance Requirements and Standards

The LBNL Quality Assurance Program, as documented in its Operating and Quality Management Plan (OQMP), PUB-3111, Rev. 8, conforms to all requirements identified in the Contractor Requirements Document (CRD) of DOE Order 414.1C, *Quality Assurance*. The LBNL OQMP also integrates the principles and practices of ANSI/ISO/ASQ Q 9001-2000 so as to conform as applicable and practicable to the international quality assurance standard.

DOE O 414.1C, Attach. 2, CRD	10 CFR 830, Subpart A	ANSI/ISO/ASQ Q 9001-2000	LBNL OQMP (PUB-3111, Rev. 8)
2.a Quality Assurance Program Development and Implementation Assign and identify a senior management position responsible for the development, implementation, assessment, and improvement of a QAP that does the following: (1) Use the graded approach (2) Use national or international consensus standards (3) Apply additional voluntary standards (4) Integrate with other quality or management systems		5.1 Management Commitment 5.2 Customer Focus 5.3 Quality Policy 5.4.1 Quality Objectives 5.4.2 Quality Management System Planning 5.5.2 Management Representative 6.1 Provision of Resources	Statement of Laboratory Policy (pg vi) Objectives and Applicability (pg. viii) Offices of Institutional and Contract Assurance (pg. viii) Section 2, Management Systems and Process Controls Section 2.6.3, Consensus Standards Appendix A, The Graded Approach Methodology at LBNL
2.b Quality Assurance Program Approvals and Changes (1) Submit a QAP to DOE for approval (2) Implement the QAP (3) Indicate any third-party certification (4) Revise an existing QAP to address enhancements required by this CRD (5) Regard a QAP as approved by DOE 90 calendar days after DOE receipt, unless approved or		4.1 Quality Management System - General Requirements 4.2.1 Documentation Requirements - General 4.2.2 Quality Manual	PUB 3111, Rev. 8, is the LBNL documented quality management and quality assurance manual.

DOE O 414.1C, Attach. 2, CRD	10 CFR 830, Subpart A	ANSI/ISO/ASQ Q 9001-2000	LBNL OQMP (PUB-3111, Rev. 8)
rejected by DOE at an earlier date (6) Submit QAP changes made the previous year annually to DOE for review and approval			
2.c Quality Guidance Usage The Contractor must consider QA guidance in developing and implementing a QAP			LBNL uses for its QA guidance: *DOE G 414.1-1A, DOE P 450.4, DOE P 450.5, DOE G 414.1-2A, DOE G 414.1-3, DOE G 414.1-4.*
3.a Management/Criterion 1—Program. (1) Establish an organizational structure, functional responsibilities, levels of authority, and interfaces for those managing, performing, and assessing work. (2) Establish management processes, including planning, scheduling, and providing resources for work.	1. Management/ Program	5.5.1 Responsibility and Authority 5.5.3 Internal Communication 6.4 Work Environment	Section 1.1, Organizational Structure Section 1.2, Planning Section 2.1, Managing Principles
3.b Management/Criterion 2—Personnel Training and Qualification. (1) Train and qualify personnel to be capable of performing assigned work. (2) Provide continuing training to personnel to maintain job proficiency.	2. Management/ Personnel Training and Qualification	6.2.1 Human Resources - General 6.2.2 Competence, Awareness, and Training	Section 1.3, Staff Proficiency
3.c Management/Criterion 3—Quality Improvement. (1) Establish and implement processes to detect and prevent quality problems. (2) Identify, control, and correct items, services, and processes that do not meet established requirements. (3) Identify the causes of problems, and include prevention of recurrence as a part of corrective action planning. (4) Review item characteristics, process implementation, and other quality-related information to	3. Management/ Quality Improvement	8.5.1 Continual improvement 8.5.2 Corrective Action 8.5.3Preventive action	Section 3.3, Continuous Improvement Section 3.3.1, Quality Improvement Section 3.3.2, Corrective Action Section 3.3.3, Lessons Learned

DOE O 414.1C, Attach. 2, CRD	10 CFR 830, Subpart A	ANSI/ISO/ASQ Q 9001-2000	LBNL OQMP (PUB-3111, Rev. 8)
identify items, services, and processes needing improvement.			
3.d Management/Criterion 4—Documents and Records. (1) Prepare, review, approve, issue, use, and revise documents to prescribe processes, specify requirements, or establish design. (2) Specify, prepare, review, approve, and maintain records.	4. Management/ Documents and Records	4.2.3Control of documents 4.2.4Control of Records	Section 2.7, Document and Records Management
3.e Performance/Criterion 5—Work Processes. (1) Perform work consistent with technical standards, administrative controls, and hazard controls adopted to meet regulatory or contract requirements using approved instructions, procedures, etc. (2) Identify and control items to ensure proper use. (3) Maintain items to prevent damage, loss, or deterioration. (4) Calibrate and maintain equipment used for process monitoring or data collection.	5. Performance/ Work Processes	6.3 Infrastructure 7.1 Planning of product realization 7.2.1 Determination of requirements related to the product 7.2.2 Review of requirements related to the product 7.2.3 Customer communication 7.5.1 Control of production and service provision 7.5.2 Validation of processes for production and service provision 7.5.3 Identification and traceability 7.5.4 Customer property 7.5.5 Preservation of product 8.2.4 Monitoring and measurement of product 8.3 Control of nonconforming product 8.4 Analysis of data	Section 2.1, Managing Principles Section 2.2, Safety Management Section 2.3, Environmental Management Section 2.4, Safeguard and Security Management Section 2.5, Other LBNL Management Systems Section 2.6.1, Core Functions Section 2.6.2, Written Procedures, Instructions, and Drawings Section 2.6.3, Consensus Standards Section 2.6.6 Function-Specific Controls Section 2.6.7, Facility-Specific Controls
3.f Performance/Criterion 6—Design. (1) Design items and processes using	6. Performance/ Design	7.3.1 Design and development planning 7.3.2 Design and	Section 2.6.6.A, Function-Specific Controls, Design

DOE O 414.1C, Attach. 2, CRD	10 CFR 830, Subpart A	ANSI/ISO/ASQ Q 9001-2000	LBNL OQMP (PUB-3111, Rev. 8)
sound engineering/scientific principles and appropriate standards. (2) Incorporate applicable requirements and design bases in design work and design changes. (3) Identify and control design interfaces. (4) Verify/validate the adequacy of design products using individuals or groups other than those who performed the work. (5) Verify/validate work before approval and implementation of the design.		development inputs 7.3.3 Design and development outputs 7.3.4 Design and development review 7.3.5 Design and development verification 7.3.6 Design and development validation 7.3.7 Control of design and development changes	
3.g Performance/Criterion 7—Procurement. (1) Procure items and services that meet established requirements and perform as specified. (2) Evaluate and select prospective suppliers on the basis of specified criteria. (3) Establish and implement processes to ensure that approved suppliers continue to provide acceptable items and services.	7. Performance/ Procurement	7.4.1 Purchasing process 7.4.2 Purchasing information 7.4.3 Verification of purchased product	Section 2.6.6.B, Function-Specific Controls, Procurement
3.h Performance/Criterion 8—Inspection and Acceptance Testing. (1) Inspect and test specified items, services, and processes using established acceptance and performance criteria. (2) Calibrate and maintain equipment used for inspections and tests.	8. Performance/ Inspection and Acceptance Testing	7.6 Control of Monitoring and Measuring Devices 8.1 Measurement, Analysis, and Improvement - General 8.2.1Customer satisfaction	Section 2.6.6.C, Function-Specific Controls, Manufactured Items Inspection and Testing Section 2.6.6.D, Function-Specific Controls, Construction Inspection and Testing
3.i Assessment/Criterion 9—Management Assessment. Ensure that managers assess their management processes and identify and correct problems that hinder the	3. Management/ Quality Improvement 9. Assessment/ Management	5.6.1 Management Review - General 5.6.2 Review input 5.6.3 Review output 8.2.3 Monitoring and	Offices of Institutional and Contract Assurance (pg. viii) Section 3.1, Management Assessment Section 3.3.2, Corrective Action

DOE O 414.1C, Attach. 2, CRD	10 CFR 830, Subpart A	ANSI/ISO/ASQ Q 9001-2000	LBNL OQMP (PUB-3111, Rev. 8)
organization from achieving its objectives.		measurement of processes	
3.j Assessment/Criterion 10—Independent Assessment. (1) Plan and conduct independent assessments to measure item and service quality, to measure the adequacy of work performance, and to promote improvement. (2) Establish sufficient authority and freedom from line management for independent assessment teams. (3) Ensure that persons conducting independent assessments are technically qualified and knowledgeable in the areas to be assessed.	10. Assessment/ Independent Assessment	8.2.2 Internal audit	Section 3.2, Independent Assessment
4.a Supplemental Quality Management System Requirements for Suspect / Counterfeit Items An S/CI prevention process must be developed and implemented as a part of the contractor's QAP and must be commensurate with the facility/activity hazards and mission impact.	8. Performance/ Inspection and Acceptance Testing		Section 2.6.4 Suspect/Counterfeit Items Appendix C, Policy and Procedure for Controlling Suspect/Counterfeit Items
4.b Work Process Controls Work processes must be developed and implemented using available S/CI information.			Appendix C, Policy and Procedure for Controlling Suspect/Counterfeit Items
5. Safety Software Quality Requirements Work processes involving safety software must be developed and implemented.	6. Performance/ Design		Section 2.6.5, Safety Software Quality Assurance

Appendix 9: Cel Analyitical Laboratory QAP

cel analytical, inc.
water, wastewater & sludge Laboratory Services

Laboratory *Quality* Assurance/Quality Control Manual

Version 5
Effective Date: 04/12/2011

cel analytical, inc.
water, wastewater & sludge Laboratory Services

Laboratory *Quality* Assurance/Quality Control Manual

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Acknowledgement

This Quality Assurance Manual (QA) is prepared by Cel Analytical, Inc. to describe in detail the Policies and identify Standard Operating Procedures (SOPs) pertinent to day-to-day activities in the laboratory. The manual incorporates the Environmental Protection Agency's good laboratory practice regulations and guidelines designed to meet the Safe Drinking Water Act and Clean Water Acts quality requirements. . The QA manual is periodically reviewed and updated with new methods and procedures as they become available. Cel-A requires thorough familiarity with the contents of this manual from all laboratory personnel. The QA Plan is also available to our clients upon request.

Each version includes, addition of new Field of Testing, updates on reference manuals, personnel updates, inter and intra laboratory audits.

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Revision 2: 12-04-2008 New FOTs ---------- *Date*----------

Reviewed by:

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Revision 3:1/17/2009 New FOTs: Shell Fish Recreational Water &General Chemistry FOT- WW

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Revision 4:09/01/201New FOTs-Trace metals DW/WW

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Revision 5:04/12/2011 New FOTs-Trace metals /Chromium VI-hazardous waste

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Table of Content

Table of Content

FIGURES:

LIST OF TABLES:

APPENDICES:

APPENDICES:

FIGURES:

LIST OF TABLES:

APPENDICES:

APPENDICES:

1.0 INTRODUCTION

Cel Analytical Inc. (Cel-A) is an environmental testing laboratory based in San Francisco, CA, focusing on microbiology and inorganic chemistry of water, wastewater and sludge. Cel-A has the capability and expertise to identify indicator bacteria, yeast, selected pathogens and bacteriophages and to test for various inorganic compounds in drinking water (DW), surface water (SW) and wastewater (WW). Cel-A provides accurate analytical data through established industry standards and methods or in-house developed rapid methods for contracts obtained through commercial or industrial facilities or governmental agencies.

As a contract laboratory, we hold ourselves to high quality testing through the commitment, support and participation of every member of our well-trained and educated staff. We adhere to deliver results in a timely manner and offer the technical expertise and advice of our trained laboratory personnel directly involved in the testing when needed. Cel-A participates in method development studies, as well as testing the applicability of new rapid microbiological methods for environmental analysis.

We hold memberships to the American Society of Microbiology and AOAC International and subscribe to a number of scientific journals to stay current with new trends in the analytical sciences.

Cel-A is committed to ensure high quality laboratory performance. Our Quality Assurance & Quality Control (QA/QC) manual was developed to provide laboratory personnel and our clients with a description of the laboratory's quality control practices and quality assurance program. This manual ultimately dictates the quality and integrity of data generated at Cel A .

2.0 PURPOSE AND OBJECTIVES

The QA/QC Plan herein describes the standard operating procedures required for day-to-day operations at Cel-A to establish compliance with quality assurance and quality control guidelines for laboratories as described in EPA Manual for The Certification of Laboratories Analyzing Drinking Water, 5th edition 2005 [2]; Manual of Environmental Microbiology, ASM press 2004; Standard Methods for Examination of Water and Waste Water (APHA 20th Edition)[3] EPA SW846 solid waste hazardous waste test methods manual 3rd edition; and fulfills the requirements of the State and Federal Regulatory Programs [7,8,9].

This manual identifies the management personnel and laboratory staff and their responsibilities, provides a description of the facility and its operation capabilities and outlines a frame work for Cel-A personnel to establish good laboratory practices and standard operating procedures that secure sound scientific data. The QA/QC plan will be updated with new regulations and methodologies as they become available and will include revisions of specific requirements and a written amendment to document all

1.0 INTRODUCTION

Cel Analytical Inc. (Cel-A) is an environmental testing laboratory based in San Francisco, CA, focusing on microbiology and inorganic chemistry of water, wastewater and sludge. Cel-A has the capability and expertise to identify indicator bacteria, yeast, selected pathogens and bacteriophages and to test for various inorganic compounds in drinking water (DW), surface water (SW) and wastewater (WW). Cel-A provides accurate analytical data through established industry standards and methods or in-house developed rapid methods for contracts obtained through commercial or industrial facilities or governmental agencies.

As a contract laboratory, we hold ourselves to high quality testing through the commitment, support and participation of every member of our well-trained and educated staff. We adhere to deliver results in a timely manner and offer the technical expertise and advice of our trained laboratory personnel directly involved in the testing when needed. Cel-A participates in method development studies, as well as testing the applicability of new rapid microbiological methods for environmental analysis.

We hold memberships to the American Society of Microbiology and AOAC International and subscribe to a number of scientific journals to stay current with new trends in the analytical sciences.

Cel-A is committed to ensure high quality laboratory performance. Our Quality Assurance & Quality Control (QA/QC) manual was developed to provide laboratory personnel and our clients with a description of the laboratory's quality control practices and quality assurance program. This manual ultimately dictates the quality and integrity of data generated at Cel A .

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changes made to the plan. The laboratory QA/QC manager will put into practice this QA plan and its requirements.

Laboratory QA/QC practices are described here with reference to the internal SOPs that are available in the laboratory.

3.0 ORGANIZATION & RESPONSIBILITIES

Cel Analytical constitutes a permanent laboratory with organizational structure and staff responsibilities described below:

Director/QC Manager: Oversees daily operation and validates results produced in the laboratory. Implements and puts into action all quality control measures and quality assurance policies in this manual, providing information and training to staff. She also participates in research and development of novel methods.

Principal Analyst/QC Officer: Works at the bench and is involved in all aspects of the analysis. She/He also prepares and organizes final reports for each project and ensures that equipment is properly maintained and calibrated and orders supplies.

Field Analysts: Responsible for all aspects of sample collection, preservation and delivery to the lab. He/She consults with analysts on the type of sample containers needed per project and records appropriate field observations on field logs along with sample information in the chain of custody documentation.

Administrator and Financial Officer: Manages the administrative and financial aspects of the laboratory, including accounts payable, accounts receivable, and responds to clients' correspondence.

Safety Officer: Assists in overseeing the safety requirements to work in the laboratory. Trains personnel on safety and regulatory issues; maintains an up to date safety manual including Material Safety Data Sheets for all chemicals used in the laboratory.

Combined Positions: meeting the provided qualifications and responsibilities of each position, an individual may carry out more than one position, as long as all analytical work is reviewed by a second qualified person.

Cel Analytical Laboratory currently employs:

- A PhD Microbiologist/Biochemistry serving as Laboratory Director/Quality Assurance Manager/Principal Analyst.
- A PhD Biochemist serving as Quality Control Manager/Analyst
- Analyst/QA/QC Manager
- Laboratory Analyst (Three)
- Two Field Analysts trained in sample collection, and recording appropriate field observation(s) along with sample information on the chain of custody documents.

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Once the laboratory is certified and a client list established, Cel Analytical will hire trained professionals to fill additional positions described above.

Sub-consulting services: Cel-A obtains the services of Envirosurvey, Inc., a health, safety and environmental consulting firm in San Francisco, on a contract basis for the preparation of compliance plans for operations in the laboratory such as the health and safety plan including the Injury and Illness Prevention Program and Hazard Communication Program in accordance with Cal-OSHA regulations.

We are committed to making sure that all management and personnel are free from any undue pressure that may adversely affect the quality of their work at all times. Cel-A upholds our sub-consulting firms to the same high level of professionalism and data quality practiced in-house.

4.0 LABORATORY FACILITY

4.1 Facility Floor Plan

The laboratory facility as depicted in Figure 1, is approximately 1200 square feet (ft^2) of work space consisting of:

1) 500 (ft^2) - primary work area and some of the equipment required for a microbiology and chemistry laboratory. A list of all laboratory equipment is presented in Appendix D. Maintenance and calibration frequencies of selected equipment are described in section (10.1) of this manual;

2) 200 (ft^2) - office, adjacent to the laboratory, where the laboratory personnel can document sample receiving and sample logging;

3) 500 (ft^2) (approx.) - lower laboratory area that is accessed by the stairs adjacent to the laboratory office area. This area houses a glassware storage cabinet, a freezer, a refrigerator, an autoclave; Stations for chemical analysis.

4) There are also separate reception and eating areas on the main floor. The main laboratory work area has an access door to the outside that can be used as an emergency exit.

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Figure 1. Facility Floor Plan

4.2 Security and Safety

Entry to the laboratory is strictly limited to qualified personnel. The access doors to the offices and lobby are closed at all times.

For safety purposes Cel Analytical rigorously implements and ensures at all times that:

- Laboratory biosafety level 2 guidelines are attained and kept to.
- All personnel wear appropriate lab coats while working in the lab.
- Personnel in the laboratory do not engage in mouth pipetting.
- Personnel do not consume or store food or drinks in the laboratory.
- Laboratory access doors have signs classifying biohazards.
- All laboratory personnel receive laboratory safety training and are made aware that when isolating microorganisms, pathogens may be present in the environmental samples.
- All laboratory personnel are made aware that water samples may contain toxic substances and that they need to wear the appropriate safety equipment when sampling and working with these samples.
- The laboratory's biological waste disposal program meets the terms of the City and County of San Francisco, Department of Public Health and the State of California Regulations on biological waste disposal.

Safety equipment such as an eyewash station and fire extinguishers are readily accessible to all personnel. All personnel are required to have a copy of the laboratory health and safety plan and must become familiar with its content prior to engaging in any laboratory work activity.

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5.0 LABORATORY SAMPLE MANAGEMENT

5.1 Sampling Procedures

The legitimacy and accuracy of the analytical process relies not only on good laboratory practices but also on proper sampling. Whether the sample collector is a Cel-A field personnel or an outside source, they should have adequate training in sampling procedures, especially aseptic sampling for microbiology samples. As part of the in-house training, field sampling technicians will be trained for proper sampling, field observation, adequate sample recording and field safety issues. A list of SOPs referenced in this section is available upon request. The sampling protocols, depending on the water type are outlined below and described in SOP#SP-00. For clients collecting their own samples, we recommend that they adhere with these sampling procedures.

5.1.1 Microbiology

In general, grab sampling technique is employed for water unless specified otherwise. Water samples are collected singly or in multiples into 1L or 500mL sterile bottles with ample head space (~ 2 inches) to facilitate mixing and sample preparation at the laboratory. Sampling must be conducted as outlined in The Standard Methods for the Examination of Water and Wastewater APHA 20th ed. (section 1060 for chemistry samples and section 9060 for microbiology samples). All water samples are collected in clean, pre-sterilized borosilicate glass or plastic bottles with the appropriate preservatives when required.

Drinking/Potable water: If water is collected from the distribution system, select a tap from the service pipe directly connected to the intake point. Do not take tap water collected in a storage tank. Allow the tap water to run for 2-3 min and using aseptic techniques collect the sample. If sampling is conducted to determine the integrity of the tap first, disinfect the faucet inside and out with a solution of sodium hypochlorite (NaOCl) at a concentration of 100mg/L. Then run the water for 2-3 minutes before collecting the sample. Make sure that there are no aerators, strainers, hose attachments, and purification devices in the water taps used for sampling. If sampling from a mix faucet (containing hot and cold water) remove the screen first, then run hot water for 2 minutes, followed by cold water for an additional 2-3 minutes before sample collection.

Samples from well water can be collected through a pump after pumping water from the well for 5-10 minutes and the temperature of the water has stabilized. If no pump is available, lower the sample bottle (attach a weight to the base) into the well deep enough to avoid surface contaminants.

Raw water/Source water: Raw water that is a source of supply to consumers may include rivers, streams, lakes, reservoirs and springs. Grab water samples are collected as representative samples of the supply source at a sensible distance from the bank/shore and not very far from the draw off.

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Surface water: may include streams, lakes and runoff. Grab water samples will be collected at designated locations, 50 feet upstream or downstream of the stream flow or recreational area. Attention must be given to the location of nearby waste water treatment plants or municipal water facilities. Sampling frequency for recreational water may be seasonal. Sampling maybe daily for water supply intake and hourly if treated waste water effluent is discharged into critical areas.

Bathing Beaches: Sampling should be conducted in locations upstream and downstream of bathing areas (preferably the most populated areas) and should include locations adjacent to nearby drains. Samples are normally collected from a depth of 1m and may include sand-water mixtures where children are playing.

Sludge/Biosolid: Sewage/sludge generated from wastewater treatment is sampled in accordance with sampling procedures described in the US EPA Pathogen Testing Guidelines. Briefly, if the sample is less than 7% biosolids (viscous fluid), it may be sampled in wide mouth glass or plastic sterile container bottles as they are being transferred from one vessel to another. Appropriate preservatives if required must be added prior to sample collection. For sludge containing over 7% biosolids a hand -auger is used to collect representative samples in a sterile glass jar. For dry sludge/compost comprised of over 30% biosolid, the sludge is preferably spread in a defined area and a grid system is established. Representative samples will be collected from each grid and composited once they arrive in the laboratory.

5.1.2 Inorganic Chemistry

A sample collection kit can be provided with all the necessary bottles, labels, seals, coolers and preservatives. In many cases Cel-A's own Field Personnel will collect or direct the collection of the Client's samples. Sample collection and handling requirements shall conform with Table 1060:I of Standard Method of Examination of Water and Wastewater APHA 20th edition.

5.1.3 Grab vs composite sampling

Grab samples represent a snapshot in time and are the required method of sampling for pH, residual chlorine and any other analyte shown to be unstable over time. Composite sampling gives information about the source over the duration of the sampling time which can be over an hour or over the course of a day. As such, composite sampling provide a more heterogeneous analysis of the sample however, this type of sampling can also dilute out analytes. Refer to the SOP for the particular analyte to determine sampling requirements.

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5.1.4 Sample Plan

Develop a sampling plan prior to collecting the first sample. Identify the method of sampling and exact location of sampling. If clients are sampling on their own we recommend they discuss their sampling plan prior to collecting samples.

5.1.5 Source for water sampling

Distribution systems

If possible, flush the lines with 3 to 5 pipe volumes or if this volume is too high, flush for at least 2 to 3 minutes before sampling. Ignore the flush volumes if the sample represents a "first draw sample".

Well water

Collect samples only after the well has been purged to ensure that the sample is representative of groundwater. Purge all stagnant water.

River or stream

Sampling location is critical to ensure the most accurate sample analyte representation. If possible, sample midstream at mid-depth for grab samples especially. If this is not possible, sample from both sides of the stream, equi-distant from the shore. Avoid areas of increased turbulence and sample by placing submerging the bottle with the opening directed toward the current to avoid collecting scum unless sampling for oil and grease.

Collection

Collect samples in a wide-mouth plastic or glass bottle. Do not pre-rinse the bottle with sample as this can lead to false high levels of analyte due to sticking to the bottle surface. Leave approximately 1% of the container volume as air space. If the bottle contains preservative be sure to fill it to the proper levels to avoid diluting out the preservatives.

5.2 Sample Preservation & Sample Handling

Sample containers that have preservative added will be noted on the label as tothe chemical nature of the preservative. Preservation techniques are guided bythe method being applied. Guidance for sample preservation and holding times is available in such references as: Standard Methods for the Examination of Water and Wastewater, American Society for Testing Materials (ASTM) Environmental Protection Agency (EPA) Methods for Chemical Analyses ofWater and Waste, 40 Codes of Federal Regulations (CFR), Part 136. Appendix F presents guidance for preservatives and holding times extracted from the above mentioned references.

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Microbiology

Samples suspected to contain halogens (e.g. chlorine) or heavy metals such as zinc or copper require the addition of the appropriate preservative to the sample container prior to sampling. Protocol for recommended preservation methods are provided in SOP# SP-01.

Inorganic Chemistry

Sample preservation requirements vary depending on the inorganic parameters to be tested. The appropriate preservation protocols are provided in SOP# IC-01.

Field measurements such as pH, temperature and conductivity are made on a separate sample which is then discarded in order to avoid contaminating samples for additional laboratory analysis. Conductivity measurements should also be done on a sample other than the one used for pH measurement because residual potassium chloride from the pH probe may affect the conductivity reading.

5.3 Sample Acceptance, Storage & Disposal Policies

Sample Custody: Cel-A requires that all samples be accompanied by a chain of custody form (SOP # SP-02) and a sample log sheet (SOP # SP-03). Should samples be obtained from an outside source, the laboratory necessitates proof of the sample's reliability. Samples should be transported preferably in an ice cooler at < 10 °C, away from dust, dirt and fumes as soon as possible to the laboratory.
Sample acceptance policy is outlined in Table 1. Any sample that does not meet Cel-A's outlined policy is flagged and the character and matter of the disparity is defined and documented.

Table 1 Acceptance Criteria

1. Accurate and extensive documentation (i.e. sample identification, location, date/ time of collection, collector's name, preservation type, sample type, and remarks).
2. Correct labeling of samples (i.e. durability of labels, use of permanent ink) containing their unique identification.
3. Utilization of proper sample containers.
4. Observance of required holding times.
5. Sufficient sample volume.
6. In case of damage and/or contamination, appropriate procedures followed.
7. Client notification of sample receipt via email, fax or mail.

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Should the sample not meet the above-specified acceptance criteria, the laboratory shall **consult with the client** in an immediate and timely manner. Protocol for sample receipt is presented in SOP# SP-04.

***Sample Tracking*:** Cel-A documents and individually identifies each item (including samples, sub-samples, or dilutions) to be tested to prevent any mix-ups or errors with respect to the item's identity. Upon receipt, each sample is given its own unique laboratory identification code, with links to its field ID code or client project number. Identification codes can be found on the chain of custody form upon receipt at the laboratory and on sample container(s) in the form of a durable label. All subsequent activities (i.e. preparation, calibration etc.) are documented by the laboratory using identifiable by codes.

Sample Storage: All samples received by the laboratory are kept in a designated and secure storage area, away from standards, reagents, food, and otherwise potential contaminating sources. All samples will have ample refrigerator space, and will be handled by minimal personnel. Once the samples are received the QC manager will sign the sample receipt and samples are stored at the appropriate temperature. It is the responsibility of the QC manager to assure that all microbiological samples are correctly stored and preserved at 1°C to 4 °C. All personnel performing the required analysis obtain samples solely from the QC manager or his/her representative.

Sample Holding Time: Cel-A conducts microbiological of testing of samples as soon as they have been collected and received. If travel and processing time exceeds an hour, an ice cooler or thermos-type insulated sample bottle is utilized during transportation. Cel-A strongly encourages the transportation time of all samples to ***not exceed 6 hours***. Should this not be a possibility, the use of temporary field laboratories is recommended.

For chemical parameters, that need to be analyzed immediately within 15 min, such as pH, dissolved oxygen, dissolved sulfide, samples are analyzed as soon as received by the laboratory. For other parameters, once the appropriate preservative has been added to the sample, it can be refrigerated for a finite amount of time as defined in the regulations prior to analysis.

Upon receipt, all samples are refrigerated and subsequently processed within a 2-hour time frame.. Storage time and temperature is documented, and later taken into account in the interpretation of results. After analysis is complete, the remainder is kept in storage for at least 3 weeks prior to disposal.

Sample Disposal: Upon completion of sample analysis and the required storage time, the laboratory discards the samples in an environmentally responsible way. Table 2 contains sample types and disposal methods. Identification labels along with laboratory records are forwarded to the QC manager, and only disposed of with the approved signature of the laboratory director.

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For chemical parameters, that need to be analyzed immediately within 15 min, such as pH, dissolved oxygen, dissolved sulfide, samples are analyzed as soon as received by the laboratory. For other parameters, once the appropriate preservative has been added to the sample, it can be refrigerated for a finite amount of time as defined in the regulations prior to analysis.

Upon receipt, all samples are refrigerated and subsequently processed within a 2-hour time frame.. Storage time and temperature is documented, and later taken into account in the interpretation of results. After analysis is complete, the remainder is kept in storage for at least 3 weeks prior to disposal.

Sample Disposal: Upon completion of sample analysis and the required storage time, the laboratory discards the samples in an environmentally responsible way. Table 2 contains sample types and disposal methods. Identification labels along with laboratory records are forwarded to the QC manager, and only disposed of with the approved signature of the laboratory director.

Table 2 Disposal Methods

Sample Type	Method of Disposal
RCRA Waste - sample, extracts, digests	Waste disposal contractor.
Acid/base preserved samples not hazardous or toxic	Neutralized in the laboratory and removed by the liquid waste hauler.
Bacterial waste	Medical waste management facility.
Samples – not preserved	No regulated constituent shall be disposed into sanitary sewer

In accordance with the provisions of the San Francisco Health Code, Part II, Chapter V, Article 25 Cel-A laboratory is certified by the Hazardous Material Unified Program Agency as a small quantity generator of medical waste, and has received a California EPA Identification Number from the Department of Toxic Substances Control (DTSC). Copies of HMUPA and Cal-EPA Identification documents are provided in Appendix D.

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6.0 ANALYTICAL METHODS

The laboratory thoroughly documents all analytical techniques, including descriptions of microorganisms, the preparation of media, the test procedures and any required confirmatory tests. A reference file available to all staff in the laboratory contains all methods used in the laboratory. In addition, all methods are made available to the lab personnel in hard copy binder.

With the development of new methods, test procedures are changed and replaced with the consent of the laboratory director when proven to be equivalent to or better than the old. New tests are validated and their performance assessed against the old.

6.1 Microbiology:

Cel Analytical has adopted the following methodologies to ensure quality of the laboratory results:

1) Published general microbiology methods pertinent to basic day to day techniques employed at the laboratory.

2) Validated referenced analytical methodologies as required by the Total Coliform Rule and the Surface Water Treatment Rule including:

- Microbiological methods published by USEPA Microbiology Website[1]
- Standard Methods of Examination of Water and Wastewater (APHA 20th edition)[2]
- EPA Analytical Methods for Biological Pollutants in Ambient Water (July 2003[3]
- USEPA Pathogen Testing Guidelines for Sewage Sludge[4]

3) Additional methods used as references in the laboratory include:

- Electronic Compilation of Analytical Methods℠ (e-CAM℠) published by AOAC International [5]
- Manual of Environmental Microbiology 2nd Edition[6]
- Bacteriological Manual (BAM-USFDA) for shellfish meat and shellfish growing water

We record all details of procedures for preparing suspensions of test organisms (for validation purposes) and the practical details of how validation trials are conducted. Methods also include reference to the organisms used as positive and negative controls, employed in isolation procedures and confirmatory tests, as well as quality control tests for assessing accuracy and precision of the methods in use.

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6.2 Inorganic Chemistry

Cel-A uses EPA or Standard Methods approved tests for all its inorganic testing (see Tables 6.3-6.6). SOPs for each compound are on file including sampling handling, test method, calibration standards and data analysis. Equipment SOPs and QC documentation is also on file.

All reagents are American Chemical Society Grade (ACS) or better. Calibration for the majority of inorganic instrumentation employs the use of external standards.
The establishment and verification of the calibration curve for inorganic instrumentation is follows the EPA required minimum-point calibrations for inorganic analytes measured by laboratory instrumentation.

6.3 SOP List

SOP lists are provided in Table 6.1 through Table 6.6. Each SOP is identified by a laboratory SOP number, and the referenced standard method number where available.

Each SOP contains descriptions of laboratory methods, particulars of methods scope, equipment required, preparation of media and reagents, full analytical procedures, calculation and reporting requirements when applicable. SOPs when necessary will cross reference other SOPs by standard method numbers or internal reference numbers.

Cel-A has developing standard operating procedures for performing molecular analysis, particularly quantitative polymerase chain reaction (qPCR) on DNA obtained from food samples samples in accordance to QA/QC Control Guidance of Bacteriological manual and EPA manual for laboratories performing PCR (October 2004),

Table 6.1 General Method SOPs

INTERNAL SOP #	ANALYTICAL METHODS
GAM-01	Methods for Preparation of Dilutions
GAM-02	Culture Transfer Techniques
GAM-03	Techniques for Isolation of Pure Cultures
GAM-04	Preparation of Bacterial Smear
GAM-05	Staining Procedures (Gram Stain)
GAM-06	Staining procedures (Acid Fast)
GAM-07	Mo Bio Laboratory Water DNA Purification Kit

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GAM-07	Mo Bio Laboratory Water DNA Purification Kit

Table 6.2 SOPs for ELAP/EPA Approved Microbiological Methods Certified FOTs Drinking Water, Source Water, Recreational Water, Shellfish growing water & Wastewater

ANALYTE	CEL A SOP#[1]	METHOD FORMAT	TECHNOLOGY/MEDIUM	EPA-SM REF. METHOD[2]
Total Coliform (TC)	TC-010	Membrane Filter Technique (MF) –	m-Endo or m-endo LES broth or agar; coliform verification in LTB & BGLB media Compliance: drinking water MI agar for simultaneous detection of TC/ E.Coli Official method: all waters	SM 9222 A,B,C; EPA-1604
	TC-011	Most Probable Number (MPN) or Multiple Tube Fermentation (MTF)	LTB; 24hr gas(+) or acid(+) confirmed on BGLB medium; completed phase	SM 9221 A,B,C
	TC-012	Presence/Absence (P/A)	Presumptive phase P-A Broth; confirmation in BGLB medium	SM 9221D
	TC-013	Enzyme substrate (Chromogenic/Fluorogenic)	Colisure® (Official method: all water) Colilert™® 18 MTF (Compliance: drinking water)	IDEXX Lab
Fecal Coliforms (FC)	FC-020	Membrane Filter Technique (MF)-	A1 medium Direct ; 3hrs @ 35.5 then transfer tubes to water bath @44.5 for 21hrs.gas (+) growth	SM9222D; 9221E
	FC-021	Most Probable Number (MPN)	P-A broth ; confirmation in EC broth	SM 9221E
	FC-022	Most Probable Number (MPN)-Shellfish Meat	LTB; 24hr gas(+) or acid(+) confirmed on BGLB medium; completed phase, EC	
Escherichia Coli	E-031	Most Probable Number (MPN)	Inoculated simultaneously from positive LTB to BGLB and EC-Medium /EC-MUG confirmation phase for drinking water; official method: drinking water and ground water	SM9221F
	SFW-E031	Membrane Filtration for themotolerent Ecoli –Shellfish growing water	m TEC Agar is used for isolating, differentiating and rapidly enumerating thermotolerant Escherichia coli from water bymembrane filtration and an in situ urease test to identify yellow-brown colies	APHA
Heterotrophic Bacteria[sm] (HB)	HB-040	Heterotrophic Plate Count (HPC)	Pour Plate Method incubated for 48hr	SM9215B
	HB-041	Heterotrophic Plate Count (HPC)	SimPlate, count blue colonies under UV Light after 48hr incubation	IDEXX Lab Simplate

Table 6.2 SOPs for ELAP/EPA Approved Microbiological Methods Certified FOTs Drinking Water, Source Water, Recreational Water, Shellfish growing water & Wastewater

ANALYTE	CEL A SOP#[1]	METHOD FORMAT	TECHNOLOGY/MEDIUM	EPA-SM REF. METHOD[2]
Total Coliform (TC)	TC-010	Membrane Filter Technique (MF) –	m-Endo or m-endo LES broth or agar; coliform verification in LTB & BGLB media Compliance: drinking water MI agar for simultaneous detection of TC/ E.Coli Official method: all waters	SM 9222 A,B,C; EPA-1604
	TC-011	Most Probable Number (MPN) or Multiple Tube Fermentation (MTF)	LTB; 24hr gas(+) or acid(+) confirmed on BGLB medium; completed phase	SM 9221 A,B,C
	TC-012	Presence/Absence (P/A)	Presumptive phase P-A Broth; confirmation in BGLB medium	SM 9221D
	TC-013	Enzyme substrate (Chromogenic/Fluorogenic)	Colisure® (Official method: all water) Colilert™® 18 MTF (Compliance: drinking water)	IDEXX Lab
Fecal Coliforms (FC)	FC-020	Membrane Filter Technique (MF)-	A1 medium Direct ; 3hrs @ 35.5 then transfer tubes to water bath @44.5 for 21hrs.gas (+) growth	SM9222D; 9221E
	FC-021	Most Probable Number (MPN)	P-A broth ; confirmation in EC broth	SM 9221E
	FC-022	Most Probable Number (MPN)-Shellfish Meat	LTB; 24hr gas(+) or acid(+) confirmed on BGLB medium; completed phase, EC	
Escherichia Coli	E-031	Most Probable Number (MPN)	Inoculated simultaneously from positive LTB to BGLB and EC-Medium /EC-MUG confirmation phase for drinking water; official method: drinking water and ground water	SM9221F
	SFW-E031	Membrane Filtration for thermotolerent Ecoli –Shellfish growing water	m TEC Agar is used for isolating, differentiating and rapidly enumerating thermotolerant Escherichia coli from water bymembrane filtration and an in situ urease test to identify yellow-brown colies	APHA
Heterotrophic Bacteria[sm] (HB)	HB-040	Heterotrophic Plate Count (HPC)	Pour Plate Method incubated for 48hr	SM9215B
	HB-041	Heterotrophic Plate Count (HPC)	SimPlate, count blue colonies under UV Light after 48hr incubation	IDEXX Lab Simplate

Table 6.2-Continued

SOP List for Analysis of Commonly Used Indicators in Water Samples Based on ELAP/EPA Approved Methods

ANALYTE	CEL-A SOP#	METHOD FORMAT	TECHNOLOGY/MEDIUM	EPA-SM REF. METHODS[2]
Enterococci/Fecal Steptococci	EN-060-050	Membrane Filter Technique	mEI medium for direct counts of colonies with blue halo after 24hr incubation at 41° (primarily recreational water but applies to other waters) MF/m-Enterococcus agar; at 35.5 for 48hrs. -MF/ ME ; verify on Brain-heart infusion agar .	EPA 1600/SM9230C
	EN-061	Fluorogenic Substrate	Enterolert (recreational water) P/A	IDEXX Lab
Salmonella	SAL-070	Membrane Filtration followed by enrichment And colony isolation	Extension of Total coliform : growth on M-Endo agar, followed by enrichment in tetrathionate broth and differential colony isolation on brilliant green agar (BGA)	SM 9260 B.
Staphylococci	STA-100	Membrane Filter method	Growth on Baird Parker Agar base as slate grey of black smooth colonies, verify by commercial multi-test system	9213-(section 6)

Table 6.2-Continued

SOP List for Analysis of Commonly Used Indicators in Water Samples Based on ELAP/EPA Approved Methods

ANALYTE	CEL A SOP#	METHOD FORMAT	TECHNOLOGY/MEDIUM	EPA-SM REF. METHODS[2]
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	EN-061	Fluorogenic Substrate	Enterolert (recreational water) P/A	IDEXX Lab
Salmonella	SAL-070	Membrane Filtration followed by enrichment And colony isolation	Extension of Total coliform : growth on M-Endo agar, followed by enrichment in tetrathionate broth and differential colony isolation on brilliant green agar (BGA)	SM 9260 B.
Staphylococci	STA-100	Membrane Filter method	Growth on Baird Parker Agar base as slate grey of black smooth colonies, verify by commercial multi-test system	9213-(section 6)

Table 6.3 SOP Lists for Inorganic Methods FOTs for Wastewater

Analyte	CEL-A SOP#[1]	Method Format	Technology/Medium	EPA-SM Ref. Methods[2]
Acidity	IC-02.00	Potentiometric Titration/Colorimetric	Brompohenol,blue or Phenolphthalein indicator is used to titrate the sample to a colorimetric endpoint	Hach Method 8201-8202
Alkalinity	IC-02.21	Potentiometric Titration	Potentiometric titration to end-point pH	SM 2320 B
Aluminum	IC-02.11	Colorimetric	Eriochrome cyanine R method Measure OD at 535 nm	SM 3500-Al B
Biochemical Oxygen Demand (BOD)	IC-02.17	Lamotte 5 day BOD Titration/colorimetric	Compare the dissolved oxygen measured at time 0 and after 5 day incubation at 20 °C using titration	SM5210B
Chemical Oxygen Demand	IC-02.7	Colorimetric	Measure dichromate reduction at O.D 600 nm	SM5220D
Chlorine (residual)	IC-02.22	Colorimetric	N,N-diethly-p-phenylenediamine (DPD) method. Measure at O.D 515 nm	SM4500-Cl G
Chloride	IC-02.12	Colorimetric	Ferric ion reacts with thiocyanate ion to produce an orange-brown thiocyanate complex in proportion to the chloride concentration. Measure OD at 455 nm	SM 4500-Cl
Conductivity	IC-02.3	Conductivity meter	Conductivity cells with Platinum electode to measure specific conductance	SM2510B
Dissolved Oxygen	IC-02.9	1.Winkler Method/ 2.Indigo carmine method (Field method)	1. Convert manganese(II) hydroxide into manganese(III) hydroxide, and then analyzing for the latter by titration 2.The reduced form of indigo carmine reacts with D.O. to form a blue product. The indigo carmine methodology is not subject to interferences from temperature, salinity, or dissolved gases such as sulfide, which plague users of D.O. meters. Results are expressed as ppm (mg/L) O2.	4500-O C. as out lined in Lamott BOD5/ ASTM D 888-87, Colorimetric Indigo Carmine ASTM D 888-87,

Table 6.3 SOP Lists for Inorganic Methods FOTs for Wastewater

Analyte	CEL-A SOP#[1]	Method Format	Technology/Medium	EPA-SM Ref. Methods[2]
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Aluminum	IC-02.11	Colorimetric	Eriochrome cyanine R method Measure OD at 535 nm	SM 3500-Al B
Biochemical Oxygen Demand (BOD)	IC-02.17	Lamotte 5 day BOD Titration/colorimetric	Compare the dissolved oxygen measured at time 0 and after 5 day incubation at 20 °C using titration	SM5210B
Chemical Oxygen Demand	IC-02.7	Colorimetric	Measure dichromate reduction at O.D 600 nm	SM5220D
Chlorine (residual)	IC-02.22	Colorimetric	N,N-diethly-p-phenylenediamine (DPD) method. Measure at O.D 515 nm	SM4500-Cl G
Chloride	IC-02.12	Colorimetric	Ferric ion reacts with thiocyanate ion to produce an orange-brown thiocyanate complex in proportion to the chloride concentration. Measure OD at 455 nm	SM 4500-Cl
Conductivity	IC-02.3	Conductivity meter	Conductivity cells with Platinum electode to measure specific conductance	SM2510B
Dissolved Oxygen	IC-02.9	1.Winkler Method/ 2.Indigo carmine method (Field method)	1. Convert manganese(II) hydroxide into manganese(III) hydroxide, and then analyzing for the latter by titration 2.The reduced form of indigo carmine reacts with D.O. to form a blue product. The indigo carmine methodology is not subject to interferences from temperature, salinity, or dissolved gases such as sulfide, which plague users of D.O. meters. Results are expressed as ppm (mg/L) O2.	4500-O C. as out lined in Lamott BOD5/ ASTM D 888-87, Colorimetric Indigo Carmine ASTM D 888-87,

Table 6.3 SOPs Inorganic Methods FOTs (E108, E109) for Wastewater & Toxic elements of Hazardous Waste FOT E114

Analyte	CEL-A SOP#[1]	Method Format	Technology/Medium	EPA-SM Ref. Methods[2]
Iron	IC-02.10	Colorimetric	Phenanthroline Method measures ferrous phenanthroline orange-red complex at OD 510 nm	3500 Fe-B
Nitrate as N	IC-02.13	Cadmium reduction	Measure at O.D 543	SM4500-NO3 E
Nitrite as N	IC-02.19 IC-02.13	Colorimetric/Cadmium reduction	Measure at O.D.543	Hach 8507/SM4500-NO3E
Ammonia as N	IC-02.14	Distillation/ISE Electrode	Ion selective eectrode	4500-NH3 B & 4500-NH3D
Phosphate, Total Phosphate, ortho	IC-02.20	Reduction by ascorbic acid to form molybdenum blue	Measure at O.D. 880	Hach 8190/Hach 8048
pH	IC-02.4	pH meter	Measure H^+	SM4500-H^+ B
Sulfide	IC-02.8	Colorimetric	Reaction of sulfide with dimethyl-p-phenylenediamine produces methylene blue Measure at O.D. 664	SM4500-S-D
Total Dissolved Solids (TDS)	IC-02.16	Filter/conductivity probes	Filter, dry at 103180 °C and weigh sample	SM2540C
Total Suspended Solids	IC-02.6	Filter	Filter, dry at 103-105 °C and weigh sample	SM2540D
Turbidity	IC-02.5	Nephelometric	Measure light scatter relative to a standard	SM2130B
Nitrite	IC-02.19 IC-02.13	Colorimetric/Cadmium reduction	Measure at O.D.543	Hach 8507/SM4500-NO3E
Trace metals/Digestions	IC-03.20 3050B	Sample digestion and reflux in Acid followed by -ICP-OES	Acid digestion/Optical Emission Spectroscopy	EPA 200.7/6030B EPA 3050B
Minerals (Ca, K, Na, Silica)	IC-03.20 3050B			
Hexavalent Chromium (Cr6+)	IC-027	Colorimetric analysis of Hexavalent Chromium	Measure OD of color reaction at 540 nm	SM9

6.3.1 SOP Lists for Drinking water Chemistry

The following Tables 6.4 and 6.5 list New inorganic or general chemistry methods using existing or New SOPs for which the laboratory is seeking certification through ELAP.

Table 6.3 SOPs Inorganic Methods FOTs (E108, E109) for Wastewater & Toxic elements of Hazardous Waste FOT E114

Analyte	CEL-A SOP#[1]	Method Format	Technology/Medium	EPA-SM Ref. Methods[2]
Iron	IC-02.10	Colorimetric	Phenanthroline Method measures ferrous phenanthroline orange-red complex at OD 510 nm	3500 Fe-B
Nitrate as N	IC-02.13	Cadmium reduction	Measure at O.D 543	SM4500-NO3 E
Nitrite as N	IC-02.19 IC-02.13	Colorimetric/Cadmium reduction	Measure at O.D.543	Hach 8507/SM4500-NO3E
Ammonia as N	IC-02.14	Distillation/ISE Electrode	Ion selective eectrode	4500-NH3 B & 4500-NH3D
Phosphate, Total Phosphate, ortho	IC-02.20	Reduction by ascorbic acid to form molybdenum blue	Measure at O.D. 880	Hach 8190/Hach 8048
pH	IC-02.4	pH meter	Measure H^+	SM4500-H^+ B
Sulfide	IC-02.8	Colorimetric	Reaction of sulfide with dimethyl-p-phenylenediamine produces methylene blue Measure at O.D. 664	SM4500-S-D
Total Dissolved Solids (TDS)	IC-02.16	Filter/conductivity probes	Filter, dry at 103180 °C and weigh sample	SM2540C
Total Suspended Solids	IC-02.6	Filter	Filter, dry at 103-105 °C and weigh sample	SM2540D
Turbidity	IC-02.5	Nephelometric	Measure light scatter relative to a standard	SM2130B
Nitrite	IC-02.19 IC-02.13	Colorimetric/Cadmium reduction	Measure at O.D.543	Hach 8507/SM4500-NO3E
Trace metals/Digestions	IC-03.20 3050B	Sample digestion and reflux in Acid followed by-ICP-OES	Acid digestion/Optical Emission Spectroscopy	EPA 200.7/6030B EPA 3050B
Minerals (Ca, K, Na, Silica)	IC-03.20 3050B			
Hexavalent Chromium (Cr6+)	IC-027	Colorimetric analysis of Hexavalent Chromium	Measure OD of color reaction at 540 nm	SM9

6.3.1 SOP Lists for Drinking water Chemistry

The following Tables 6.4 and 6.5 list New inorganic or general chemistry methods using existing or New SOPs for which the laboratory is seeking certification through ELAP.

Table 6.4-Drinking water/Source Water FOT 102 &103

Analyte	CEL-A SOP#1	Method Format	Technology/Medium	EPA-SM Ref. Methods2
Alkalinity	IC-02.21	Potentiometric Titration	Potentiometric titration to end-point pH	SM2320B
Conductivity	IC-02.3	Conductivity meter	Conductivity cells with Platinum electode to measure specific conductance	SM2510B/EPA 120.1
Nitrite	IC-02.13	Cadmium reduction	Measure at O.D 543	SM4500-NO3 E
Nitrate	IC-02.13	Cadmium reduction	Measure at O.D 543	SM4500-NO3 D,E
Phosphate, Ortho	IC-02.20	Titation		SM4500-P E
Total Dissolved Solids (TDS)	IC-02.16	Filter/conductivity probes	Filter, dry at 103 & 180 °C and weigh sample	SM2540C
Total Suspended Solids (TSS)	IC-02.6	Filter	Filter, dry at 103-105 °C and weigh sample	SM2540D
Total Solids (TS)	IC-02.6	Filter	Reduce volume at 80, dry at 103-105 °C and weigh sample	SM2540B
Turbidity	IC-02.5	Nephelometric	Measure light scatter relative to a standard	SM2130B
pH	IC-02.4	pH meter	Measure H^+	SM4500-H^+ B/EPA
Color	IC-02.23	(Colorimetric-Platinum-Cobalt)	Color is measured by visual comparison of the sample with platinum-cobalt standards. One unit of color is that produced by 1 mg/L platinum in the form of the chloroplatinate ion.	EPA 110.2

Table 6.4-Drinking water/Source Water FOT 102 &103

Analyte	CEL-A SOP#1	Method Format	Technology/Medium	EPA-SM Ref. Methods2
Alkalinity	IC-02.21	Potentiometric Titration	Potentiometric titration to end-point pH	SM2320B
Conductivity	IC-02.3	Conductivity meter	Conductivity cells with Platinum electode to measure specific conductance	SM2510B/EPA 120.1
Nitrite	IC-02.13	Cadmium reduction	Measure at O.D 543	SM4500-NO3 E
Nitrate	IC-02.13	Cadmium reduction	Measure at O.D 543	SM4500-NO3 D,E
Phosphate, Ortho	IC-02.20	Titation		SM4500-P E
Total Dissolved Solids (TDS)	IC-02.16	Filter/conductivity probes	Filter, dry at 103 & 180 °C and weigh sample	SM2540C
Total Suspended Solids (TSS)	IC-02.6	Filter	Filter, dry at 103-105 °C and weigh sample	SM2540D
Total Solids (TS)	IC-02.6	Filter	Reduce volume at 80, dry at 103-105 °C and weigh sample	SM2540B
Turbidity	IC-02.5	Nephelometric	Measure light scatter relative to a standard	SM2130B
pH	IC-02.4	pH meter	Measure H^+	SM4500-H^+ B/EPA
Color	IC-02.23	(Colorimetric-Platinum-Cobalt)	Color is measured by visual comparison of the sample with platinum-cobalt standards. One unit of color is that produced by 1 mg/L platinum in the form of the chloroplatinate ion.	EPA 110.2

7.0 LABORATORY DATA REDUCTION, VALIDATION AND REPORTING

Analytical data produced in Cel-A Lab is recorded, reported, reviewed, and archived. The laboratory preserves all documentation regarding sample analysis for a minimum of five years, or until the next certification, data audit is concluded. All clients are given notice prior to the disposal of their specific records. All reports include quality control data, signature of the analyst performing the analysis and the signature of the reviewer. Depending on our client's preference, we provide the results in hard copy, electronic copy or both.

7.1 Data Reduction

Each analyst records raw laboratory data consisting of project name, laboratory ID number, method procedures, dilutions, calculations, and QC measures in his/her laboratory note book in permanent ink. Any changes in data shall be marked with a line through in a manner that the initial account is evident. All changes are initialed and dated. The analyst reduces the raw data and the QC results into the laboratory reportable format (SOP#DR-01), which then becomes part of the laboratory's documentation system. A second analyst must review the data generated at the bench.

7.2 Data Validation

The analyst is responsible for data accuracy and completeness, for calibration and performance verification of instruments, and for analyzing the correct type and quantity of quality control samples, which must meet the laboratories, pre-determined control limits. A validation checklist as presented in Table 7.2.1 (SOP# DR-01) below must be completed. Should these limits not be met, analytical results are flagged, documented, and justified by the analyst. The laboratory director will review all available data and provide corrective action if deemed necessary.

Table 7.2.1

Validation Checklist **Lab Project ID#**

- ❑ Correct data entry (decimals, figures, measures)
- ❑ Correct data tracking documentation (name of analyst and date of analysis)
- ❑ Complete quality control information (QC sample analysis, media sterility check, dilution/rinse water blank, analyst counting variability)
- ❑ Review of reports to establish whether data is satisfactory
- ❑ Once these checks have been established, the data report is signed by the analyst and a second qualified person as described in the following section.

7.0 LABORATORY DATA REDUCTION, VALIDATION AND REPORTING

Analytical data produced in Cel-A Lab is recorded, reported, reviewed, and archived. The laboratory preserves all documentation regarding sample analysis for a minimum of five years, or until the next certification, data audit is concluded. All clients are given notice prior to the disposal of their specific records. All reports include quality control data, signature of the analyst performing the analysis and the signature of the reviewer. Depending on our client's preference, we provide the results in hard copy, electronic copy or both.

7.1 Data Reduction

Each analyst records raw laboratory data consisting of project name, laboratory ID number, method procedures, dilutions, calculations, and QC measures in his/her laboratory note book in permanent ink. Any changes in data shall be marked with a line through in a manner that the initial account is evident. All changes are initialed and dated. The analyst reduces the raw data and the QC results into the laboratory reportable format (SOP#DR-01), which then becomes part of the laboratory's documentation system. A second analyst must review the data generated at the bench.

7.2 Data Validation

The analyst is responsible for data accuracy and completeness, for calibration and performance verification of instruments, and for analyzing the correct type and quantity of quality control samples, which must meet the laboratories, pre-determined control limits. A validation checklist as presented in Table 7.2.1 (SOP# DR-01) below must be completed. Should these limits not be met, analytical results are flagged, documented, and justified by the analyst. The laboratory director will review all available data and provide corrective action if deemed necessary.

Table 7.2.1

Validation Checklist **Lab Project ID#**

- ❑ Correct data entry (decimals, figures, measures)
- ❑ Correct data tracking documentation (name of analyst and date of analysis)
- ❑ Complete quality control information (QC sample analysis, media sterility check, dilution/rinse water blank, analyst counting variability)
- ❑ Review of reports to establish whether data is satisfactory
- ❑ Once these checks have been established, the data report is signed by the analyst and a second qualified person as described in the following section.

7.3 Data Reporting

The final report of the data is generated only after all the internal review and validations are completed and signed by the analyst and the laboratory director. Results for each method of testing, whether single or multiple samples are analyzed, are provided on separate pages with each page containing the client information, source of the sample, laboratory ID, the method used with detection limits when applicable and the laboratory reporting limits (SOP# DR-02). The QA/QC information for each method is provided on a separate page. A comment section is included with the QA/QC result that would allow the analyst to document any deviations from pre-established reporting limits, or sample holding times. These notes are called "data qualifier" with a standard set of codes that identify them:

- **ND** Not detected - no contaminant is detected above the laboratory detecting limit
- **J** the number is an estimated concentration; something in the sample interfered with the analysis
- **>>TNTC** Too-numerous to count or confluent
- **R** The data is unusable, re-sampling and re-analysis are requested
- **P/A** **P**=present; **A**= absent
- **MDL** Method detection limit
- **DIL** Dilution has been applied prior to sample analysis

The final report includes all of this pertinent information with a transmittal or a cover letter and is released solely to the client with the accompanying chain of custody documentation. Requests for copies of analytical reports made by a third party are available only upon written consent from the client.

All sample submissions must indicate on the Chain-of-Custody if the results are to be submitted to the State. For more information on when state notifications are required, visit their website at www.dhs.ca.gov/ps/ddwem.

Microbiology Reporting Requirements

In accordance to the Total Coliform Rule, and Codes of Federal Regulations (CFR) 141.21(b) and 40 CFR 141.31, the laboratory will notify the proper authorities of a positive total coliform, fecal coliform or E. coli result found in drinking water or source water samples, including confirmatory tests conducted to determine the positives within 24hrs of finding. Similarly per 40 CFR, 141.21 (c) (2) results of water samples that indicate non-coliforms have interfered with coliform analysis, are reported to the proper authorities.

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The final report of the data is generated only after all the internal review and validations are completed and signed by the analyst and the laboratory director. Results for each method of testing, whether single or multiple samples are analyzed, are provided on separate pages with each page containing the client information, source of the sample, laboratory ID, the method used with detection limits when applicable and the laboratory reporting limits (SOP# DR-02). The QA/QC information for each method is provided on a separate page. A comment section is included with the QA/QC result that would allow the analyst to document any deviations from pre-established reporting limits, or sample holding times. These notes are called "data qualifier" with a standard set of codes that identify them:

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Nitrate/Nitrite Reporting Requirements: Notification of proper authority (facility managers) of MCL Exceedance for Nitrate (10mg/L) and Nitrite (1 mg/L) is REQUIRED and should be performed within 24hrs of the finding. Ensure all informations, and telephone number of the facility managers are at hand, for appropriate follow up actions (example repeat samples) can be conducted per CFR 141.202.

7.3 Data Recording

Cel-A records and maintains documentation of all laboratory activities ranging from original observation, calculations and derived data to calibration records and copies of the test report. Records are maintained in a manner as to allow historical reconstruction of all laboratory activities. Records include:

The laboratory preserves all documentation regarding analysis, along with all equipment maintenance and repair records for a minimum of five years, or until the next certification data audit is concluded. All clients are given notice prior to the disposal of their specific records. In response to our client's possible requests, Cel-A provides raw data, and calculations. Depending on our client's preference we provide either hard copy, or electronic (with back up) copies.

Records are maintained in a manner as to allow historical reconstruction of all laboratory activities. Records and data storage checklists are summarized in Tables 7.3.1 and 7.3.2 (SOP#DR-03):

Table 7.3.1

Document Recording Checklist

- ❑ Identity of personnel involved in sampling, preparation, calibration, or testing.
- ❑ Records pertinent to equipment, test methods, related laboratory activities, sample receipt, sample preparation, and data verification.
- ❑ Inspection and verification of records.
- ❑ Staff's initials or signature on documentation entries.
- ❑ All records entered in a legible manner and in permanent ink.
- ❑ All changes are initialed or signed, and are done in a manner as to allow visibility of the original entry.
- ❑ A copy of the raw laboratory data, including calculations, notes pertinent to each project along with the final report and chain of custody documentation shall be filed under the project name for easy access and retrieval.

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Table 7.3.2

Data Storage Checklist

- ☐ All electronic records have corresponding hardware and software copies available for retrieval.
- ☐ Computer records have hard copy or write-protected backup copies.
- ☐ Storage of records includes laboratory notebooks, instrument logbooks, standard logbooks, and records for data reduction, validation, storage, and reporting.
- ☐ A logbook is kept by the laboratory, recording name and reason for access to archived information.
- ☐ Records are maintained in a manner as to protect them against, theft, loss, environmental deterioration, and vermin.

8.0 INTERNAL QUALITY CONTROL CHECK

To ensure the accuracy of the analytical process quality control samples are routinely used from the time of sample collection to sample analysis. An overview of the types of QC samples used by Cel-A laboratory is described below.

8.1 Field Quality Controls

As part of the sample collection activities and depending on the source and/or regulatory criteria for sample analysis, Cel-A field personnel are required to collect the following QC samples:

1) Field Blank (FB) - generally consists of laboratory de-ionized water in a sample bottle taken to the field and processed the same way as the field samples through sample handling and analytical procedures.

2) Field Duplicate samples (FD) - are collected from the same source, at the same time and processed in parallel throughout the analytical procedures. Results of duplicate sample analysis provide added precision from the time of sample handling, preservation to sample analysis.

3) Split Samples (SS) - consist of aliquots of samples taken from the same sample container and analyzed separately for verification purposes and to document intra-laboratory precision.

4) Blind Samples (BS) - may include a duplicate sample, a blank sample, or proficiency sample with its source unknown to the analyst.

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8.2 Analytical Quality Controls

Depending on the methods used for analysis, laboratory analysts incorporate the appropriate QC samples to estimate the precision of sample data, to verify calibration of equipment(s) used and identify method disparity or sample interferences that can affect the quality of the data. For methods that are not standard, routine QC requirements may not be practical. Then, the most effective QC measures must be determined through trial and error.

The following outlines the general QC routines employed during sample analysis. The analyst must refer to specific methods to identify the appropriate QC samples required for that method.

8.2.1 Microbiology

1) **Duplicate analyses** - to provide analytical precision. Duplicate samples are analyzed on 10% of the samples in an uninterrupted test run or at least one sample if less than 10 samples are processed per week.
2) **Replicates analysis.** A lab replicate is a sample that is split into sub-samples at the lab. Each sub-sample is then processed and analyzed. Lab replicates are used to obtain an optimal number of bacterial colonies on the filters for counting purposes. Usually, sub-samples of 100, 10, and 1 milliliter (mL) are filtered to obtain bacterial colonies on the filter that can be reliably and accurately counted (usually between 20 and 80 colonies). The plate with the count between 20 and 80 colonies is selected for reporting the results, and the count is expressed as colonies per 100 mL. Replicate samples are also used to test results through an alternate method for validation purposes and spiking samples for method accuracy.
3) **Control cultures** - analytical method is checked by parallel testing and processing of pure cultures of a known positive and a known negative at the time of sample analysis. A predetermined quantity of bacteria is added to the reagent water, or spiked in a replicate test sample. Analysis of pure culture or spiked sample recovered should conform to a known result, within an acceptable margin of error.
4) **Sterility check**. Blank media, filters, buffer dilution and rinse water are routinely included in the test runs to verify sterility. Sterile reagent water is included as a blank sample in the test run to monitor the sterility of the equipment. Media sterility is checked by incubating a representative batch (identified by batch reference number), at the appropriate temperature for 24 to 48 hr and observed for growth. Sterility of filters is checked by placing a blank processed filter on the media and incubating with the test samples at the appropriate temperature for 24 to 48 hrs. There should be no bacterial growth on the filters or broth after incubation.

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8.2.2 Inorganic

1) **Quality Control Samples**: Analyze a quality control sample for analytes every 3 months. This standard should come from a source other than the source of the daily standards.
2) **Blanks**: A laboratory reagent blank should be carried through the full analytical procedure with every sample batch and should not exceed the method's MDL.
3) **Laboratory Fortified Sample Matrix**: A known quantity of analytes will be added to at least 10% of the test samples if applicable to determine any possible sample interference. The quantity of known sample should not be less than the background concentration of sample unless specified in the method. If the sample concentration is unknown, choose a concentration based as a percentage of the MCL or mid point in the calibration range. **Over time, samples from all routine sample sources should be fortified as much as is practical.** If these results are not within the expected range, and control samples are, this data must be identified as suspect due to matrix effects.
4) **Laboratory Fortified Blanks:** Used to evaluate laboratory performance and analyte recovery in the blank matrix. A reagent water sample that has been fortified with a known concentration of analytes of interest. Include at least one LFB or up to 5% LFB (which ever is more) per analysis. The LFB should contain at least 10 times the MDL, the midpoint of the calibration curve or other level as specified in the method. Prepare the LFB from a different source than the calibration standards. Record the LFB for percent recovery of the added analytes. Calculate the percent recovery for these samples. This result reflects any interference in detection of that particular analyte.
5) **Control Charts**: Used to define accuracy. Based on the results from the Laboratory Fortified Blanks, calculate the mean percent recovery (x) and the standard deviation (s) of the percent recovery for the QC checks. Calculate:

   Upper control limit = x + 3s (upper warning limit + 2s)

   Lower control limit = x – 3s (lower warning limit -2s)

   Use the control limits specified in the method until data is acquired for 20 to 30 samples.

6) **Quality Control Samples** for Instrument analysis (Spectrophotomers/Inductively Couple Plasma-ICP-AES) Include:

   6.1) Calibration Standard (CS) A standard containing known quantities of target analyses, prepared from traceable stock materials of known, certified quality obtained from a reliable source or sources. Used to
   calibrate analytical instrument response.

   6.2) Initial (ICV) An initial calibration verification (ICV) is performed to check the accuracy of the calibration curve immediately after it

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is prepared. The ICV comes from a different source than the initial calibration standard. Normally the ICV concentration obtained from the calibration curve should agree within ±5% of the true value of the ICV solution. Failure to obtain acceptable ICV results may be due to:

- Concentrations of either the calibration standard(s) or ICV solution are not accurate.
- There is a problem with the calculation of the calibration curve.
- The instrument developed a problem between the completion of the calibration and the analysis of the ICV.
- An instrument problem which existed during the calibration was corrected prior to the ICV analysis.
- The analysis of the ICV was botched.

6.3) The continuing calibration verification (CCV) is used to determine that the initial calibration is holding. The source of the CCV should be a standard containing known quantities of target analyses, prepared from traceable stock materials of known, certified quality obtained from a reliable source or sources independent from those associated with the corresponding calibration standards. (See also *Laboratory Control Standard.*)
The minimum frequency for the CVV will be specified by the method but at least once per use.

7. **Initial Demonstration of Capability**: Must be performed for each method prior to analyzing compliance samples. IDC should be performed for each analyst and each piece of equipment. Precision, accuracy should be determined for each analyst. IDC includes demonstration of ability to achieve low background, the precision and accuracy required by the method, and satisfactory performance on an unknown sample. The IDC should be performed again if there is a change of analyst, instrument or significant change to the method.

   IDC for Trace Metal analysis in drinking water and wastewater is provided in Appendix E

8. **Periodic MDL calculation**: The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte accordance with the procedure given in 40 CFR 136 Appendix B. The procedure requires a complete, specific, and well defined analytical method and may vary as a function of sample type.

9. **Low Level Quantitation (LOQ)**: is the level above which quantitative results may be obtained with a specified degree of confidence. The LOQ is mathematically defined as equal to 10 times the standard deviation of the results

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for a series of replicates used to determine a justifiable **limit of detection**. Limits of quantitation are matrix, method, and analyte specific.

10. **Instrument Detection Limit (IDL)** is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise by a particular instrument. The IDL should always be below the **method detection limit**, and is not used for compliance data reporting, but may be used for statistical data analysis and comparing the attributes of different instruments. The IDL is similar to the "critical level" and "criterion of detection" as defined in the literature. (Standard Methods, 20th edition)

11. **Limit of Detection (LOD)** or detection limit, is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence). The LOD is typically determined to be in the region where the **signal to noise ratio** is greater than 5. Limits of detection are **matrix**, method, and analyte specific. (ss. NR 140.05(12) & 149.03(15)

Note: For the purposes of laboratory certification, the LOD is approximately equal to the **MDL** for those tests, which the **MDL** can be calculated.

9.0 PERFORMANCE AND SYSTEM AUDITS

As part of Cel-A's QA/QC program the following audits are conducted:

- Internal and external system audits to evaluate and validate qualitatively the laboratory's methods and overall performance, to identify any problems, and to provide technical support to the laboratory staff.
- Performance audits consisting of intra-laboratory performance evaluation and participation in the environmental laboratory proficiency testing sponsored by recognized authorities at the California Department of Health Services, Bureau of Environmental Laboratory Management (ELAP program).
- In addition, for all tests conducted at the laboratory the analyst(s) must show initial demonstration of capability (IDC) for the method(s) in question prior to analysis of environmental samples. At Cel Analytical we highly commend and encourage our staff to remain current with advances in technology and methodology by attending workshops, seminars, and educational courses and to participate in-house in new method development or training for new methods.

9.1 Internal Audit Program

Cel-A's internal audit program is designed to evaluate the staff's observance of the quality assurance plan and determine possible weaknesses in the plan itself. These audits are conducted annually by the Quality Assurance Manager and consist of a general review of all aspects of sample management, sample analysis, calculation and reporting. Notebooks and SOPs are reviewed to ensure protocols as outlined in SOPs are being followed. If any errors are discovered through the internal audit, corrective action goes

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into effect without delay and is documented. The laboratory director must inform clients in writing if errors affect the integrity of the laboratory's results.

9.2 External Audit Program

Cel-A is audited every other year by the California Department of Health Services Environmental Laboratory Accreditation Program (ELAP). The external audit program assesses the overall effectiveness of the laboratory quality assurance program and provides suggestions to correct any deficiencies that may be observed during site visits and document reviews. Cel-A's QA/QC manager is responsible for implementing the recommended corrective actions.

Furthermore, if desired, clients can conduct their own audit to ensure fulfillment of their specific quality control requirements or contract specifications.

9.3 Performance Audit and Initial Demonstration of Capability

Cel-A Lab stands committed to establishing compatibility levels of routine analytical results by participating in Proficiency Testing (PT) and accreditation programs. Laboratory staff will analyze reference material(s) obtained through authorized vendors where their performance is evaluated through inter-laboratory comparisons. The performance audit results are then reviewed by ELAP authorities and accreditation status is granted upon successful analysis of referenced material. Accreditation status, once granted must be renewed through the same process every 2 years.

As part of the general QC requirements of Cel-A, the laboratory director and individual analysts participate in an in-house initial demonstration of capabilities (IDC) test for each method SOP, prior to engaging in field sample analysis. Samples used for IDC testing will be either water of known microbial or analyte content, or blind samples. Blind samples are water samples with a known quantity of a particular organism or analyte yet unknown to the analyst. Results of each IDC test will be recorded in such a way that trends such as variability, reproducibility and accuracy of methods are detectable and reviewed by statistical techniques described in section 11 of this manual.

10.0 PREVENTIVE MAINTENANCE

10.1 Equipment Maintenance, Calibration Procedures and Frequency

The Cel Analytical laboratory QA/QC manager sets policies for routine checks and repairs of laboratory equipment depending on the manufacturer's recommendations and prior to sample analysis when applicable. Equipment performance is monitored and logged to ensure they meet the appropriate levels of accuracy and precision. A maintenance log is kept for equipment to record all checks and repairs. QA/QC logbooks are kept in the laboratory for autoclaves, microscopes, pipettors, biological hoods, vacuum pumps, the laboratory water purification system, incubators, refrigerators,

into effect without delay and is documented. The laboratory director must inform clients in writing if errors affect the integrity of the laboratory's results.

9.2 External Audit Program

Cel-A is audited every other year by the California Department of Health Services Environmental Laboratory Accreditation Program (ELAP). The external audit program assesses the overall effectiveness of the laboratory quality assurance program and provides suggestions to correct any deficiencies that may be observed during site visits and document reviews. Cel-A's QA/QC manager is responsible for implementing the recommended corrective actions.

Furthermore, if desired, clients can conduct their own audit to ensure fulfillment of their specific quality control requirements or contract specifications.

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freezers and the PCR machine. Balances, thermometers and the pH meter are routinely calibrated to meet the NIST national standards and EPA standards.

A log is kept near each piece of equipment in accordance with the USDA/FSIS Microbiology laboratory, Guidebook 3rd Edition 1998. The logbook contains:

- Type of equipment
- Manufacturer's name and product's serial number or other identification
- Receipt and service date
- location
- Copy of manufacturer's instructions
- Copy of dates and results of calibrations and/or verification
- Copy of date of next calibration and/or verification
- Description of maintenance performed in past and scheduled for future
- Product history with respect to any damage, malfunction, modification or repair

Laboratory personnel are required to record daily temperatures of incubators and refrigerators and to ensure that all equipment is functioning properly prior to the start of any analysis. Expired maintenance schedules or equipment malfunctions must be brought to the attention of the laboratory director.

Standard operating procedures for the recording log that contains the date of use, event, corrective action taken, and the initials of the person making the entry is described below for each piece of equipment. Recordings are kept for five years past last entry.

A brief description of the operation and maintenance of the equipment currently in use at Cel-A is described below with details provided in SOPs referenced in this section. The SOPs are found in appendix C of this manual and are also are maintained electronically under the name of equipment in the equipment file (File location: C drive/Cel-A-lab file/Equipment Log forms file).

10.2 Equipment:

Calibration of all measurement devices is traceable to national standards whenever possible.

Autoclave: Operation of the autoclave must be done according to manufacturer's instructions. Each autoclave cycle is recorded in a log book (placed next to the autoclave) that indicates the date, content, sterilization time, temperature and operator's initials. With each cycle of use heat sensitive tape is placed on the items being sterilized to assure the proper temperature is reached.

Sterilization procedures should be followed as described in the SOP # EA-01. All items such as media and reagents requiring sterilization are sterilized in the autoclave at 121°C for the designated time. The minimum sterilization time is 12-15 min for most carbohydrate containing media with the exception of A-1 medium which is 10 minutes.

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Biohazard waste is sterilized for a minimum of 30 minutes. Efficiency of sterilization is monitored monthly using spore stripes or ampoules.

Maintenance records and corrective actions required along with the initial of the person making the entry should be recorded on the maintenance log form (SOP # EA-02).

Balance (top loader): Calibrated monthly using ASTM (NIST traceable) type weight (type 1, 2 and 3) and recorded in a log book. In addition, professional calibration will take place annually. The balance should be cleaned prior to and following each use. Calibrate non-reference weights with reference weights every six months. Mass measurement should be recorded daily using a single weight. A logbook displaying daily checks on cleaning, maintenance, performance deviations, and corrective actions should be recorded and kept next to the balance (Ref SOP # EB-01).

Phase/Light Microscope: The microscope is equipped with lenses to provide approximate 20X and 40X to 100X magnification. Manufacturer's protocol and maintenance is strictly followed and logged in the binder provided next to the microscope. SOPs # EM-01 & EM-02 provide detail of routine in house QC and the corrective action log respectively. The accuracy and life time of the microscope depends on proper maintenance after each use and prompt reporting of any mal-function observed during use.

Conductivity meter, pH meter: Before each use standardize the pH meter with pH 7.0, 4.0 and 10.0 standard buffers. All pH buffers (NIST Traceable) are aliquotted for single use. The original containers when opened are dated and the lot number recorded. Expired buffer must be discarded. Maintain electrodes according to the manufacturer's recommendations. Each pH meter has a corresponding log (SOP#EpH-01) containing dated entries with each use, change of buffers or electrodes, service and corrective action taken in case of performance deviation.

The conductance meter is capable of measuring conductivity within 1μ Ohm per centimeter and is used routinely for testing reagent grade water. Similar to the pH meter, it is maintained according to the manufacturer's protocol. The conductivity meter is calibrated with every use using certified standard KCl solutions as described in Standard Method of Examination of Water and Wastewater 20th edition (APHA page 2-45) and recorded in the log book (SOP# Econ-01).

If a calibration curve is being used, standard solutions should be analyzed from time to time within the required range of concentration. The ideal calibration curve is linear within the range most often used.

Class II biological hood: Serviced at installation and annually thereafter. The pressure gauge reading should correspond approximately to the same level as the annually recorded level on the calibration sticker. If pressure is significantly higher, it indicates that the filters are dirty and need to be changed. If pressure is significantly lower, it may indicate an electric problem. Monthly check of airflow; test the filters for obstruction and dirt accumulation; clean and replace as needed. A light weight paper strip attached

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to the window can also indicate the inward air flow daily. Record maintenance and corrective action taken as outlined in SOP#EH-01.

Expose open agar plates to flow and air for an hour each month. Check contamination by incubating plates at 35 degrees Celsius for 24 hours. Clean and sanitize hoods and cabinets following each use, and turned UV light on. Lift up the work surface and clean under this area periodically.

Ultraviolet lamp in the hood: Disconnect and clean monthly, with soft cloth moistened with ethanol. Replace lamp if agar spread plate containing 200 to 250 microorganisms is exposed to UV light for 2 minutes and does not show a count reduction of 99%. For sanitization (SOP# EH-02), conduct spread plate irradiation test quarterly.

Thermometers: working thermometers include bulb thermometers immersed in glycerol, full immersion thermometer for water bath and maximum registered thermometer for autoclave and hot air oven. Thermometers are NIST calibrated and are re-certified whenever they have been exposed to temperature extremes. A reference certified thermometer is used to check the working thermometer. Referenced thermometer is recalibrated every 5 years.

Incubator Units (35ºC & 41.5ºC, 44.5ºC): All incubators are maintained at their desired temperature (+ or – 0.5 degrees Celsius) or (+ or – 0.2 degrees Celsius) depending on the application. Temperatures are recorded on daily log forms twice per day 3-4 hrs difference(SOP#EI-01). Any nonconforming temperature, possible cause, and corrective action are entered in a log book (SOP#EI-02).

Refrigerator/Freezer, -20 degrees Celsius: Calibrated at installation. Freezer maintains temperature of –20 + or – 5 degrees Celsius. Refrigerators are maintained between 2-8 degrees Celsius. Place NIST calibrated thermometer bulb immersed in glycerol on the shelf near the work area. Record temperature once per day at minimum for each day it is in use (SOP#ERF-01). Normal housekeeping once a week and clean and rearrange once a month. Annually check the overall condition of freezer and refrigerator, with a corresponding log of maintenance dates, problems, and corrective action (SOP#ERF-02).

Inoculating equipment: Use sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipette tips. Metal inoculating loops and needles are made of nickel alloy or platinum. Note: for oxidase test DO NOT use nickel alloy loops as they may interfere with the oxidase tests.

Pipettes (Mechanical): Use sterile, calibrated micropipette tips to ensure delivery of the correct volume per manufacturer's recommendation. Delivery volumes (depending on the size) should be within a 2.5% tolerance. Verify delivery volumes monthly by applying mass/volume measurement is near mass/volume expected in accordance with USDA/FSIS Microbiology Laboratory, Guidebook 3rd Edition 1998 (SOP#EP-01). Recalibrate in accordance with manufacturer's recommendations if performance verification failed. Return to manufacturer for recalibration. Keep records of maintenance, service, calibration, and verification measurements (SOP#EP-02).

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J-6 Centrifuge: The maximum speed of the J-6 centrifuge is 2700 rpm with a maximum rotor capacity of 2 liters. Maximum centrifugal speed is 1520 x g. Maintain the rpm and the corresponding g-force according to the chart attached to the instrument. For each centrifuge run, log speed, time of centrifugation, run temperature, and operator's initials (SOP#ECEN-01).

Record of calibrations, services, and corrective maintenance are kept as described in SOP# ECEN-02.

Vacuum Pump: Maintain and operate per manufacturer's instructions. Following each use clean and sanitize. Check annually and record maintenance, performance deviations, and corrective actions in log (SOP#EPU-01).

Membrane Filtration Equipment: Use membrane filters approved by manufacturer for total coliform water analysis; (approval based on data from tests for toxicity, recovery, retention, and absence of growth-promoting substances). Filters: cellulose ester, white, grid marked, 47 mm diameter, 0.45 µm pore size. Use stainless steel, glass, or autoclavable plastic. Before use, make sure they are not scratched, corroded or leaking. Use a standard graduated cylinder to check the accuracy of graduation marks on clear glass or plastic funnels used to measure sample volume (tolerance: less than or equal to 2.5%; retain calibration records.) The lot number for membrane filters and date received must be logged (SPO#EMF-01).

Water Purification System for Reagent Grade Water: The Millipore water purification system utilized at the laboratory consists of three components: Elix 5 with a reverse osmosis (RO) membrane that receives tap water and feeds purified deionized water into a 5 gallon water container that feeds the high quality water into the MilliQ Cell membrane system with a molecular weight cut off of 5000 Dalton, producing pyrogen free water. MilliQ water is further purified through a 0.2 µm filter at the dispenser tip for bacteria free water. The system is maintained according to manufacturer's instructions. With each use, conductivity (>0.5 megohms-cm at 25 degrees Celsius) of the reagent grade water is tested. The DI/RO system-cartridge is replaced as recommended by the manufacturer. A daily log of the conductivity reading (SOP# EW-01) and record of any scheduled maintenance or corrective action taken (SOP#EW-02) are maintained.

Spectrophotometer: The wavelength range of the SP 830 plus spectrophotometer is 330 to 999 nm. Allow the instrument to warm up for 30 minutes prior to use. Follow manufacturer's instructions for instrument validation and lamp adjustment. Record lamp use routine maintenance, instrument validation, lamp adjustment or any corrective action taken in the logbook located near the spectrophotometer (SOP SP-01). Standards are also employed to verify the accuracy of the optical densities at each given wavelength in use.

Inductive Couple Plasma Atomic Emission Spectroscopy ICP AES: An inductively coupled plasma is a plasma that contains a sufficient concentration of ions and electrons to make the gas electrically conductive. The plasmas used in spectrochemical analysis are essentially electrically neutral, with each positive charge on an ion balanced by a free

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One of the most frequent forms of routine maintenance is changing out sample and waste tubing on the peristaltic pump, as these tubes can get worn fairly quickly resulting in holes and clogs in the sample line, resulting in skewed results. Other parts that will need regular cleaning and/or replacing are sample tips, nebulizer tips, sample cones, skimmer cones, injector tubes, torches and lenses. It may also be necessary to change the oil in the interface roughing pump as well as the vacuum backing pump, depending on the workload put on the instrument. The ICP is due for an annual maintenance check by the instrument provider (Varian-Agilent Technology).

10.3 Laboratory Reagents and Supplies

Reagent and supplies are routinely checked for expiration dates when applicable, contaminant levels when applicable, and inventory checks to ensure all reagents and supplies are in stock and in good condition at all times. SOPs referenced in this section are found in appendix D of this manual.

Reagent Water ($dIII_20$): Cel Analytical uses only pyrogen free MilliQ ultrapure water (dIH_20) to prepare media, reagents, and dilution rinse water. Reagent grade water is microbiologically suitable (MS) water, free from traces of dissolved metal, bactericidal, and inhibitory compounds. Water quality shall conform to the EPA standards as shown in

Table 10.2 EPA Standards for Quality of Reagent Water

EPA Standards for Quality of Reagent Water

Parameter*	Limits	Frequency
Conductivity	<2 microhms/cm at 25°C	Daily
Pb, Cd, Cr,	No greater than 0.05 mg/L/ contaminant	Annually
Cu, Ni, Zn	collectively, no greater than 0.1 mg/L	Annually
Total Chlorine Residual	<0.1 mg/L	Monthly
Heterotrophic Plate Count	<1000 CFU/mL	Monthly

If a test requires bacterial free water, the MilliQ ultrapure deionized water suitable for the application since a 0.22µ filter is already installed at the dispenser for immediate use. This water is also suitable for trace metal analysis since it has been filtered. Store deionized water in sterilized in glass bottles only for bacterial use..

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Dilution/Rinse Water: Use prepared stock buffer solution/peptone water prepared in accordance with the EPA's Standard Methods under Section 9050C in Standard Methods for the Examination of Water and Wastewater. Details of the preparation are described in the appropriate SOPs. In general use stock buffers that have been autoclaved or filter-sterilized. The prepared water must be labeled and dated on the container. Stock buffers are refrigerated and are checked to ensure they are free of turbidity. Sterility of each batch of dilution/rinse water is checked by adding 50 mL of water to a double strength non-selective medium such as trypticase soy or tryptose broth. Check growth by incubating at 35 degrees Celsius + or – 5 degrees Celsius for 24 hours. Discard water if growth is discovered.

2% HNO3 water: For metal analysis, standards, quality control samples, spikes and dilutions of unknown samples will be prepared by using the 0.22 micron filtered water acidified to 2% with Nitric acid (HNO3).

Media: Dehydrated media or commercially prepared media are purchased from Fisher Scientific. Powder media in use should not be more than a year old. Therefore, media are ordered in smaller containers from the suppliers to reduce shelf-life. Document the expiration dates, when received and opened, lot number, type, quantity, and appearance of each media in a log book (SOPRSM-01). In general prepared media will be purchased from manufacturer/suppliers with the certificate of quality assurance and quality control for the batch received. Once received at the lab, the information is recorded in the media log book. Ensure that lot number, date opened and the expiration dates are clearly marked on the media. New lots of dehydrated or commercially prepared media are checked before use with positive and negative control cultures. In addition, laboratory prepared media should include positive and negative culture controls with each batch. These organisms are purchased commercially as disks impregnated with organisms or are available in ampoule form.

Quality control pertaining to each media is further described in detail in analytical method SOPs.

Disposable Culture Dishes: Plastic dishes are certified by the manufacturer to be clean, sterilized, and accurate. Incubate loose-lid petri dishes in tight-fitting containers, such as a plastic crisper containing a moistened paper towel to prevent dehydration of membrane filter and media. Dispose of opened packs of disposable culture dishes between uses.

Glassware and plastic ware: Prior to use examine, (and if necessary discard) glassware and flasks to ensure they are not chipped, broken or etched. Check glassware and flasks following washing to ensure water beads are not excessive on the surface. Always use pre-sterilized plastic ware and ensure plastic items do not contain foreign residues and that they contain visible gradations. **For metal analysis, a dedicated set of sample containers or disposable conical tubes shall be used. Plastic or glass containers shall be rinsed in acid-water to ensure all residues have been removed prior to a final rinse.**

Sample containers: All sample containers, whether glass or plastic are tested to ensure their sterility through random selection of at least one sample container of a set of sterile

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2% HNO3 water: For metal analysis, standards, quality control samples, spikes and dilutions of unknown samples will be prepared by using the 0.22 micron filtered water acidified to 2% with Nitric acid (HNO3).

Media: Dehydrated media or commercially prepared media are purchased from Fisher Scientific. Powder media in use should not be more than a year old. Therefore, media are ordered in smaller containers from the suppliers to reduce shelf-life. Document the expiration dates, when received and opened, lot number, type, quantity, and appearance of each media in a log book (SOPRSM-01). In general prepared media will be purchased from manufacturer/suppliers with the certificate of quality assurance and quality control for the batch received. Once received at the lab, the information is recorded in the media log book. Ensure that lot number, date opened and the expiration dates are clearly marked on the media. New lots of dehydrated or commercially prepared media are checked before use with positive and negative control cultures. In addition, laboratory prepared media should include positive and negative culture controls with each batch. These organisms are purchased commercially as disks impregnated with organisms or are available in ampoule form.

Quality control pertaining to each media is further described in detail in analytical method SOPs.

Disposable Culture Dishes: Plastic dishes are certified by the manufacturer to be clean, sterilized, and accurate. Incubate loose-lid petri dishes in tight-fitting containers, such as a plastic crisper containing a moistened paper towel to prevent dehydration of membrane filter and media. Dispose of opened packs of disposable culture dishes between uses.

Glassware and plastic ware: Prior to use examine, (and if necessary discard) glassware and flasks to ensure they are not chipped, broken or etched. Check glassware and flasks following washing to ensure water beads are not excessive on the surface. Always use pre-sterilized plastic ware and ensure plastic items do not contain foreign residues and that they contain visible gradations. **For metal analysis, a dedicated set of sample containers or disposable conical tubes shall be used. Plastic or glass containers shall be rinsed in acid-water to ensure all residues have been removed prior to a final rinse.**

Sample containers: All sample containers, whether glass or plastic are tested to ensure their sterility through random selection of at least one sample container of a set of sterile

bottles. 25mL of sterile, non-selective broth is added and then incubated for 24 hours at 35 degrees Celsius, + or – 5 degrees Celsius. The growth is subsequently checked and where growth is detected re-sterilized. Sufficient amounts of the appropriate preservatives must be added to sample bottles prior to sterilization if water samples are suspected to contain chlorine or heavy metals. Refer to sample collection SOP.

Dyes and Stains: Use organic chemicals as indicators (e.g., phenol red) and microbiological stains (e.g., gram stains) certified by the Biological Stain Commission.

Chemical and Reagents: Chemicals employed are ACS or an equivalent grade to prevent impurities and undesirable reactions. All reagents and mixtures are labeled as shown in Table 10.2.4 and 10.2.3. Also see SOP#Chem-01 & Chem-02.

Table 10.2.2
Labels for Reagents:

Identity:
Concentration:
Date Opened: Initials: Expiration Date:
Storage Conditions: RT/4°C/-20°C

Table 10.2.3
Labels for Solutions:

Identity:
Concentration:
Date Prep.: Initials: Expiration Date:
Storage Conditions: RT/4°C/-20°C

An inventory list of all chemicals with the associated **material safety data sheets** (MSDS) are maintained in a hard copy binder on a shelf in the lab. In addition, an inventory list shall be maintained electronically that includes the chemical name, lot number, date purchased and reordering dates. The laboratory holds the most current versions as hard copies and in electronic format.

Glassware Washing: Wash glassware with special laboratory detergent. Thoroughly rinse glassware with tap water. Use pyogen free MilliQ ultrapure distilled/deionized water purified through a reverse osmosis filter for the final rinse.

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Glassware Washing: Wash glassware with special laboratory detergent. Thoroughly rinse glassware with tap water. Use pyogen free MilliQ ultrapure distilled/deionized water purified through a reverse osmosis filter for the final rinse.

Microbiology:

Complete the glassware inhibitory residue test in accordance with EPA's Standard Methods for the Examination of Water and Wastewater under section 9020B (internal SOP#GW-01).

Chemistry:

Consult individual parameter testing method for special glassware handling procedures. Maintain separate glassware for nitrate analysis. Some parameters require acid rinse to remove dissolved metals that may interfere with analysis.

10.4 Laboratory Cleanliness

Cel-A lab commits itself to maintaining high standards of cleanliness. The laboratory is scheduled for weekly cleaning, and removal of non-hazardous garbage. Floors are wet-moped and washed with disinfectant, and at no time swept or dry-moped. The laboratory air is tested on a weekly base to screen bacterial counts in the work area, as well as a background area. Biological wastes are autoclaved in the laboratory prior to disposal. Disinfection of laboratory benches and biological cabinets are performed before and after each use by the laboratory personnel. A monitoring log sheet is used to record and document the routine autoclaving of the biological waste in the laboratory.

11.0 LABORATORY PROCEDURES USED TO ASSESS PRECISION, ACCURACY AND COMPLETENESS OF DATA

Statistical methods outlined below are used for determining precision and accuracy of data. These methods are based on established EPA guidelines for statistics in the inorganic[3] or microbiology[11] testing laboratory. The methods outlined below help to establish trends and quality control limits that will improve the quality of the data generated by the laboratory.

11.1 Precision

Precision of quantitative methods is a measure of reproducibility of data through analysis of replicate samples, method blanks, and control samples. Precision is checked by running duplicate analyses and expressing results as a range (difference between duplicate analyses). For each different sample such as drinking water, waste water or surface water the precision of its duplicate analysis is determined, using the most recent values.

Microbiology:

Complete the glassware inhibitory residue test in accordance with EPA's Standard Methods for the Examination of Water and Wastewater under section 9020B (internal SOP#GW-01).

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11.1.1 Range

Range is commonly used to evaluate an analyst's precision for methods and sample types commonly used in the laboratory. Range is determined using the following protocol:

1. Duplicate samples are analyzed on the first 15-20 sample types with positive responses, preferably over time, depending how many samples are processed per week in the laboratory. Duplicate samples are designated D1 and D2.

2. The results are log- transformed for subsequent statistical calculations because logarithmic transformation of data tends to normalize the skewed distribution of results from duplicate/replicate analysis. This will even-out the deviation normally observed among samples with different microbial densities. If either of a set of duplicate results is <1, add 1 to each value prior to transforming to the logarithm value.

   The logarithm (Log_{10}) of results:

   $L1 = Log_{10}$ of D1
   $L2 = Log_{10}$ of D2

3. Calculate the range (R) for each pair of transformed duplicates as the mean (R) range as follows:

   a) Calculate the range difference between duplicates:
   $$R_{log} = (L2 - L1)$$
   b) Calculate the sum of all log transformed duplicates:
   $$\sum \text{ of } R_{log}$$
   c) Calculate R (mean range):

   $\bar{R} = \sum R\ Log_{10}/n$, where n is the number of samples.

5. Control limits for sample variability are established using the first 15 duplicate samples by

   Multiplying the mean range ($\bar{R}$) by the student "t" factor value of 3.27 for a 95% confidence interval:

   Control Limit = R x 3.27

   Any duplicate range that exceeds the set control limit would indicate an error has occurred.

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   Any duplicate range that exceeds the set control limit would indicate an error has occurred.

6. Control limits and precision calculations are conducted and compared with the initial established limits for 10% of sample types analyzed routinely. If the range is greater than 3.27(R) then this variability has to be investigated. The initial control limits are periodically updated by repeating the procedures using the most recent set of 15 duplicate samples.

The QC manager will track the precision of each method by evaluating historical data for any indication of variability caused by the analyst's performance, change in reagents/supplies or the need for equipment maintenance.

11.2 Accuracy

While precision measures the closeness of duplicate sample analysis, it does not ensure the accuracy of the data generated at the laboratory. Accuracy of data reflects the degree to which the analytical measurement represents the actual concentration of the tested sample or its "true value".

Microbiology

Microbial samples pose a special problem of reproducibility due to natural variations in the number of organisms found from sample to sample. Apart from blank samples (laboratory blank or field blank), additional control samples (described below), are utilized to determine accuracy when deemed necessary to establish method performance:

a) Control samples: Water samples inoculated with a known number of organisms from a referenced culture (obtained from commercial sources, American Type Culture Collection-ATCC grade) are analyzed simultaneously. Recovery from the Control sample is compared with Buffered water samples on the non-selective medium and expressed as Percent Recovery (P).

Inorganic Chemistry

Laboratory Fortified Blanks: A reagent water sample that has been fortified with a known concentration of analytes of interest is a LFB. Calculate the percent recovery (P) of the analyte in the Laboratory Fortified Blanks (LFB).

$$P = \frac{(\text{LFB} - \text{Sample Result})}{\text{Known LFB conc. added}} \times 100$$

When duplicate spiked samples are run along with unspiked samples (particularly for drinking water samples) the relative percent difference (RPD) between the control spiked samples can be calculated:

$$\text{RPD} = \frac{\text{(Spiked Sample A) - (Spiked Sample B)}}{\text{(Spiked A + Spiked B)/2}} \times 100$$

b) For Instrument analytical methods where known reference with known values or "true values" are available commercially, accuracy is established by comparing the relative percent difference (RPD) between the laboratory measured value and the known "true value".

$$\%\ \text{RPD} = \frac{\text{True Value - Measured Value}}{\text{(True Value + Measured Value)/ 2}} \times 100$$

11.3 Mean & Standard Deviation

To establish control limits and determine variations expected in the laboratory data the mean and standard deviation are often calculated. Approximately 20 control replicate samples are analyzed over time, for each test method to determine an estimate of the central tendency or "the mean value" of the data set. Any shift or drift from the mean value may compromise the accuracy of the method and lead to errors. Control limits are updated by periodically calculating the mean and standard deviation using the most recent set of 20 replicate samples.

Mean

In microbiology, the data often does not show a normal statistical distribution. The data is skewed because the frequency of low bacterial counts is favored. In order to obtain a more symmetrical distribution of the data, the values are converted to their logarithms. The preferred method to calculate the mean of the dataset is the **geometric mean** $\bar{x}_{(g)}$ instead of **arithmetic mean** $\bar{x}_{(a)}$ (used for normal distribution) since it is less influenced by outliers (extreme values) occasionally observed in datasets. The geometric mean may be calculated using either procedure described below:

1) Calculate the anti log of the arithmetic mean of log transformed data:

a) $\log \bar{x}_{(g)} = (\sum \log x_i)/n$ $\log \bar{x}_{(a)}$ = logarithm of mean calculated value

$\sum \log x_i$ = sum of individual log transformed values

$= (\text{Log } x_1 + \log x_2 + \log x_3, \ldots + \log x_n)$

n = total number of values

b) $\bar{x}_{(g)} = \text{antilog}\ (\log \bar{x}_{(g)})$

2) Calculate the nth root of the product of the results:

$$\bar{x}_{(g)} = \sqrt[n]{(x_1)\ (x_2)\ (x_3) \ldots .\ (x_n)}$$

The same results can be obtained using either (1) or (2).

Note: arithmetic mean may be used if required by regulatory bodies

$\bar{x}_{(a)} = \sum xi\ /n$ where $xi = x1 + x2 + x3 \ldots . + .xn$

Standard Deviation

Once the mean is calculated, the standard deviation is determined by taking the difference of each control result from the mean, squaring that difference, dividing by n-1, then taking the square root. All these operations are shown in the following equation:

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{(n - 1)}}$$

where **s**= is the standard deviation,
$\sum$ means summation of all the $(xi - \bar{x})^2$ values,
x_i = an individual control result,
$\bar{x}$= the mean of the control results,
n = the total number of control results included in the group.

A larger standard deviation (s) reflects a greater dispersion of data and an increased frequency of random error. A smaller standard deviation (s) reflects narrower and sharper distribution of data and a lower frequency of random error.

For a measurement procedure, it is generally expected that the distribution of control results will follow log normal distribution such that control limits and warning limits can be established:

Warning Limit= Observed result ± 2(s)

This limit indicates that with 95% confidence limit, the observed result is within +/- 2 standard deviations of the mean.

Control limit = Observed result ± 3(s)

Establishes the acceptance limit with 99% confidence such that the observed result falls within plus/minus 3 standard deviations of the mean.

These limits provide monitoring of the accuracy of the results for the methods used at Cel-A. If variation is observed beyond these limits, the data is rejected and the cause of dispersion is investigated.

The mean and standard deviation are calculated using the EPA software ProUCL (software to calculate upper confidence limits).

11.4 Completeness of Data

Completeness of data is established by the percentage of laboratory results backed up by accepted quality assurance data. Completeness of data however relies also on the sampling process, transportation and treatment of samples prior to receipt by laboratory in addition to the analytical procedures.

11.5 Method Detection Limit (MDL)

MDL Calculation: Three important things to remember about calculating MDLs for inorganic parameters are:
1) use the sample standard deviation
2) use the correct Student's t-value and
3) use all significant figures.

The sample standard deviation, s, must be used when calculating MDLs. One of the most common mistakes is using the population standard deviation, s.

The EPA's MDL procedure derives the 95% confidence interval
estimates for the method detection limit using the percentiles of the chi square over the degrees of freedomdistribution.

For seven replicates, the 95% confidence intervals listed in 40 CFR 136 are:
Lower Critical Limit (LCL) = 0.64 x MDL
Upper Critical Limit (UCL) = 2.20 x MDL

These limits vary depending on the number of replicates. For the purposes of determining whether multiple MDL determinations are equivalent, the laboratory calculates the UCL and LCL for the lowest MDL value. If the other MDLs fall below the UCL of the lowest MDL, it is acceptable to report the highest MDL value as the detection limit, as long as the highest MDL meets all of the necessary regulatory requirements.

MDL is calculated as shown in the following equation:

Control limit = Observed result ± 3(s)

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MDL is calculated as shown in the following equation:

$$MDL = (s)(t\text{-value})$$

Where: MDL = Method Detection Limit
s = Sample stadnadard deviation
t-value = Student t values provide in the EPA MDL method for the number of tests performed

THE FIVE POINT CHECK

In addition to analyst experience, the calculated MDL should be evaluated using several checks to determine if it will meet all of the necessary criteria. The following five items, which will be referred to as the "FivePoint Check" are simple ways to check a calculated MDL.

1. Does the spike level exceed 10 times the MDL? If so, the spike level is high.
2. Is the MDL higher than the spike level? If so, the spike level is too low.
3. Does the calculated MDL meet regulatory requirement for the necessary program(s)?
4. Is the signal/noise (S/N) in the appropriate range?
5. Are the replicate recoveries reasonable?

If a particular analyte does not have a maximum required MDL the third item can be disregarded. For environmental programs setting maximum MDLs, it is important to check the appropriate Administrative Code or analytical methods for the current requirements. Items 4 and 5 are not required, but are useful for evaluating the data used to generate the MDL.

Other ways to evaluate whether or not a calculated MDL is a good estimate of the detection limit exist. The MDL procedure in 40 CFR 136 gives an iterative procedure, utilizing pooled standard deviations, forevaluating the MDL. This procedure is found in Appendix D. Another validation method is the analysis of serial dilutions.

The S/N Test - The MDL procedure in 40 CFR Part 136 recommends that the detection limit be estimated somewhere in the range where the signal to noise ratio is 2.5 to 5. The S/N is not only useful for estimating the initial detection limit, but it is also useful for evaluating the final MDL determination. The signal to noise ratio describes the effect of random error on a particular measurement, and estimates the expected precision of a series of measurements. Samples spiked in the appropriate range for an MDL determination typically have a S/N in the range of 2.5 to 10. A signal to noise ratio less than 2.5 indicates that the random error in a series of measurements is too high, and the determined MDL is probably high. In this instance, the samples should be spiked at a higher level to increase the signal. If the signal to noise ratio is greater than 10, the spike concentration is *usually* too high, and the calculated MDL is not necessarily representative of the LOD. In this case, the samples should be spiked at lower level. In some instances, especially with highly precise analytical techniques, the S/N may always be higher than ten. Again, the analyst's experience is crucial for determining when the S/N ratio is too high. The S/N ratio for a series of measurements can be **estimated** by:

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$$(S/N)_{est} = X_{ave}/s$$

Where
Xave = the average of either the calculated concentrations or analytical signals for the replicates
S= the sample standard deviation for the replicates.

The S/N ratio is a useful test for MDL validity, but a high signal to noise ratio does not necessarily indicate that the MDL is invalid.

Percent Recoveries Notes - One of the drawbacks of the MDL procedure is that it doesn't take into account the effects of high or low bias in a series of measurements. The effects of a bias are usually most notable in samples other than reagent water, due to matrix interferences. Bias can be measured by the average percent recovery of a series of samples. In order for an MDL to be realistic, the average percent recovery for the samples should be reasonable. A reasonable
recovery is subjective, and will be defined differently for different situations. Since MDL calculations involve low level analysis, the recoveries may not be comparable to samples spiked somewhere well within the "quantification" region of the calibration curve. An analyst familiar with the analytical system should be able to judge whether or not the average percent recovery falls within the expected range for low level samples. The average percent recovery can be calculated using the following equation:

$$Ave.\%R = (X_{ave}/spikelevel)x100\%$$

Where X ave = the average concentration of the samples
spike level=the initial spike concentration.

Some analytical methods specify appropriate control limits for low-level precision and accuracy samples. If a method does not specify appropriate low level control limits, it is often useful to evaluate the average percent recovery using previously established control limits. The appropriate control limits for reagent water spikes could be the limits for fortified blank recovery. If the determination was performed in a matrix other than reagent water, use the control limits specific for the matrix spikes. The results for low-level analysis may not always fall within the acceptable range, but if a calculated MDL is questionable, evaluating the recoveries in this manner may reveal a bias which could affect the calculation.

11.6 Laboratory Reporting Limits (RL)

The RL is the in-house determination of control limits described in the previous sections which take into account the precision and the accuracy of the data. The RL is reported to clients for each method, when applicable. The Method Detection Limit (MDL) is the lowest level of detection determined by inter-laboratory collaborative studies for certain methods. Where the MDL is available it is reported along with the laboratory reporting limit (RL).

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In general, results below the reporting limit are interpreted as either

a) the level of contaminants is very low or
b) not present in the sample.

Results above the RL mean the contaminant is not only there, but the lab is very confident of the reported result.

If the results are too high, such as too-numerous to count as in membrane filtration method, results are reported with data qualifiers defined at the bottom of the report with an explanation of the results and pertinent information about the sample or analytical procedures that may have affected the reported results. The laboratory often requests additional samples to repeat the analysis.

For inorganic parameters, Reporting limits are not acceptable substitutes for detection limits unless specifically approved by the Department for a particular test.

12.0 CORRECTIVE ACTION

12.1 Laboratory Procedures

If a problem is identified at the laboratory, corrective action is initiated by the QA/QC officer. In general most problems are associated with equipment, sample management, analytical results and data reporting where the associated internal SOPs outline what to do when anomalies are observed. Corrective actions affecting the integrity of the analytical results must be brought to the attention of the client, in writing by the laboratory director. The effectiveness of the corrective action must be established by follow up procedures and an internal audit and verified by the QA/QC manager. SOP CA-1 outlines the laboratory procedure for corrective actions.

12.2 Customer Complaint

At all times our company's policy promises to provide customers with the best possible service in a courteous and thoughtful manner. Possible complaints are reviewed and taken seriously, as we are open to feedback from clients who may suggest areas of improvement. Potential customer complaints are forwarded immediately to the laboratory director, who ensures the execution of corrective action to prevent recurring failures, and who communicates findings to the customer in a good faith effort to resolve the complaint. With the customer's satisfaction and approval, the dispute will be marked as resolved. A complaint investigation form (SOP CA-2) will be maintained in the customer file.

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b) not present in the sample.

Results above the RL mean the contaminant is not only there, but the lab is very confident of the reported result.

If the results are too high, such as too-numerous to count as in membrane filtration method, results are reported with data qualifiers defined at the bottom of the report with an explanation of the results and pertinent information about the sample or analytical procedures that may have affected the reported results. The laboratory often requests additional samples to repeat the analysis.

For inorganic parameters, Reporting limits are not acceptable substitutes for detection limits unless specifically approved by the Department for a particular test.

12.0 CORRECTIVE ACTION

12.1 Laboratory Procedures

If a problem is identified at the laboratory, corrective action is initiated by the QA/QC officer. In general most problems are associated with equipment, sample management, analytical results and data reporting where the associated internal SOPs outline what to do when anomalies are observed. Corrective actions affecting the integrity of the analytical results must be brought to the attention of the client, in writing by the laboratory director. The effectiveness of the corrective action must be established by follow up procedures and an internal audit and verified by the QA/QC manager. SOP CA-1 outlines the laboratory procedure for corrective actions.

12.2 Customer Complaint

At all times our company's policy promises to provide customers with the best possible service in a courteous and thoughtful manner. Possible complaints are reviewed and taken seriously, as we are open to feedback from clients who may suggest areas of improvement. Potential customer complaints are forwarded immediately to the laboratory director, who ensures the execution of corrective action to prevent recurring failures, and who communicates findings to the customer in a good faith effort to resolve the complaint. With the customer's satisfaction and approval, the dispute will be marked as resolved. A complaint investigation form (SOP CA-2) will be maintained in the customer file.

13.0 QUALITY ASSURANCE REPORT TO MANAGEMENT

The QA/QC officer is responsible for gathering all quality control information pertinent to the day to day laboratory operations monthly in addition to a yearly report and to present them to the laboratory director for her review. The QA/QC reports in general include:

- Any updates and revisions to the QA/QC manual with dates and revision number
- Performance audit results and rating, including annual performance evaluation
- Annual internal audit report for each analysis conducted at the laboratory
- Precision and accuracy report of each method of testing
- Revisions or any updates on SOPs
- Corrective action reports

Annual Report Contains:

- Evaluation and revision of the laboratory's Quality Assurance Manual.
- Assessment and modification of the laboratory's Standard Operating Procedures.
- Review of the internal quality assurance audits.
- Synopsis of deviations and remedial action, including analyst non-compliance with laboratory policies
- An abstract of instrument calibration and maintenance.
- Review of temperature monitoring data for incubators and refrigerator up keep.
- Assessment of reagent water quality and maintenance schedules
- Assessment of performance results.
- Evaluation of external audit results

The laboratory director shall review all the reports, comment on any irregularities and ensure implementation of corrective action(s) when needed in a timely manner.

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General References:

The following sources were used in preparation of this quality assurance manual and the internal standard operating procedures:

[1] US EPA Microbiology Home Page: http://www.epa.gov/nerlcwww/

[2] US EPA Publication G-065: Manual for the Certification of Laboratories Analyzing Drinking Water -5th edition, January 2005, On line copy: http://www.epa.gov/safewater/methods/pdfs/manual_labcertification.pdf

[3] Standard Methods for the Examination of Water and Waste Water 20th edition 1998. Published jointly by American public health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF)

[4] Guidelines Establishing Test Procedures for the Analysis of Pollutants: Analytical Methods for Biological Pollutants in Ambient Water; Final Rule Fact Sheet (EPA-821-F-03-009) July 2003; http://www.epa.gov/ost/methods/biological/fsbio.htm

[5] USEPA Control of Pathogen and Vector Attraction in Sewage Sludge http://www.epa.gov/ORD/NRMRL/Pubs/1992/625R92013appF.pdf

[6] Association of Official Analytical Chemists. (AOAC) https://ecam.commer.net/aboutecam.asp

[7] Total Coliform Rule: http://www.epa.gov/OGWDW/tcr/pdf/qrg_tcr_v10.pdf

[8] Surface Water Treatment Rule: http://www.epa.gov/OGWDW/therule.html#Surface

[9] California Department of Health Division of Drinking Water and Environmental Management http://www.dhs.ca.gov/ps/ddwem/beaches/Beaches_appendices/

[10] Efiok, B., Eduok, E. 2000: Basic Calculations for Chemical and Biological Analysis, Second Edition. AOAC international publication

[11]. US EPA publication 600/878017 "Microbiological Methods for Monitoring the Environment, Water and wastes" Part IV Section B: Statistics for Microbiology, 1978

[12] US EPA Publication SW846 3rd edition- Solid waste Hazardous Waste - Test Methods

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[11]. US EPA publication 600/878017 "Microbiological Methods for Monitoring the Environment, Water and wastes" Part IV Section B: Statistics for Microbiology, 1978

[12] US EPA Publication SW846 3rd edition- Solid waste Hazardous Waste - Test Methods

[12] The U.S. Environmental Protection Agency Method Detection Limit (MDL) procedure found in Title 40 Code of Federal Regulations Part 136 (40 CFR 136, Appendix B, revision 1.11

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Appendix 10: Sonoma County Laboratory Bacteroides Analysis QAP

Bacteroides Quality Assurance Program (QAP)

5/12/11

The Sonoma County Public Health Laboratory performs molecular assays on recreational water samples for the quantitative determination of Human (HuBac), Bovine (Bobac) and Total (Allbac) bacteroides species.

Organization of the laboratory

The Sonoma County Public Health Laboratory staff is currently comprised of four public health microbiologists, three laboratory technicians a secretary and a laboratory director. The microbiologists are certified by the State of California and perform numerous assays in accordance with CLIA, ELAP and the CDC. Job descriptions and responsibilities are on file.

Molecular Testing Responsibilities

All of the molecular assays are performed by certified public health microbiologists. As members or the laboratory response network (LRN) each microbiologist and the lab director's molecular technique and skills are reviewed at a minimum of every other month. This review includes extraction, PCR, interpretation and final reporting of results. These competency reviews are observed and critiqued as part of our routine bio-terrorism readiness.

Safety

The entire staff receives annual safety training. The trainings include both biological agent and chemical hygiene. The microbiologists also annually review the proper use of all personnel protective equipment (PPE). This includes the proper use and care of PAPR's used inside the BSL 3, molecular laboratory.

All trainings consist of, but are not limited to:

1. Blood borne pathogens
2. Personnel Protective Equipment
3. Incident response
4. MSDS information
5. Select agent reviews
6. Chemical Hygiene

Data Quality Objectives:

The objective of the Sonoma County Public Health Laboratory is to produce good quality, reproducible data. Measures in place to ensure that the reported data is of the highest quality include; quality control procedures, system controls, assay controls, technical competency reviews, corrective action program and participation in numerous laboratory proficiency testing programs.

Sample receiving and handling

Water samples are collected by the submitting agency, business, organization or private clients. Once the samples arrive at the laboratory, the paper work and or chain of custody is dated and time stamped in. The temperature of the sample(s) is taken with a NIST traceable infra-red thermometer and recorded onto the sample log sheet. The samples are immediately placed into a refrigerator at 4 degrees centigrade. All samples are given a laboratory number and hand written onto a log sheet. The log sheet will capture at a minimum the date, the time, the temperature, the submitter and the name of the employee logging in the sample. Additional information may be required (chain-of-custody or location of sample) and would be captured at this time. Sample processing will begin as soon as possible once the samples arrive and are logged in. Any sample received that does not meet acceptable standards will be flagged on the log sheet and the final report may be qualified.

Performance and System Audits:

Audits are performed annually or whenever there is a change in SOP. The performance audit consists of checking SOP protocols against actual practice. The performance audit will ensure that the analysts are following the written SOP procedures.

The systems audit entails following archived records of a sample from receipt of the sample into the laboratory to the final report. The system audit ensures that protocols are followed, thermometers used were certified, molecular reagents employed were not out of date, the assay controls fell within their normal range, the system control gave an expected result and that the final report was accurate and issued within an acceptable time frame.

Corrective Action:

Corrective action is taken whenever there is a known deviation from the SOP or when the assay controls or system controls fail or fall outside the normal range of acceptable performance. Corrective action involves a critical analysis of "what went wrong". Was the problem procedural or technical in nature? Was there human error? Once the problem is diagnosed a corrective action is employed to rectify the problem. Additional measures may be taken to prevent the problem from happening again. All corrective actions are recorded and archived in the laboratory.

Proficiency Testing:

The laboratory subscribes to a proficiency testing program through the College of American Pathologists (CAP) and we receive unscheduled proficiencies from the Centers for Disease Control (CDC) that involve all aspects of our molecular testing protocols. The results are graded and reviewed.

Preventative Maintenance of Instruments and Equipment.

Our ABI 7500 fast dx instrument is used for the bacteroides qPCR assay. This instrument is maintained and serviced by ABI technical service representatives. As partners in the federal Laboratory Response Network for bio-terrorism and bio-watch we are required to have current service contracts in place for all of our technical equipment.

All of our molecular testing is performed in a BSL 3 level lab. The room maintains a continuous negative air pressure. The BSL 3 also houses two biological safety cabinets (BSC) for safety and preservation of samples. The room and the BSC are annually re-certified by a private contractor. Records are available for review.

Temperatures of freezers, refrigerators and incubators housed within the BSL 3 are taken daily. Thermometers are calibrated to meet NIST traceable standards.

There are two ***Clean-Spots*** TM used for molecular testing. One *clean-spot* is dedicated for the preparation of master mix only. The second is dedicated for the addition of template only. Both of these clean spots are wiped before and after use with a DNase product to minimize carry-over. By dedicating clean-spots in this way we reduce the potential of molecular carry-over.

All disposable components in our molecular lab are certified by our vendors to be DNase free.

System Controls and Assay Controls:

Each molecular run will contain both a system control as well as positive and negative assay controls. The system control is designed to detect carry over (false positives) from any component used in the assay from extraction (manual or auto) through the making of master mix. This system control goes through the entire protocol and should yield a negative result. This ensures that the "system" is working and that no singular component is contaminated with template. System controls can also include a known DNA target inserted into the sample to detect any possible molecular inhibition (false negatives) in the sample itself.

The assay controls are simply known positive and negative templates. This ensures the instrument and the microbiologist followed proper protocols and reagent sequences to yield the correct expected results.

If any combination of these controls does not yield the correct expected result the run is in invalid. Once the problem has been identified a corrective action would be employed and the run repeated.

With quantitative assays, the standards are run in duplicate to ensure reasonable reproducibility. Each sample is run in duplicate to also demonstrate reasonable reproducibility.

If applicable outdates and lot numbers of molecular reagents are checked each day of use and recorded.

Assessment of Data Precision, Accuracy, Validation and Reporting:

The laboratory director or designee reviews worksheets and test data for any discrepancies. Mathematical calculations are reviewed for accuracy. The final report is reviewed to ensure the correct interpretation has been made. All raw data and worksheets are archived and available for future review. Upon request copies of all raw data, worksheets and control performances will be submitted along with the final report.

Richard Green 5/12/11

Appendix 11: Approval Sheet Signatures

Appendix 11: Title and Approval Sheet

Steve Butkus, Project Manager – Data Manager – Contract Manager
North Coast Regional Water Quality Control Board

Steve Butkus 16 NOV 2011

Signature Date

Appendix 11: Title and Approval Sheet

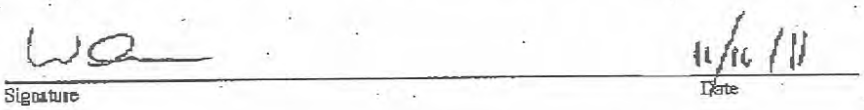
Rich Fadness, Project QA Officer
North Coast Regional Water Quality Control Board

11-16-11

Signature Date

Appendix 11: Title and Approval Sheet

William Ray, Board Quality Assurance Officer
California State Water Resources Control Board



Signature Date

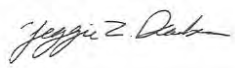
Appendix 11: Title and Approval Sheet

Gary Anderson, Lab Director
Lawrence Berkeley National Laboratory (LBNL)

Signature Date

Appendix 11: Title and Approval Sheet

Yeggie Dearborn, Lab Director
Cel Analytical Laboratories



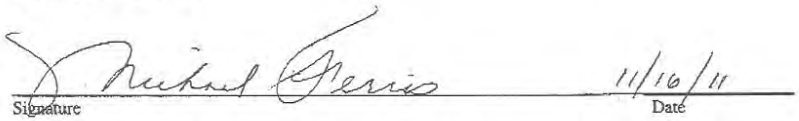
11.16.2011

Signature Date

Appendix 11: Title and Approval Sheet

Contract Laboratory Director

Michael Ferris, Sonoma County Public Health Laboratory, Lab Director
County of Sonoma



Signature 11/16/11 Date