Regional Water Quality Control Board
North Coast Region

Russian River Pathogen Indicator Bacteria TMDL

Quality Assurance Project Plan

Version 1.0

Originated by:

Rich Fadness
Steve Butkus

North Coast Regional Water Quality Control Board

(May 19, 2011)

Group A: Project Management

A1: Title and Approval Sheet

Project Title: Russian River Pathogen Indicator Bacteria TMDL

Lead Organization: North Coast Regional Water Quality Control Board
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Primary Contact: Steve Butkus
Project Manager
North Coast Regional Water Quality Control Board
Phone Number: 707-576-2834
Email Address: SButkus@waterboards.ca.gov

Effective Date: May 19, 2011

Approvals

See Appendix 6: Section A-1 Approval Sheet Signatures. Originals are kept on file by the Regional Water Quality Control Board - North Coast Region (Region 1) Microbiology Laboratory Director.

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

See Appendix 6: Section A-1 Approval Sheet Signatures _____ _____
Signature Date

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

See Appendix 6: Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Charles Reed, Region-1 Microbiology Laboratory, Co-Lab Director
North Coast Regional Water Quality Control Board

See Appendix 6: Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Caryn Woodhouse, Region-1 Microbiology Laboratory, Co-Lab Director
North Coast Regional Water Quality Control Board

See Appendix 6: Section A-1 Approval Sheet Signatures _____ _____
Signature Date

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

See Appendix 6: Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Beverly van Buuren, SWAMP QA Officer
Quality Assurance Research Group, Moss Landing Marine Laboratories

See Appendix 6: Section A-1 Approval Sheet Signatures _____ _____
Signature Date

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A3: Distribution List

Table 1: QAPP Distribution List Primary Contact Information

Contact Information	Organization's Mailing Address
Project Manager; **Data 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; **Contract Manager** 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
Region-1 Microbiology Laboratory – **Co-Laboratory Director 1** Main Contact: Charles Reed Phone: 707-576-2752 Email: CReed@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Region-1 Microbiology Laboratory – **Co-Laboratory Director 2** Main Contact: Caryn Woodhouse Phone: 707-576-2701 Email: CWoodhouse@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
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
SWAMP Quality Assurance Officer Main Contact: Beverly van Buuren Phone: 206-297-1378 Email: bvanbuuren@mlml.calstate.edu	Quality Assurance Research Group Moss Landing Marine Laboratories 7544 Sandholdt Rd Moss Landing, CA 95039

A4: Project/Task Organization

A monitoring study has been initiated by the North Coast Regional Water Board. The North Coast Regional Water Board will be conducting laboratory analysis to include *E. coli*, total coliform, and *Enterococcus,* and has contracted with the Sonoma County Public Health Laboratory to conduct laboratory analysis to also include *E. coli*, total coliform, and *Enterococcus.* The North Coast Regional Water Board will be responsible for the collection of samples, to include field measurements, and the analysis of *E. coli*, total coliform, and *Enterococcus* samples. Sonoma County Public Health Laboratory will be responsible for the analysis of *E. coli*, total coliform, and *Enterococcus* 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	North Coast Regional Water Board	(707)-576-2834 (707)-523-0135 sbutkus@waterboards.ca.gov
Charles Reed	Laboratory Co-Director 1	North Coast Regional Water Board	(707)-576-2752 (707)-523-0135 creed@waterboards.ca.gov
Rich Fadness	Project QA Officer; Contract Manager	North Coast Regional Water Board	(707)-576-6718 (707)-523-0135 rfadness@waterboards.ca.gov
Caryn Woodhouse	Laboratory Co-Director 2	North Coast Regional Water Board	(707)-576-2701 (707)-523-0135 cwoodhouse@waterboards.ca.gov
Michael Ferris	Contract Laboratory Director	Sonoma County Department of Public Health Services	(707)-565-4711 (707)-565-7839 mferris@sonoma-county.org
Beverly van Buuren	SWAMP QA Officer	Moss Landing Marine Labs	(206) 297-1378 (206) 297-1378 bvanbuuren@mlml.calstate.edu

North Coast Regional Water Board

Project Manager - Data 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, reporting and data submission to the SWAMP information management system (IMS).

Region 1 Microbiology Lab Co-Director 1

Charles Reed - He is responsible for the logistical aspects of the monitoring project including organization of field crews, collection of samples, and delivery of samples to the Region 1 Microbiology Laboratory and the Sonoma County Health lab within required holding times. In addition, he is responsible for ensuring that microbiological samples are processed in accordance with the method and QA assurance requirements found in this QAPP and the Region 1 Microbiology Laboratory QAP, (see appendix 6).

Region 1 Microbiology Lab Co-Director 2

Caryn Woodhouse - She is responsible for ensuring that microbiological samples are processed in accordance with the method and QA assurance requirements found in the Region 1 Microbiology Laboratory QAP, (see Appendix 6)

Contract Manager - 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 and Lab Personnel

North Coast Regional Water Board staff will conduct all field sampling, data collection activities, and laboratory analysis. Sonoma County Public Health Laboratory personnel will conduct laboratory analysis

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 QAP, (see Appendix 7).

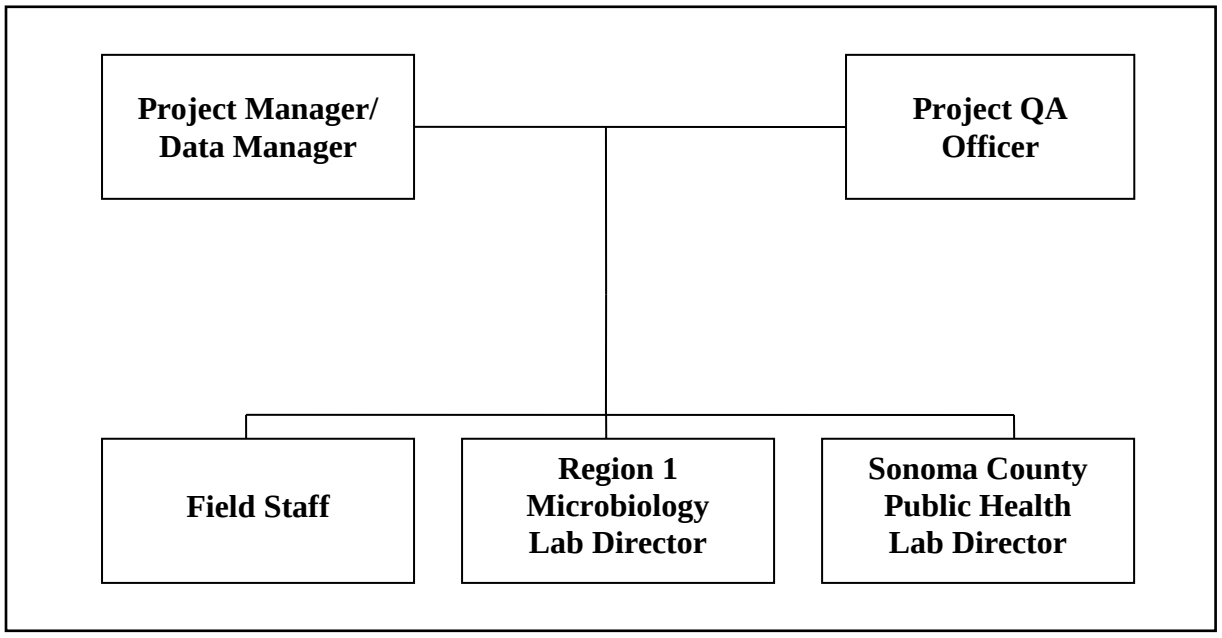

Figure 1. Project Organizational Chart

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. The impaired waterbodies are identified on Figure 2 numerically as:

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

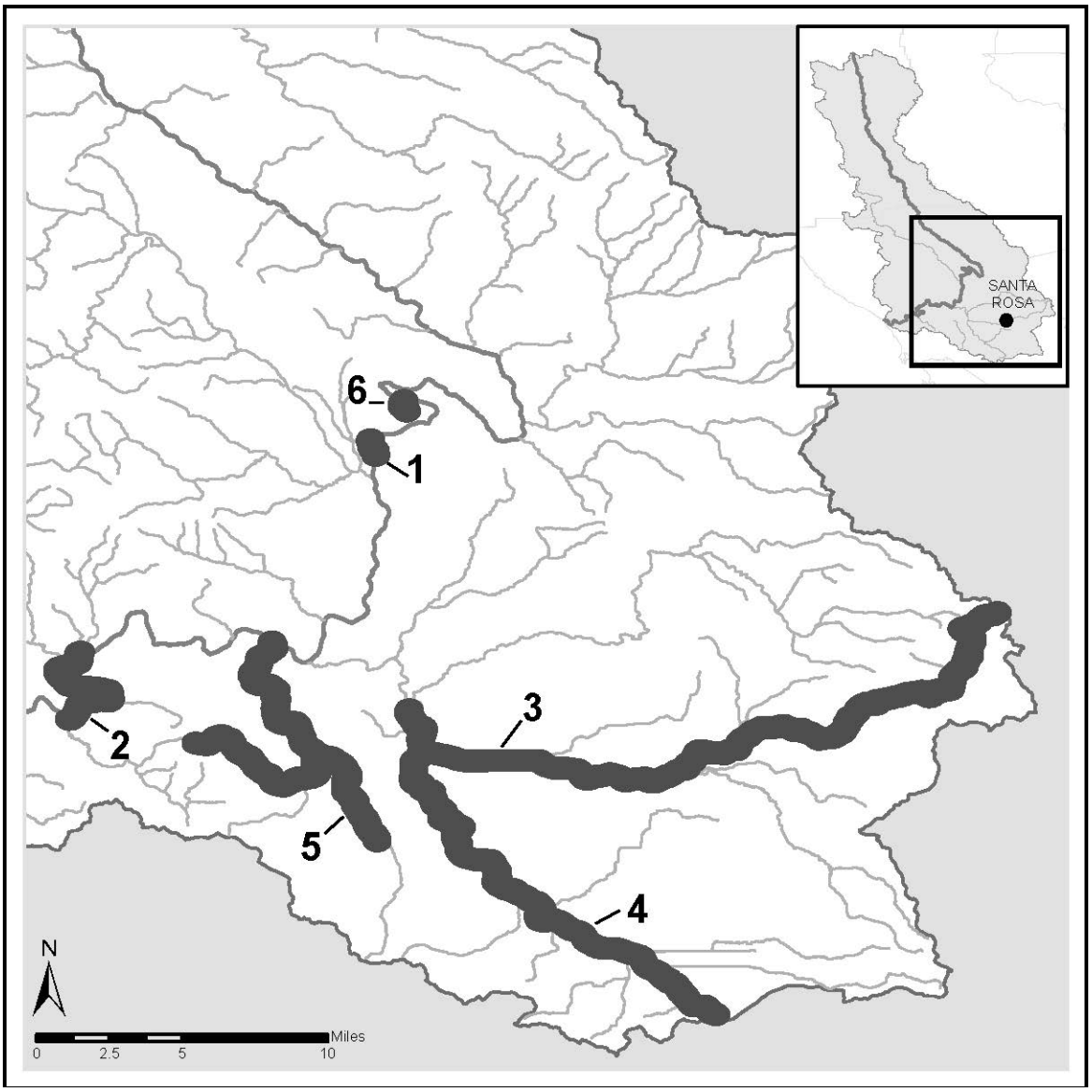

Figure 2. Surface waters impaired for REC-1.

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 U.C. Davis Aquatic Ecosystems Analysis Laboratory (Shilling et al. 2009; Viers et al. 2009). U.C. 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:

- Improve the current dry season monitoring program which is focused on monitoring points of human contact without respect to source or pathway of fecal contamination and timed for effective weekend beach closures. In addition to monitoring more frequently, such as during the weekend when most contact recreation is taking place, tributary inputs should also be monitored to provide a more comprehensive understanding of potential pathogen exposure over time and space.
- Weekly sampling is the minimum frequency recommended due to the large variation during the summer and the frequency of Water Quality Objective (WQO) exceedances.

- Monitoring more episodic events, such as storm events, late winter pulses, and holiday weekends that increase likelihood of exposure.
- Expand the monitoring program to other important seasons. The present program of monitoring from Memorial Day to Labor Day misses four important temporal windows:

    1. Low flow conditions in September: Contact recreation continues past Labor Day throughout the Russian River corridor, thus potential exposure to elevated indicator bacteria levels during this period are currently unknown.
    2. First flush during October-November: The first flush period has been shown to have elevated indicator bacteria levels.
    3. Storm events from December-April: The pilot study shows indicator bacteria levels during both the storm flow period and the recession flow period are orders of magnitude above WQOs.
    4. Inoculation during recession flows in May: The high concentrations of indicator bacteria in storm recession flows "set up" conditions downstream later in the season.

- A single grab sample collected at a specific location and measured for an indicator bacteria concentration is often considered to be representative of the actual concentration in the water. However, this assumption is often not correct. To detect change and accurately measure conditions, a minimum of three (3) samples should be taken, and preferably more. This minimum sample size allows one to estimate the central tendency the data (i.e., mean or median), which is more likely to reflect the actual concentration. Three samples also allow the calculation of variance that is needed to assess spatial or temporal differences.

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 eight management questions:

1. Are Basin Plan Water Quality Objectives for pathogenic indicator bacteria being met in the middle and lower Russian River watershed?
2. What is the sampling variability of pathogenic indicator bacteria?
3. What is the analytical laboratory variability of pathogenic indicator bacteria?
4. What is the spatial variability of pathogenic indicator bacteria?
5. What is the temporal variability of pathogenic indicator bacteria?
6. What are the most significant sources of pathogenic indicator bacteria?
7. What is the background or natural levels of pathogenic indicator bacteria?
8. Do beach areas pose a higher risk to REC-1 than non-beach reaches?

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. 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 (UAA) 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 pathogens in the Russian River.

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.

Field measurements for dissolved oxygen (DO), specific conductivity, pH, and temperature will be collected using a Yellow Springs Instrument (YSI) 600XL Datasonde (Sonde) and 650MDS (Mulitparamter Display System).

E. coli and total coliforms analysis will be conducted by the Region-1 Microbiology Laboratory, utilizing the IDEXX, Colilert®. *Enterococcus* analysis will be conducted by the Region-1 Microbiology Laboratory, utilizing the IDEXX, Enterolert®.

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, Marine Pollution Studies Laboratory - Department of Fish and Game (MPSL-DFG), 15 October 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	January 2012	Lab Data Reports	Continuous
Draft Monitoring Plan Data Report	January 2012	April 2012	Draft Report	April 2012
Final Monitoring Plan Data Report	May 2012	June 2012	Final Report	June 2012

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).

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 measurement quality objective for completeness shown in Table 3.

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. 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.

Accuracy for multi-probe measurements is tested through the pre-calibration process using standards that are within or bracket the expected measurement range and through the post-calibration process after use, using the standards to determine if the probes remained in calibration throughout the measurement period. The post-sampling checks of each unit ensure that the readings taken during use were within QC acceptance limits for each multi-probe analyte.

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. Measurement quality objectives require 90 percent completeness as shown in Table 3.

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 MQOs.

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. Measurement quality objectives for laboratory measurements.

Parameter	Method	Target Reporting Limits	Accuracy	Precision	Recovery	Completeness
E. Coli.	Colilert®	1 MPN /100 mL	Positive results for target organisms. Negative results for non-target organisms	Rlog within 3.27*mean Rlog	-	90%
Enterococcus	Enterolert®					
Enterococcus	Multi-Tube Fermentation (MTF)	2 colonies /100 mL				

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 Laboratory Quality Assurance Officer is responsible for the oversight and training of all laboratory personnel, but the Laboratory Director will provide the training for all laboratory personnel and retain documentation of all training in administrative files.

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 the Region-1 Microbiology Laboratory Lab Director in the North Coast Regional Water Board's lab.

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).

Laboratory personnel will conduct all analysis and sampling handling as outlined in each of the Laboratories SOPs (see Appendices 6 and 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 Appendix 3) for the samples collected. COC forms will accompany water samples to the appropriate laboratories for analysis.

Field data and observations will be recorded in real time on activity-specific data forms. Observations made and measurements taken in the field are recorded on standardized fieldsheets (see appendix 2). For these surveys, an individual fieldsheet is used for each station per sampling event.

Typical information required on the water quality fieldsheets 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 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. 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 generated will also be entered into the SWAMP IMS. 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 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. Tasks 1 and 2 are designed to assess indicator bacteria variability, while tasks 3 and 4 are designed to evaluate the influence of land use and recreational beach use on pathogen indicator concentrations.

Task 1: Sample Variability

Task 1 is designed to answer the following management questions:

1. Are Basin Plan Water Quality Objectives for pathogenic indicator bacteria being met in the middle and lower Russian River watershed?
2. What is the sampling variability of pathogenic indicator bacteria?
3. What is the analytical laboratory variability of pathogenic indicator bacteria?
4. What is the spatial variability of pathogenic indicator bacteria within a reach?

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. Previous Russian River indicator bacteria sample collection has been conducted using a screening level approach. This sampling focused on collecting a single grab sample to assess indicator bacteria concentrations at the sample site. Due to the nature of the sampling, little work had been conducted to assess the variability of that sample estimate. When evaluating sample variability, there are different components of that variability that need to be addressed.

Analytical Methods Variability

Multiple analytical laboratory procedures for pathogenic indicator bacteria in water samples have been approved as standard methods. Tests for coliform groups of bacteria have traditionally been conducted using the multiple-tube fermentation procedure as a most probable number index (Standard Method 9010). IDEXX's Colilert® and Enterolert® procedures have been adopted as standard methods for monitoring recreational water quality by the United States Environmental Protection Agency (IDEXX, 2001; USEPA, 2003). The Colilert® and Enterolert® methods are relatively recent MPN procedures that produce results in 24 hours. To address this component, we are collecting replicate samples at each sample location to be analyzed for *Enterococcus* using multiple-tube fermentation and IDEXX's Enterolert® procedure.

Analytical Laboratory Variability

Laboratories following standard method procedures develop quality assurance information on the variability of the laboratory procedures for indicator bacteria measurements (i.e., Standard Method 9020B.4). Variability has been observed

between split samples sent to different laboratories (Griffith et al. 2006). A small study conducted by the North Coast Regional Water Board at Monte Rio Beach has shown that there were differences between the results of split samples analyzed by different laboratories, however the sample size was inadequate to allow a statistical comparison. To address this component, we are collecting replicate samples at each sample location and having them analyzed by the Sonoma County Public Health Laboratory and the Region-1 Microbiology Laboratory. This analysis will provide an estimate of inter-laboratory variability.

Sample Temporal Variability

Samples collected at any location can vary temporally. One needs to know the variance over a short time (i.e., between samples collected subsequently at the same time and location) in order to compare between sampling events over time. The measured variation of replicate samples can be used to assess the serial variation between samples collected within the sampling time period. To address this component, we are collecting three replicate samples in rapid succession at each location to be analyzed by the Region-1 Microbiology Laboratory. This analysis will provide an estimate of temporal variability at a sample location.

Sample Spatial Variability

Samples collected at any location can vary spatially. To determine the variance across the general area of a sampling location in order to compare between sampling sites. The measured variation of replicate samples can be applied to single sample data in order to make comparisons between single sample data from various sites. To address this component, we are collecting nine spatially distributed samples at each sample location. This analysis will provide an estimate of spatial variability at a sample location.

Sample Collection

Assessment of the sample variability will be conducted by collecting samples during one sampling event at each of the three (3) listed reaches in Table 5. Field crews will locate these sampling locations, (figure 3), with the use of the reach maps in Appendix 5, or by GPS if needed.

Table 5. Sampling Reaches for Task 1

Station ID	Reach Name	Location	Latitude	Longitude
114RR2940	Healdsburg Memorial Beach	Old Redwood Hwy	38.60357	-122.85993
114RR0898	Monte Rio Beach	Bohemian Hwy	38.46604	-123.00926
114SR3260	Santa Rosa Creek	Prince Memorial Greenway	38.43501	-122.71874

Samples will be collected at each of nine (9) locations at each reach: three (3) longitudinal locations along the river channel (upstream ½ width of stream, at the site, downstream ½ width of stream) and at three (3) locations along the lateral transect (off of far bank, mid-channel, off of near bank). Samples will be collected at the downstream locations first to avoid collection of disturbed sediments in subsequent samples. Samples will be collected when there are no

swimmers in the reach (i.e., early morning) to eliminate the sampling of bottom sediments disturbed into the water column by recreation. Samples will be collected during a dry weather period.

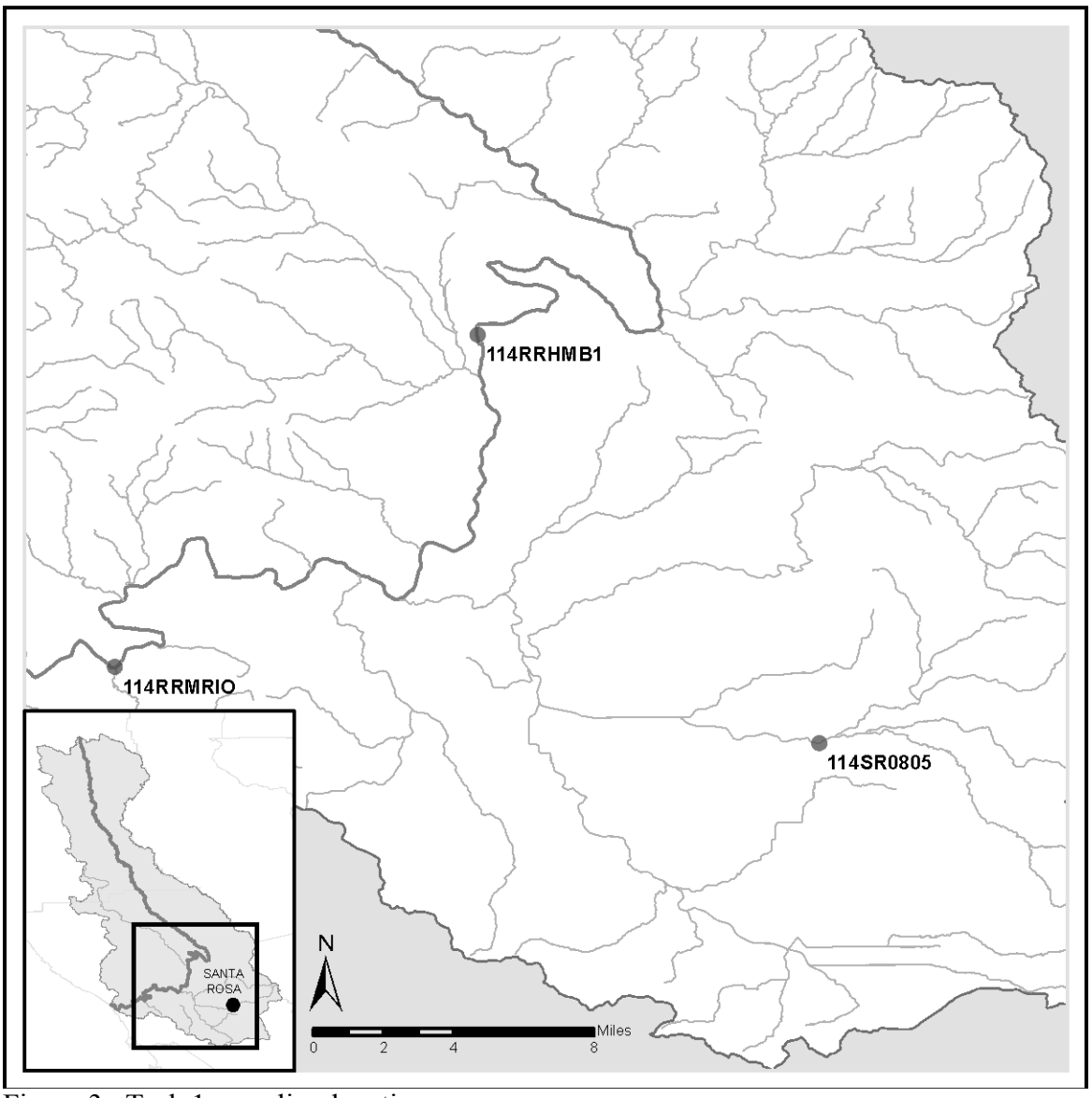

Figure 3. Task 1 sampling locations.

The resulting sample size will be a total of 405 samples. Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 81 samples to Region-1 Microbiology Laboratory for Colilert®
- 81 samples to Region-1 Microbiology Laboratory for Enterolert®
- 81 samples to Sonoma County Health Services Lab for Colilert®
- 81 samples to Sonoma County Health Services Lab for Enterolert®
- 81 samples to Sonoma County Health Services Lab for multiple tube fermentation for *Enterococcus*.

Analysis

These data will be compared using the Kruskal-Wallis nonparametric statistical hypothesis test to determine if there are significant differences between collected samples. The Kruskal-Wallis Test is a one-way analysis of variance conducted using ranked data. The non-parametric method is used for testing equality of population medians among groups. Nonparametric methods are often referred to as distribution free methods as they do not rely on assumptions that the data are drawn from a given probability distribution. Commonly used statistics like mean and standard deviation assume the data set follows a Gaussian (i.e., normal) distribution. Pathogenic indicator bacteria data rarely meet that assumption. Non-parametric methods are more robust due to the reliance on fewer assumptions.

Task 2: Site Variability

Task 2 is designed to answer the following management questions:

1. Are Basin Plan Water Quality Objectives for pathogenic indicator bacteria being met in the middle and lower Russian River watershed?
2. What is the spatial variability of pathogenic indicator bacteria in the middle and lower River?
3. What is the temporal variability in the middle and lower Russian River of pathogenic indicator bacteria between wet and dry seasons?

The Russian River Pathogens Pilot Study (see Element A5) identified the need to expand current indicator bacteria monitoring over a range of climatic conditions and to sites other than public beaches. The pilot study also recommended that a minimum of weekly sampling should be conducted throughout the summer recreational use period. Conducting weekly sampling assures that a sufficient number of samples are collected to meet the assessment requirements of the North Coast Regional Water Quality Control Board's Basin Plan Objective for the Beneficial Use support of REC-1 use requiring five (5) samples in a 30-day period.

When calculating TMDLs, numeric targets are selected to meet WQOs for surface waters and subsequently ensure the restoration and/or protection of beneficial uses. Different TMDL numeric targets and load calculations for indicator bacteria are typically established for wet and dry weather periods because the bacteria transport mechanisms are different under each weather condition.

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. Bacteria densities during a wet weather event are best represented by the single sample maximum WQOs. The Basin Plan does not contain a single sample WQO for indicator bacteria. However, U.S. EPA recommends criteria to evaluate single sample indicator bacteria concentrations (USEPA, 1986). These criteria, which will be used to advise this assessment, are the single sample concentration of *E. coli* and *Enterococcus* at 235 per 100 ml MPN and 61 per 100 ml MPN, respectively.

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. Therefore, dry season bacteria densities are usually best represented by the 30-day median WQO fecal coliform set in the Basin Plan. U.S. EPA also recommends an *Enterococcus* concentration criterion for a 5-sample, 30-day geometric mean of 33 per 100 ml MPN for freshwater recreational waters. Both of these criteria are appropriate for assessing dry season impairments to REC-1 use.

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

Dry Season

Samples will be collected on a weekly basis and analyzed by the Region-1 Microbiology Laboratory beginning on May 17 and ending September 27, for a total of 20 sampling events. Samples will be collected on Tuesdays of each week to match the weekly schedule most commonly used in previous years. Continuing the collection of samples on Tuesdays allows the data to be compared to previously collected data without introducing bias that may be introduced from weekly variation.

Wet Season

Samples will be collected during five (5) wet periods and analyzed by the Region-1 Microbiology Laboratory from October through April. 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. Field crews will locate these sampling locations, (figure 4), with the use of the reach maps in Appendix 5, or by GPS if needed.

Table 6. Sampling Location for Task 2

Station ID	Station Name	Location	Latitude	Longitude
*114RR4234	Alexander Valley Campground *	Alexander Valley Road	38.65857	-122.82969
*114RR3119	Camp Rose *	Camp Rose Road	38.61361	-122.83115
*114RR2940	Memorial Beach *	Old Redwood Hwy	38.60357	-122.85993
*114RR2036	Steelhead Beach *	Old River Road	38.50024	-122.89944
*114RR1898	River Access Beach *	River Drive	38.51073	-122.92384
*114RR1325	Johnson's Beach *	Church Street	38.49938	-122.99900
*114RR0898	Monte Rio Beach *	Bohemian Hwy	38.46604	-123.00926
114RR6273	Commisky Station	Hwy 101	38.88741	-123.05447
114RR5748	Cloverdale River Park	Crocker Road	38.82307	-123.00985
114RR4751	Geyserville Bridge	Highway 128	38.71265	-122.89553
114DB0147	Dutch Bill Creek	Fir Road	38.46482	-123.00902
114RR0066	Jenner	Jenner Boat Ramp	38.44942	-123.11572
114SR6158	Santa Rosa Creek	Los Alamos Road	38.45703	-122.63092
114SR3260	Santa Rosa Creek	Prince Memorial Greenway	38.43501	-122.71874
114LG3411	Laguna de Santa Rosa	Sebastopol Community Center	38.40804	-123.81827
114GV2455	Green Valley Creek	Martinelli Road	38.47858	-122.90793

The sample locations in Table 5 will be sampled during both wet and dry periods. To also assess sampling variability, triplicate (3) samples will be collected at the first seven (7) beach locations listed in Table 5 (and identified with a *) during the dry period sampling events (20 dry events). These beach locations were selected for replication due to the higher recreational demand.

Figure 4. Task 2 sampling locations.

The resulting sample size will be a total of 1200 dry season samples and 160 wet season samples. Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 600 Dry Season samples to Region-1 Microbiology Laboratory for Colilert®. 30 samples collected during 20 sampling events.
- 600 Dry Season samples to Region-1 Microbiology Laboratory for Enterolert®. 30 samples collected during 20 sampling events.
- 80 Wet Season samples to Region-1 Microbiology Laboratory for Colilert®. 16 samples collected during 5 sampling events.
- 80 Wet Season samples to Region-1 Microbiology Laboratory for Enterolert®. 16 samples collected during 5 sampling events.

Task 3: Land Use Variability

Task 3 is designed to answer the following management questions:

1. Are Basin Plan Water Quality Objectives for pathogenic indicator bacteria being met in the middle and lower Russian River watershed?
2. What is the variability of pathogenic indicator bacteria between different land covers?
3. What is the temporal variability of pathogenic indicator bacteria between wet and dry seasons?
4. What are the most significant sources of pathogenic indicator bacteria?
5. What is the background or natural levels of pathogenic indicator bacteria?

In the Russian River Pathogens Pilot Study (see Element A5), it was determined that different land uses exhibited different pathogenic indicator bacteria levels. The objective of this task is to assess the relative magnitude and variability of indicator 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 will be collected during three (3) dry periods from May 2011 through October 2011.

Wet Season

Samples will be collected during three (3) wet periods from 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.

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 7. Field crews will locate these sampling locations, (figure 5), with the use of the reach maps in Appendix 5, or by GPS if needed.

Table 7. Sampling Locations for Task 3

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

The sample locations in Table 6 will be sampled during both wet and dry periods. To also assess sampling variability, triplicate samples will be collected from each site during six (6) sampling events, with three (3) sample events occurring during the dry period and three (3) sample events occurring during wet periods.

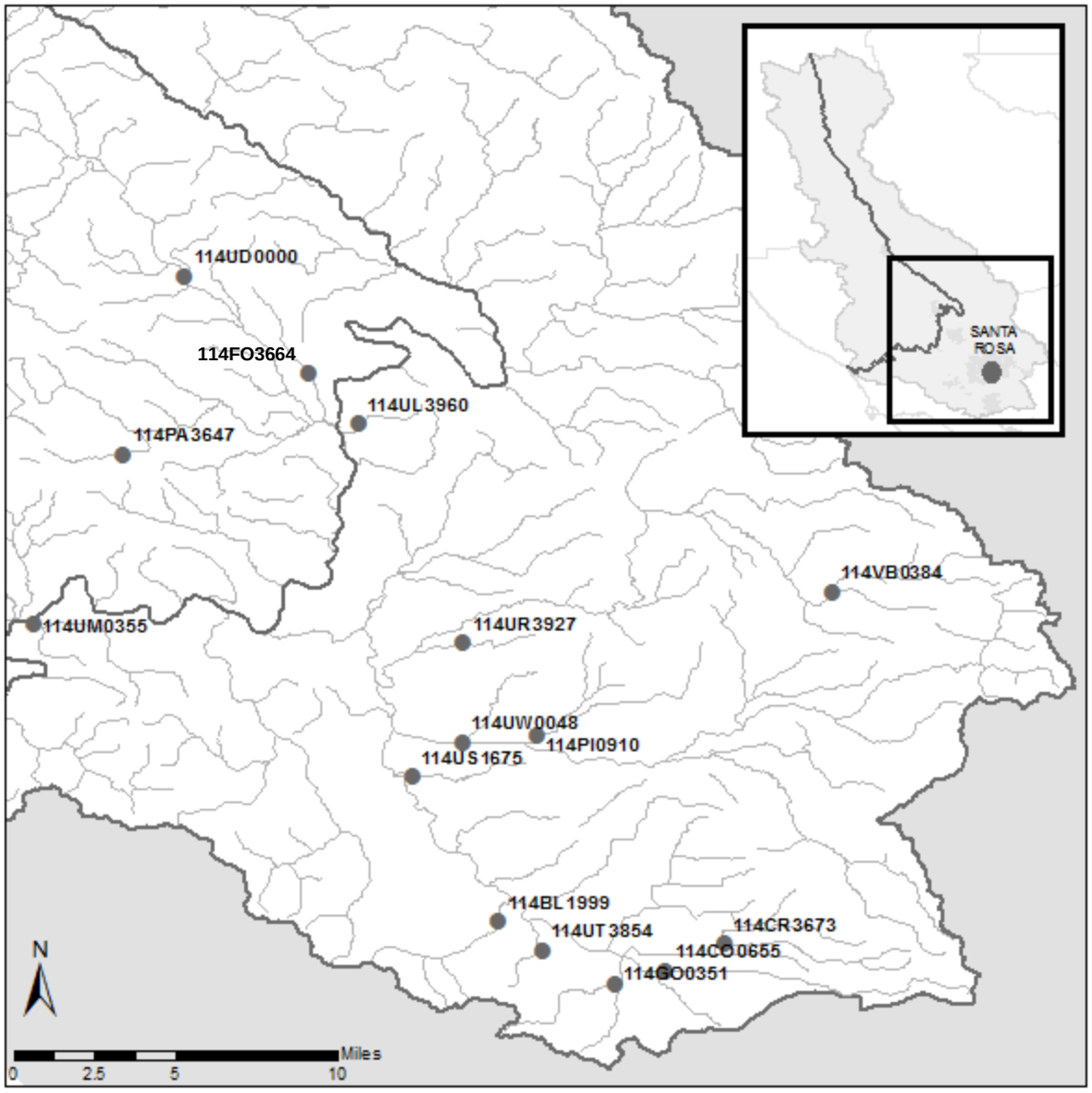

Figure 5. Task 3 sampling locations.

The resulting sample size will be a total of 90 dry season samples and 90 wet season samples. Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 45 Dry Season samples to Region-1 Microbiology Laboratory for Colilert®. 15 samples collected during 3 sampling events.
- 45 Dry Season samples to Region-1 Microbiology Laboratory for Enterolert®. 15 samples collected during 3 sampling events.
- 45 Wet Season samples to Region-1 Microbiology Laboratory for Colilert®. 15 samples collected during 3 sampling events.
- 45 Wet Season samples to Region-1 Microbiology Laboratory for Enterolert®. 15 samples collected during 3 sampling events.

Analysis

The data for each land use will be combined resulting in 18 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 4: Recreational Use Variability

Task 4 is designed to answer the following management questions:

1. Are Basin Plan Water Quality Objectives for pathogenic indicator bacteria being met in the middle and lower Russian River watershed?
2. Do beach areas pose a higher risk to REC-1 than non-beach reaches?

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. Previous Russian River indicator bacteria sample collection has been conducted using a screening level approach. This sampling focused on collecting a single grab sample on a single day to assess indicator bacteria concentrations at the sample site. Due to the nature of the sampling, little work had been conducted to assess the variability that may be associated with sample collection on any given day. 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 on weekends.

Sample Collection

Intensive sampling events will be conducted to assess the local impact of recreational activities on indicator bacteria levels at public beaches. Sampling will be conducted by collecting samples at each of the listed stations in Table 7; Healdsburg Memorial Beach and Monte Rio Beach. For the Monte Rio Beach reach, Dutch Bill Creek will also be sampled as a suspected stream source. Field crews will locate these sampling locations, (figure 6), with the use of the reach maps in Appendix 5, or by GPS if needed.

Table 8. Sampling Locations for Task 4

Station ID	Station Name	Location	Latitude	Longitude
114RR2940	Healdsburg Memorial Beach	Old Redwood Hwy	38.60357	-122.85993
114RR0898	Monte Rio Beach	Bohemian Hwy	38.46604	-123.00926
114DB0147	Dutch Bill Creek	Bohemian Hwy	38.46482	-123.00902

Due to limitations on available incubator space at the Region-1 Microbiology Laboratory, samples will be collected every other day for two weeks, (rather than every day for one week), to assess daily variability. Sample collection will bracket a summer holiday weekend (Memorial or Labor Day weekends), capture the possible variability in indicator bacteria concentrations due to the elevated recreational use. Samples will be collected ~100 meters upstream, at the beach, and ~100 meters downstream.

For each sampling day and location, three (3) samples will be collected and analyzed by the Region-1 Microbiology Laboratory for both *E. coli* and *Enterococcus* using the Colilert® and Enterolert® methods.

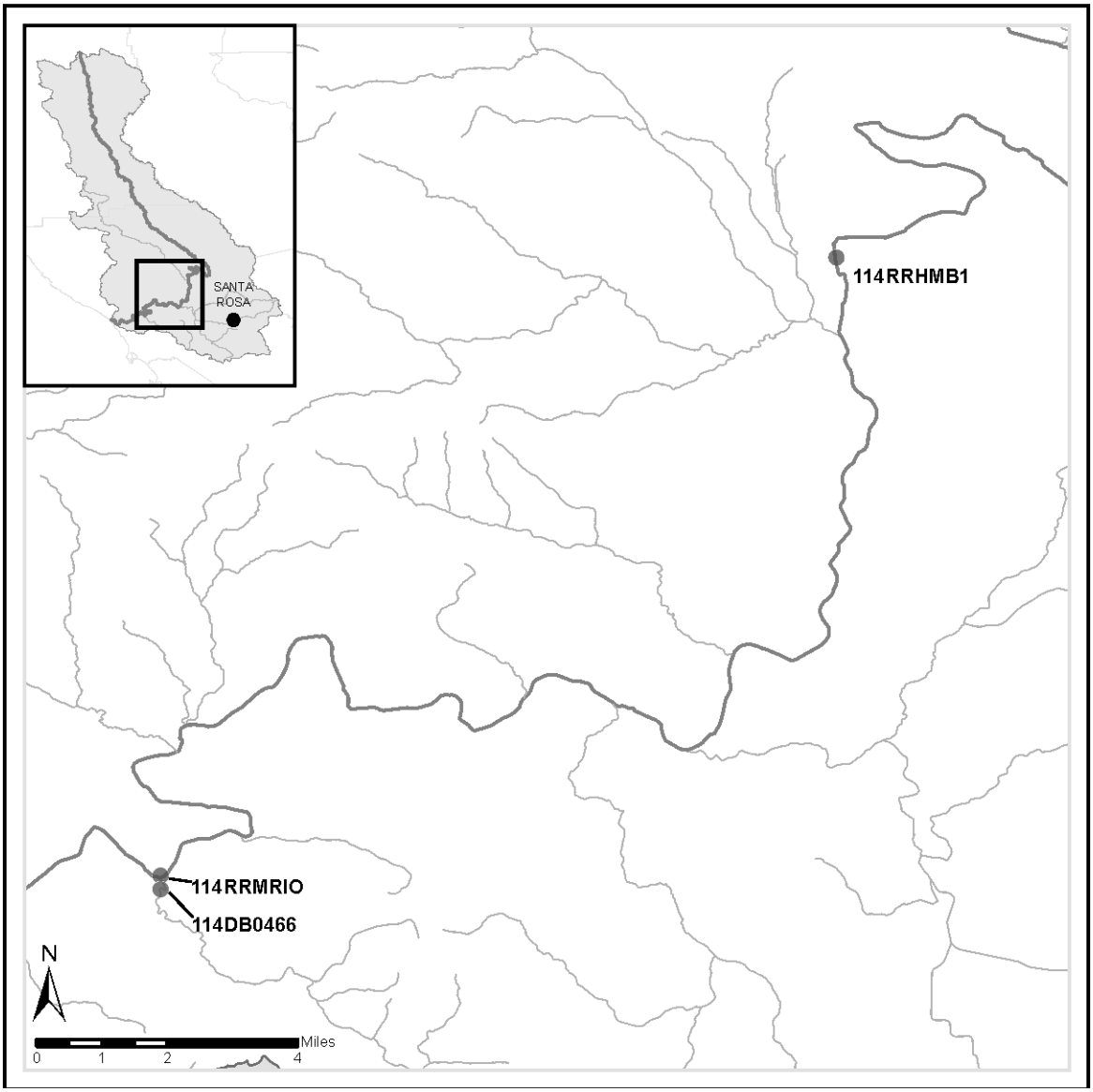

Figure 6. Task 4 sampling locations.

The resulting sample size will be a total of 126 samples. Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 63 samples to Region-1 Microbiology Laboratory for Colilert®. 9 samples collected during 7 sampling events.
- 63 samples to Region-1 Microbiology Laboratory for Enterolert®. 9 samples collected during 7 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) the SWAMP Quality Assurance Program Plan, b) 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 fieldsheets (see Appendix 2), chain of custody forms (see Appendix 3), laboratory sheets (see Appendix 3), sample labels, sample collection bottles, and verifying equipment functionality and availability. Field crews will be responsible for preparing all forms and obtaining sample bottles for sample collection from the Region-1 Microbiology Laboratory.

Sample Volume and Bottle Type

Field measurements will be taken on site and do not require a sample volume or collection bottle.

Samples collected for total coliform and *E. coli* analysis will be collected in either a 125 ml or 250 ml, factory sterilized and sealed polyethylene bottle. Sample volumes will be either 100 ml or 200 ml, respectively.

Sample Preservation and Holding Times

Samples collected for total coliform and *E. coli* will be preserved with sodium thiosulfate which has been added to the bottles by the manufacturing company.

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 8 hours after the first sample was collected.

Sample incubation times for *Enterococcus*, total coliform, and *E. coli* require an incubation time of 24 to 28 hours. For consistency, samples will be pulled from the incubator at 24 hours and quantification run immediately.

Multipurpose Datasonde:

Tracking the YSI datasondes used in the field is important for proper maintenance. Pre-calibration of the meter will be conducted within 24 hours of sampling according to the instructions, as detailed in the manufacturer's procedures manual located in the North Coast

Regional Water Board Lab. The YSI datasondes will also be post-calibrated within 24 hours of the end of the sampling run and at any time during the run if values seem not "normal." These results are then recorded on the YSI datasonde calibration forms (see Appendix 4).

Responsible Individuals

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 fieldsheet.

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. The samples collected for this study are intended for use as regulatory data. For all bacteria 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 8 hours of the time the first sample was collected.

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 Region-1 Microbiology Laboratory.

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.

Samples received by the Region-1 Microbiology Laboratory will be processed immediately upon receipt and within the full 8 hour holding time. Verification of preservation temperature will be checked and documented upon delivery to the lab as per the individual laboratory SOPs.

Table 9: Sampling and Preservation

Analyte	Units	Container	Sample Volume	Preservation	Maximum Holding Time
E. Coli	MPN /100 mL	125 mL Sterile Plastic container	100 mL	Cool to 6 ◦C in the dark.	8 hours
Enterococcus	Colonies /100 mL				
Total Coliform	MPN /100 mL				

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 fieldsheets and chain of custody forms, as needed.

Transport

Samples will be stored in the dark in coolers on ice, at a temperature below 6°C. Samples to be analyzed for *Enterococcus*, total coliform and *E. coli* will be delivered to the appropriate Laboratory:

Region 1 Microbiology Laboratory
5550 Skylane Blvd, Suite A
Santa Rosa, CA 95403
Tel: 707-576-2719

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

Field crews will deliver samples and required documentation to laboratory staff designated to receive samples. Samples collected will be verified against fieldsheets 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 fieldsheets 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

Field measurements for dissolved oxygen (DO), conductivity, pH, and temperature will be collected using the YSI 600XL Sonde and 650 MDS. A probe guard will be attached at the end of the YSI to avoid fouling and protect the probes.

The microbiological and laboratory analytical methods to be used for this project to analyze water samples in the laboratory are listed in Table 10.

Table 10. Laboratory Analytical Methods.

Analyte	Laboratory	Project Action Limit	Project Quantitation Limit	Analytical Method	MDLs
E. coli	Region-1 Microbiology Laboratory	<235 MPN /100mL	1 MPN /100mL	Colilert®	1 MPN /100mL
Enterococcus	Region-1 Microbiology Laboratory	<61 MPN /100mL	1 MPN /100mL	Enterolert®	1 MPN /100mL
Enterococcus	Sonoma County Public Health Laboratory	<61 colonies /100mL	2 colonies /100mL	SM 9230 B	1 colonies /100mL

Table 11. Field Measurements – YSI Datasonde.

Parameter	Probe	Units	Resolution	Reporting Limit	"Electronic Specs" Accuracy**
Dissolved Oxygen	Polarographic	mg/L	0.01	0.2	± 0.2
pH	Electrode	None	0.1	n/a	± 0.2
Specific Conductivity	Conductivity Cell	µS/cm	1	2	± 2
Temperature	Thermistor	°C	0.1 or 0.5	n/a	± 0.1

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 fieldsheet with the data report, along with the corrective action that was made. Additionally, corrections will be annotated in any applicable maintenance logs.

Sample Disposal Procedures

All of the Sonoma County Public Health Laboratory wastes will be handled as biohazard waste and discarded in accordance with Biohazard Waste Regulations. Samples analyzed by the

Region-1 Microbiology lab will be sealed in IDEXX Quantitrays. The Quantitrays will be delivered to the Sonoma County Public Health Laboratory, 3313 Chanate Road, Santa Rosa, CA to be autoclaved and disposed.

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 Measurements

Field measurement equipment will be checked for calibration against standards of known concentrations for pH and EC. Checks will be run at the beginning of the field run and at the end of the field run.

Duplicate field measurements will be performed for the multi-probe parameters of pH, temperature, dissolved oxygen and conductivity and will be made at least once during each sampling event. Field duplicate measurements will be made by collecting an additional sample from the stream immediately following and in an identical manner to the original sampling and measurement process. The accuracy of the field analytical equipment will be based upon proper calibration practices. Duplicate precision will be acceptable if the relative percent difference is less than 20% for low level and 10% for high level field measured parameters. When differences in duplicates are greater, a third replicate sample will be collected and field measurements will be repeated.

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 sterile 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. The field blank is filled at a sampling location. 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 both *E. coli* and *Enterococcus* analyses.

Field Duplicates

Field duplicate samples provide precision information on all steps after sample acquisition. These samples will be collected by alternately filling two sample containers for each analysis. They will be collected at a minimum of one sampling location during each sampling event. The field duplicate samples will be preserved, packaged, and sealed in the same manner described for the surface water samples. A separate sample number and will be assigned to each duplicate and the samples will be submitted to the lab.

Field duplicates shall be collected immediately following the collection of normal samples. In cases where multiple intermediate bottles are used for a single analysis, field duplicates and normal sample containers should be filled in an alternating sequence (i.e., normal-duplicate).

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. Laboratory duplicates will be conducted for both *E. coli* and *Enterococcus* analyses. They will be processed at a minimum of one laboratory blank during each sampling event. No special sampling considerations are required.

B6: Instrument/Equipment Testing, Inspection, and Maintenance

Microbiological sample bottles will be provided by the Region-1 Microbiology Laboratory prior to the sampling events. The Region-1 Microbiology Laboratory maintains sufficient quantities of fresh calibration standards for the instruments that will be used for field activities.

Field measurement equipment will be inspected to ensure proper functioning in accordance with the manufacturer's specifications prior to each trip in the field. This includes battery checks and cleaning. All equipment will be inspected for damage when first handed out and when returned from use. Field measurements are not collected if the equipment appears visibly worn or if field technicians report problems with the equipment upon returning from the field.

Field measurement equipment is calibrated and the calibration is recorded in the Calibration and Maintenance Log. Any maintenance performed on the field instruments is also recorded, such as DO probe membrane replacement. The instrument is calibrated and operated according to the manufacturer's User's Manual.

Laboratory equipment will be inspected, calibrated, and maintained according to the individual laboratories QAP, (see Appendices 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

All equipment and instruments are operated and calibrated according to the manufacturer's recommendations. Operation and calibration are performed by personnel properly trained in

these procedures. Documentation of all calibration information is recorded in the appropriate logs. If equipment is not meeting the listed criteria (Table 12) it is the responsibility of the field crews to notify the Project Manager and Project QA Officer, who will be responsible for addressing the problem. This may include repair or replacement of equipment. All corrective actions are documented in the appropriate log.

Table 12. Field Equipment Calibrations – YSI datasonde.

Parameter	Points Per Calibration	Pre-Measurement Calibration Adjustment Frequency	Accuracy Check (Post-Calibration Check) Frequency	Allowable Drift (Measurement Accuracy)
Dissolved Oxygen	1	Within 24 hours prior to beginning sampling run	Within 24 hours of the end of the sampling run	± 0.5 or 10%
pH	2			± 0.2
Specific Conductivity	1			± 4 or 10%
Temperature	2	n/a	Once annually	± 0.5 or 10%

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 Region-1 Microbiology 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 the acceptance criteria to the 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 Quality Assurance Program Plan.

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), fieldsheets, 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 fieldsheets 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 fieldsheets 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, fieldsheets will be checked for completeness and accuracy.

Region-1 Microbiology Laboratory staff will record *Enterococcus*, total coliform and *E. coli* analysis on the bacteria processing worksheet (see Appendix 3). Data approved by the Laboratory Director will be entered into an Excel worksheet and ultimately entered into the SWAMP database by staff designated by the Project Manager.

Computerized Information System Maintenance

Official electronic files will be maintained by the Project Manager once the data reports are received from the Region 1 Microbiology Laboratory and the Sonoma County Public Health

Laboratory QA Officers. The files will be located on the North Coast Regional Water Board network prior to entering the data into the SWAMP Information Management System. The North Coast Regional Water Board Information Technology unit performs backup nightly on all network drives.

SWAMP Information Management System

Field measurement, *Enterococcus*, total coliform, and *E. coli* data will be verified as meeting QA/QC requirements by the Project Manager and Project QA Officer. Once the data is verified acceptable, it will be entered into the SWAMP database by staff designated by the Project Manager.

Data in the SWAMP Database will be made available to the public through the California Environmental Data Exchange Network (CEDEN). Information on CEDEN is available at www.ceden.org.

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. Readiness reviews will consist of the following activities:

Equipment Checks

All field equipment will be clean and ready to use when it is needed. Therefore, prior to using all sampling and/or field measurement equipment, each piece of equipment will be checked to make sure that it is in proper working order.

Equipment Maintenance Records

Equipment maintenance records will be checked to ensure that all field instruments have been properly maintained and ready for use.

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 Project Quality Assurance Officer 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 Region-1 Microbiology Laboratory Lab Director and Sonoma County Public Health Laboratory Lab Director 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 13.

Table 13 – Report Due Dates

Report Type	Frequency	Responsible Party	Schedule
Data Report	Per field run	Region-1 Microbiology 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 labsheets 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 MQOs

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

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.

Checking Data

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.

The Project Manager, Project QA Officer, and SWAMP Quality Assurance Team will be responsible for the verification and validation of data going into the SWAMP IMS.

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. For data in the SWAMP IMS, the Project QA Officer will perform an independent recheck of at least 10% of these records as the validation methodology.

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. For data in the SWAMP IMS, the Project QA Officer will perform independent re-checks of at least 10% of them as 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. For data in the SWAMP IMS, the Project Manager, Project QA Officer, and SWAMP data management team (DMT) will be responsible for reporting and correcting any errors that are found in the data during the verification and validation process.

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.

During the process of verification and validation for data in the SWAMP IMS, the methods that will be used are described in the Surface Water Ambient Monitoring Program Information Plan.

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 and will be submitted to the SWAMP's IMS and the State Water Quality Control Board's Safe to Swim Portal. Subject to physical habitat limitations, 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

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Appendix 2: Field Data Sheet

SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ | *Project Code: RWB1_BACTL_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_generic) | *GPS / DGPS | Lat (dd.ddddd) | Long (ddd.ddddd)

OBSERVED FLOW: NA Dry Waterbody Bed No Obs Flow Isolated Pool Trickle (<0.1cfs) | WADEABILITY: Y / N / Unk | Target: | -

0.1-1cfs 1-5cfs 5-20cfs 20-50cfs 50-200cfs >200cfs | *Actual: | -

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

SITE ODOR: None Sulfides Sewage Petroleum Smoke Other_____ | BEAUFORT SCALE: (see back) | WIND 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_____ | (DS)

DOM. SUBSTRATE: Bedrock Concrete Cobble Gravel Sand Mud Unk Other_____ | DupID:

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 WI 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)

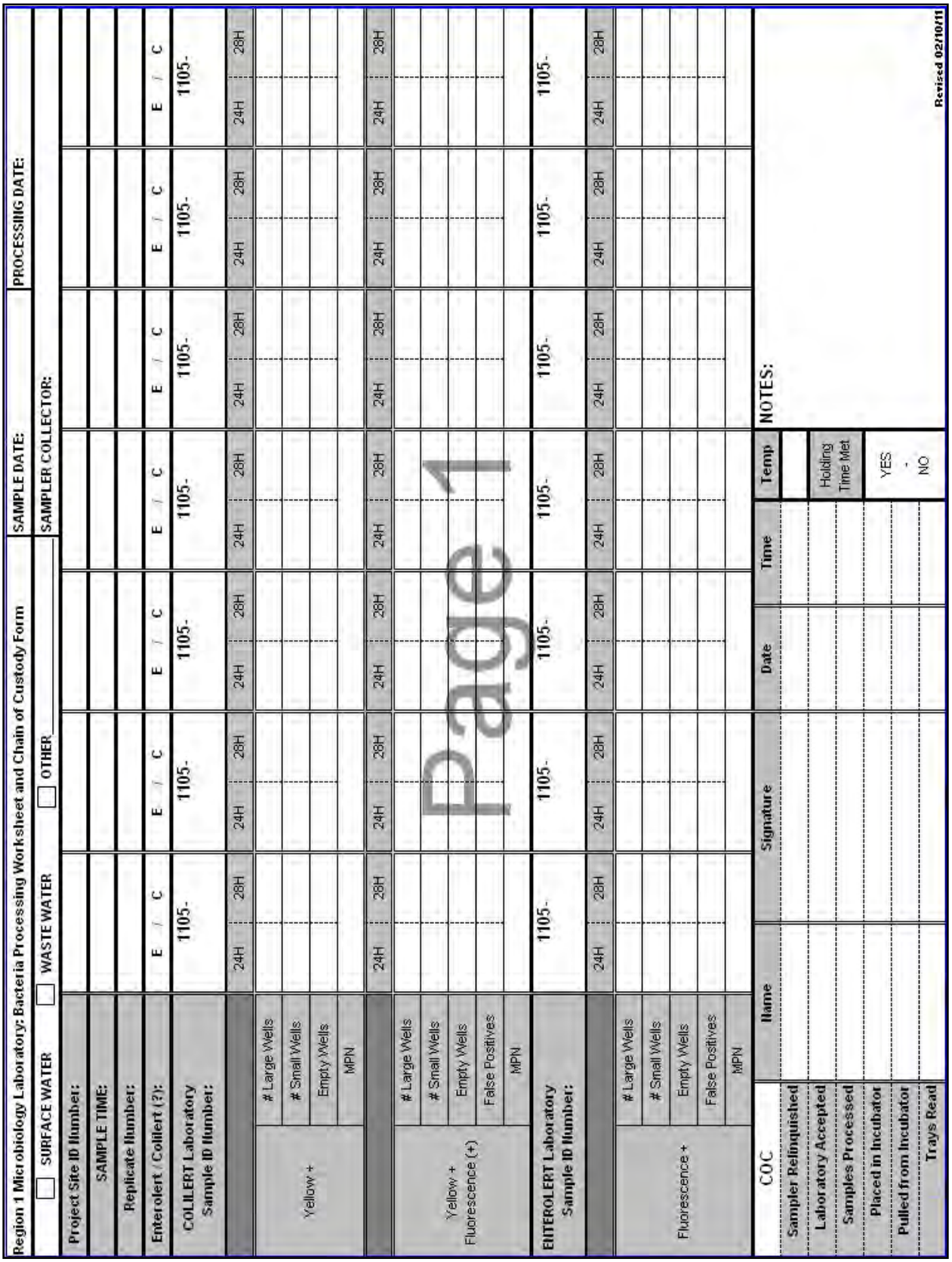
DepthCollection (m)	Air Temp (°C)	STREAM WIDTH (m):	WATER DEPTH (m):	Velocity (fps)	Water Temp (°C)	Specific Conductivity (uS/cm)	pH	O2 (mg/L)	O2 (%)		Salinity (ppt)	Barometric Pressure
0.1												
DISTANCE FROM BANK (m):		Instrument	VIS	YSI Datasonde: 600XL					S/N:	YSI 650MDS		
		Calibration Date						Calibration Sheet No:				

COMMENTS:

*Purpose: WaterChem

Rev. 12/31/2009

Appendix 3: Chain of Custody Forms



Appendix 4: YSI Datasonde Calibration Form

Pre-run Calibration

S/N:

Calibrated by:

Battery Voltage (%):

Date:

Time:

Altitude (ft):

Project:

For DO Calibration (Value of DO Standard)

Barometric Press. (mm Hg):

Pre-Calibration Recorded in Log Book?

Instrument Function	Temp. of Standard	Value of Standard	Initial Reading:	Calibrated to:
Sp.Cond. uS/cm				
pH Buffer 7.00				
pH Buffer 10.00				
Dissolved Oxygen (mg/L)				
Turbidity (DI Water)		0.0		
Turbidity (LOW)		12.7		
Turbidity (HIGH)		126.0		

Record calibration standard lot numbers where indicated

Lot #:

Lot #: pH 7 MV:

Lot #: pH 10 MV:

DO Charge:

Lot #:

Lot #:

Calibration Information Record the following diagnostic numbers AFTER calibration			** IMPORTANT ** Check DO warm-up pattern prior to first use
Conductivity Cell Constant		Range 4.5 to 5.5	☐ ☑ (Sonde Menu - Advanced - Cal Constants)
DO Gain		Range 0.7 to 1.5	☐ (Sonde Menu - Advanced - Cal Constants)
DO Charge		Range 25 to 75	☐ (Sonde Menu - immediately after DO calibration)
pH MV Buffer 7.00		Range -50 to 50 MV	☐ (Sonde Menu - immediately after pH 7 calibration)
pH MV Buffer 10.00		Range -127 to -227 MV	☐ (Sonde Menu - immediately after pH 10 calibration)
pH Slope (pH 7 MV - pH 10 MV)		Range 162 to 180 MV	☐ If slope is very near lower limit, consider changing probe before failure

DO Recalibration Note: Recalibrate DO ONLY if station to station elevation varies by more than 500 feet in altitude

Prior to 500 ft elev. change: Record the values	Temp:		**DO (mg/L): Reading**		Barometric Press. (mm Hg): (uncorrected)
After 500 ft elev. change: Recalibrate DO	Temp:		**DO (mg/L): Calibrated**		Barometric Press. (mm Hg): (uncorrected)
Prior to 500 ft elev. change: Record the values	Temp:		**DO (mg/L): Reading**		Barometric Press. (mm Hg): (uncorrected)

Post-run Calibration Check

Date:

Time:

Altitude (ft):

Post-Calibration Check by:

Battery Voltage (%):

For DO Calibration (Value of DO Standard)

Barometric Press. (mm Hg):

Instrument Function	Temp. of Standard	Value of Standard	Instrument Reading	Drift (+ -)	Post Calibration Error Limits
Sp.Cond. uS/cm					☐ ☑ +- 5%
pH Buffer 7.00					☐ +- 0.3 standard units
pH Buffer 10.00					☐ +- 0.3 standard units
Dissolved Oxygen (mg/L)					☐ +-0.5 mg/L
Turbidity (DI Water)		0.0			☐
Turbidity (LOW)		12.7			☐
Turbidity (HIGH)		126.0			☐

Notes: _____

Post-Calibration Recorded in Log Book?

Recorded in Spreadsheet?

Verified by:

Rev. 12/31/09

Appendix 5: Monitoring Location Maps

List of Figures

Figure 1: Russian River at Alexander Valley Campground.

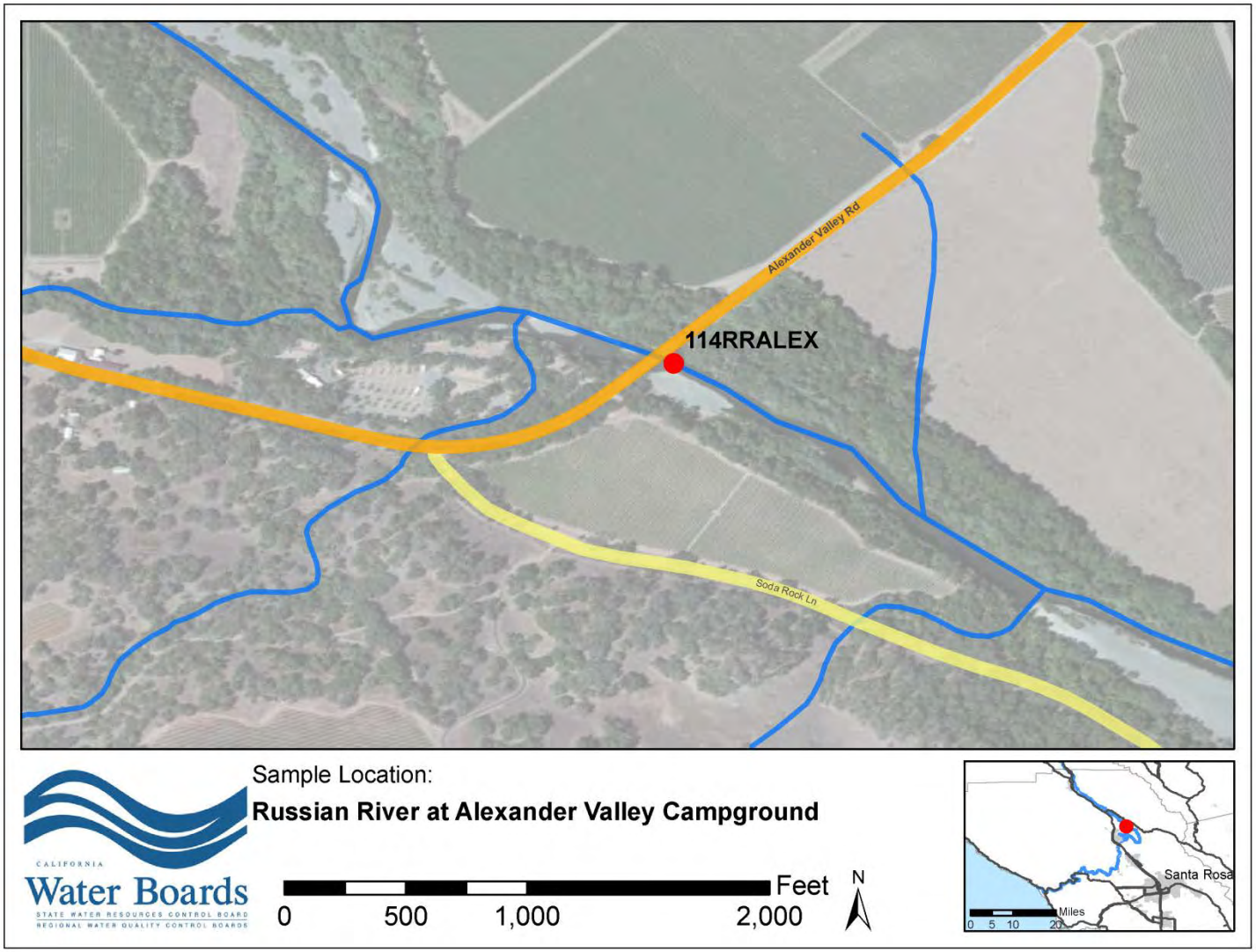


Figure 2: Russian River at Camp Rose.

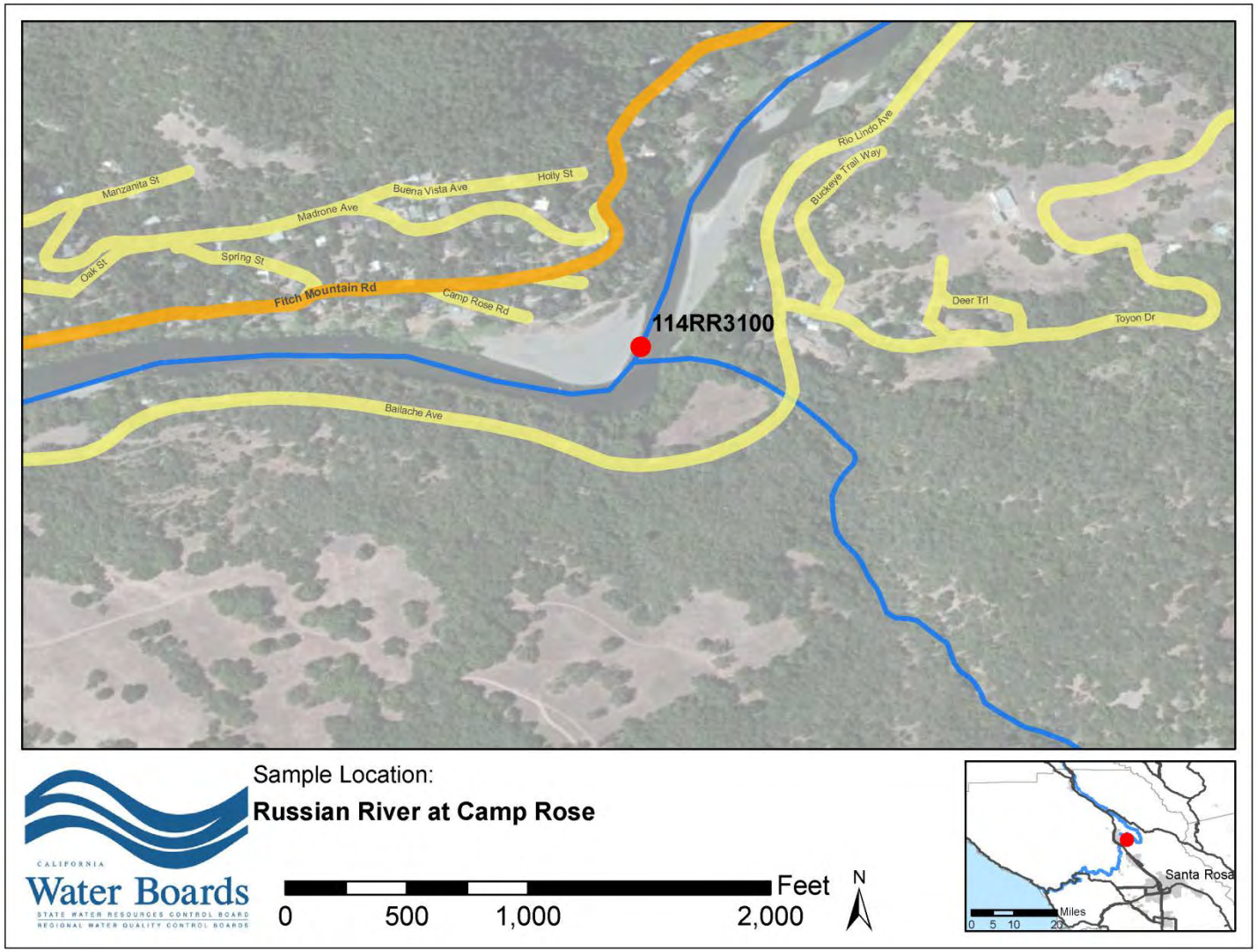


Figure 3: Russian River at Healdsburg Memorial Beach.

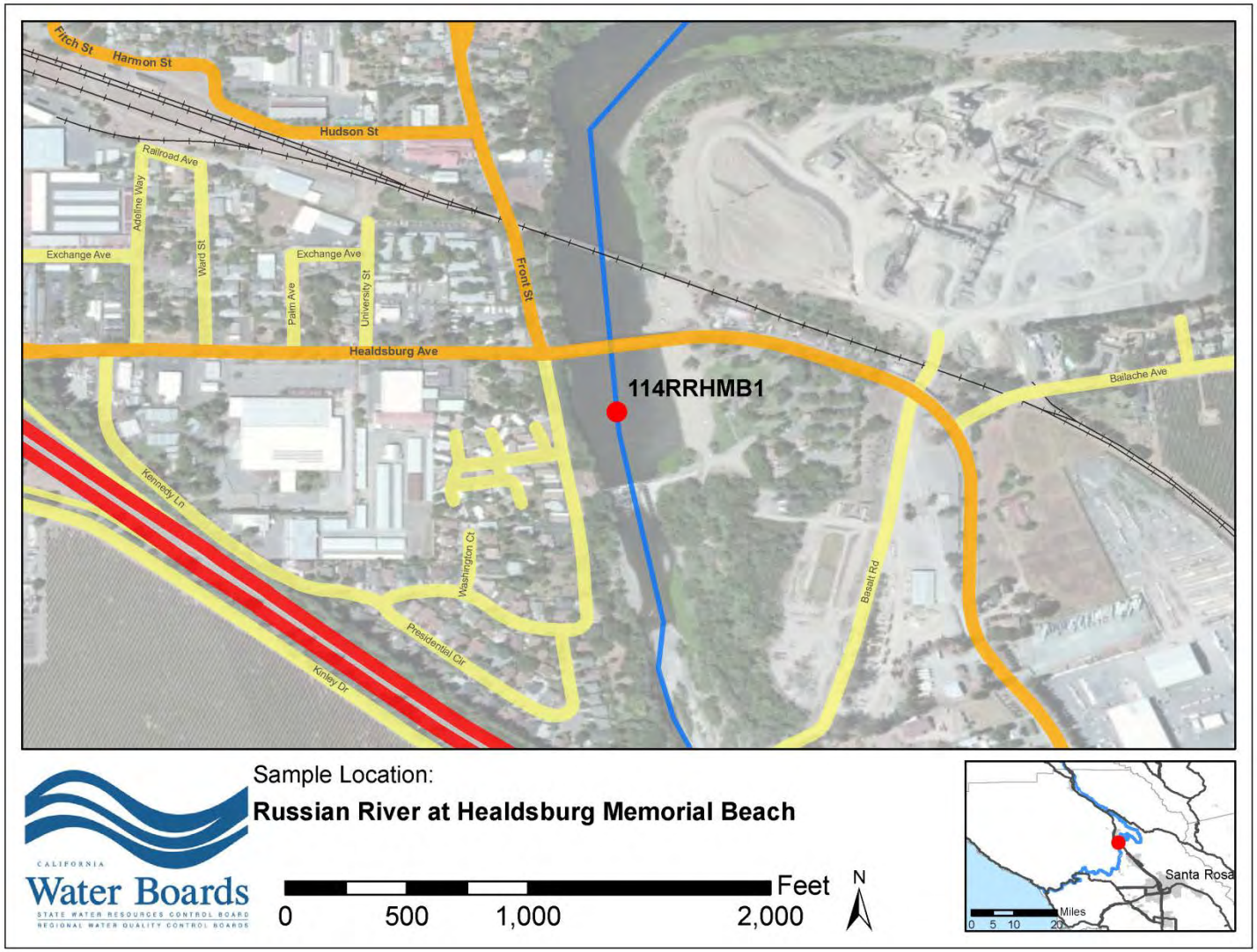


Figure 4: Russian River at Steelhead Beach.

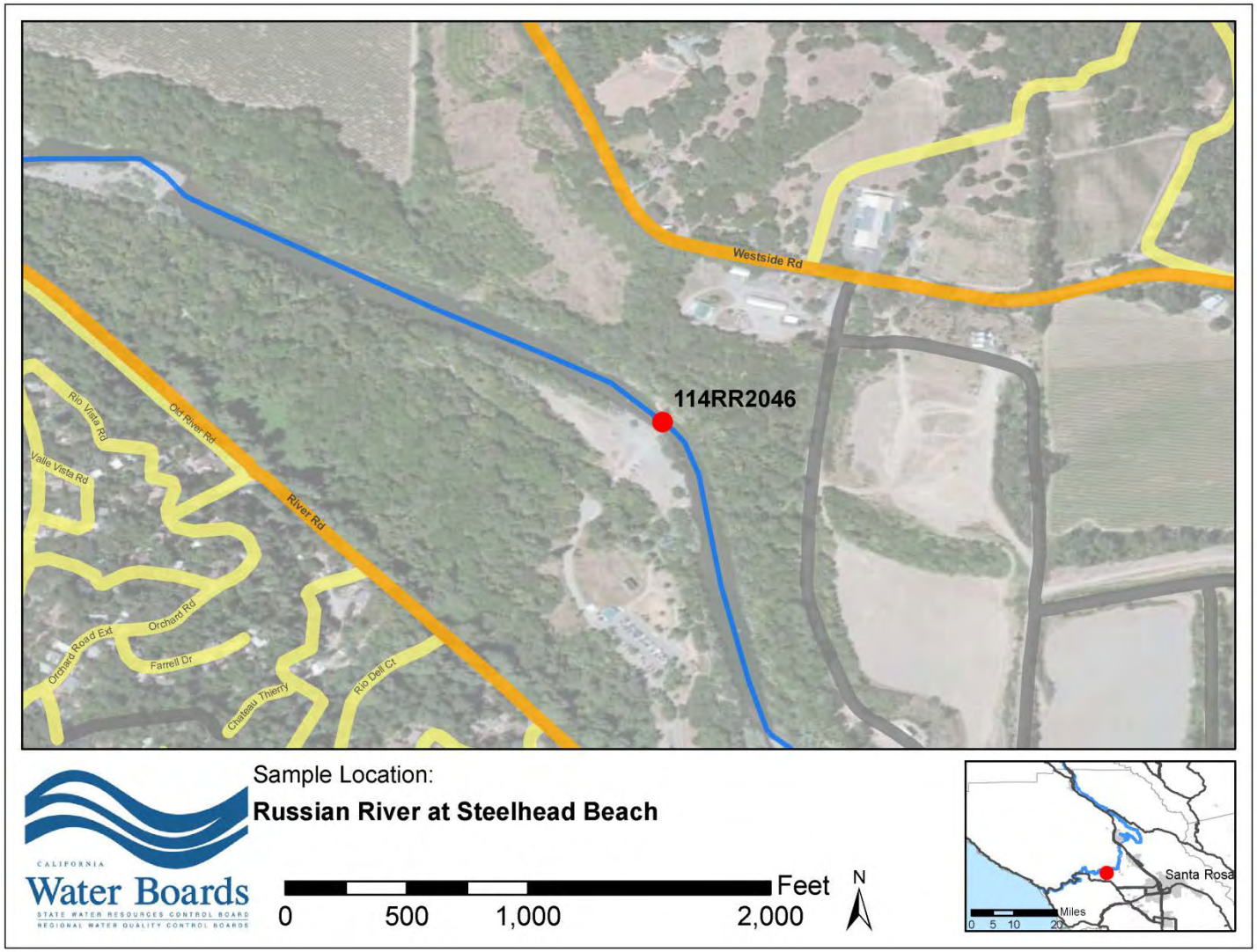


Figure 5: Russian River at Forestville River Access Beach.

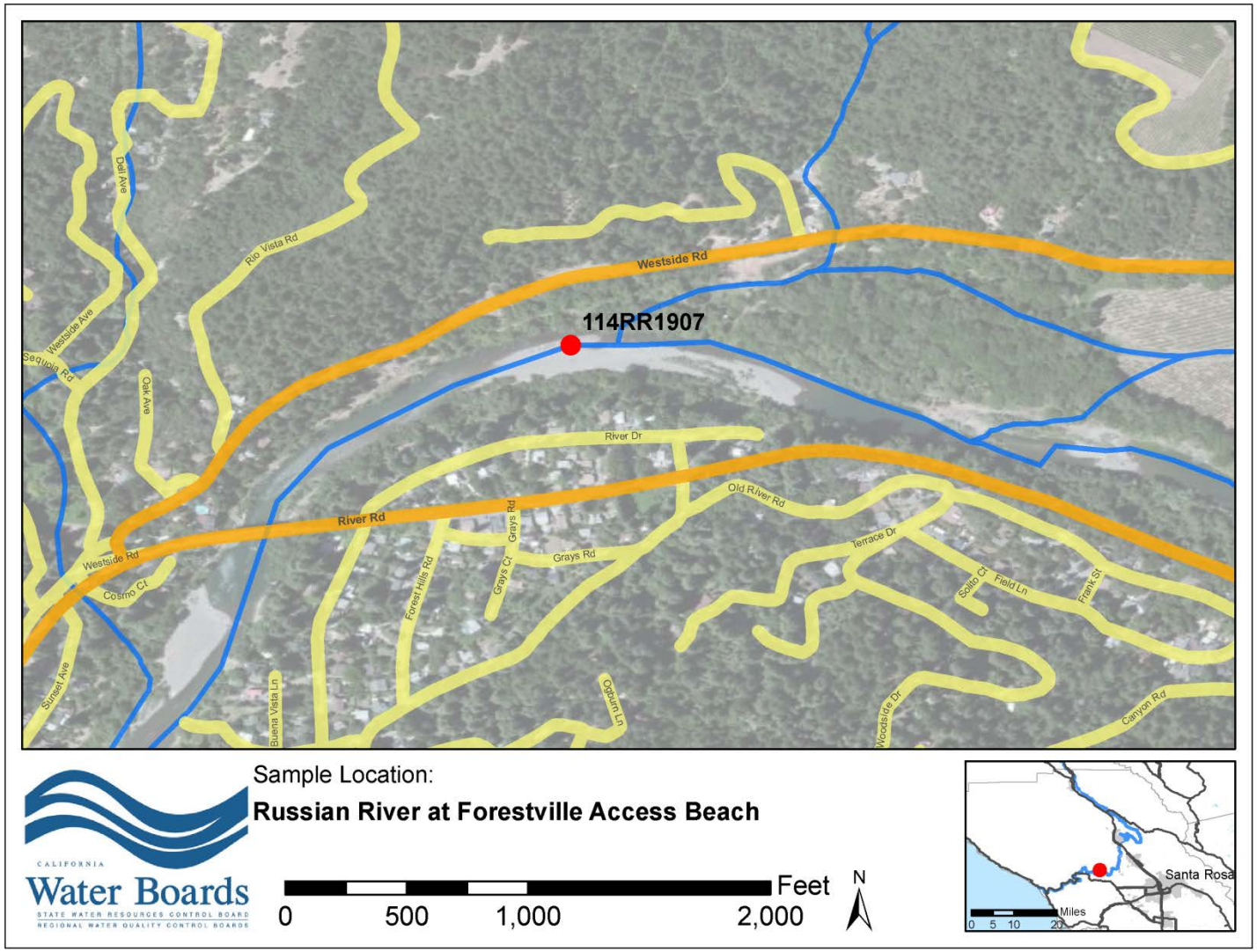


Figure 6: Russian River at Johnson's Beach.

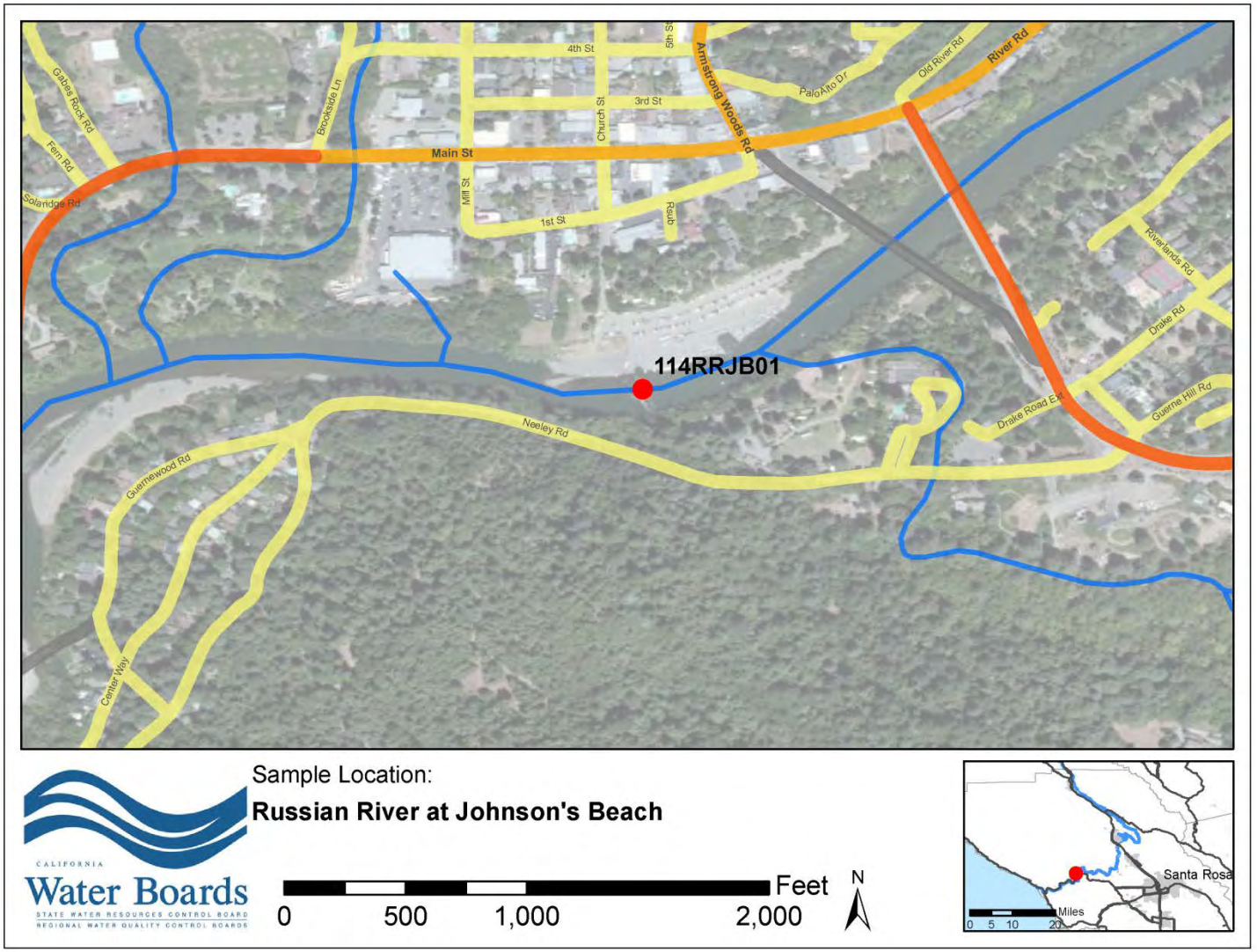


Figure 7: Russian River at Monte Rio Beach.

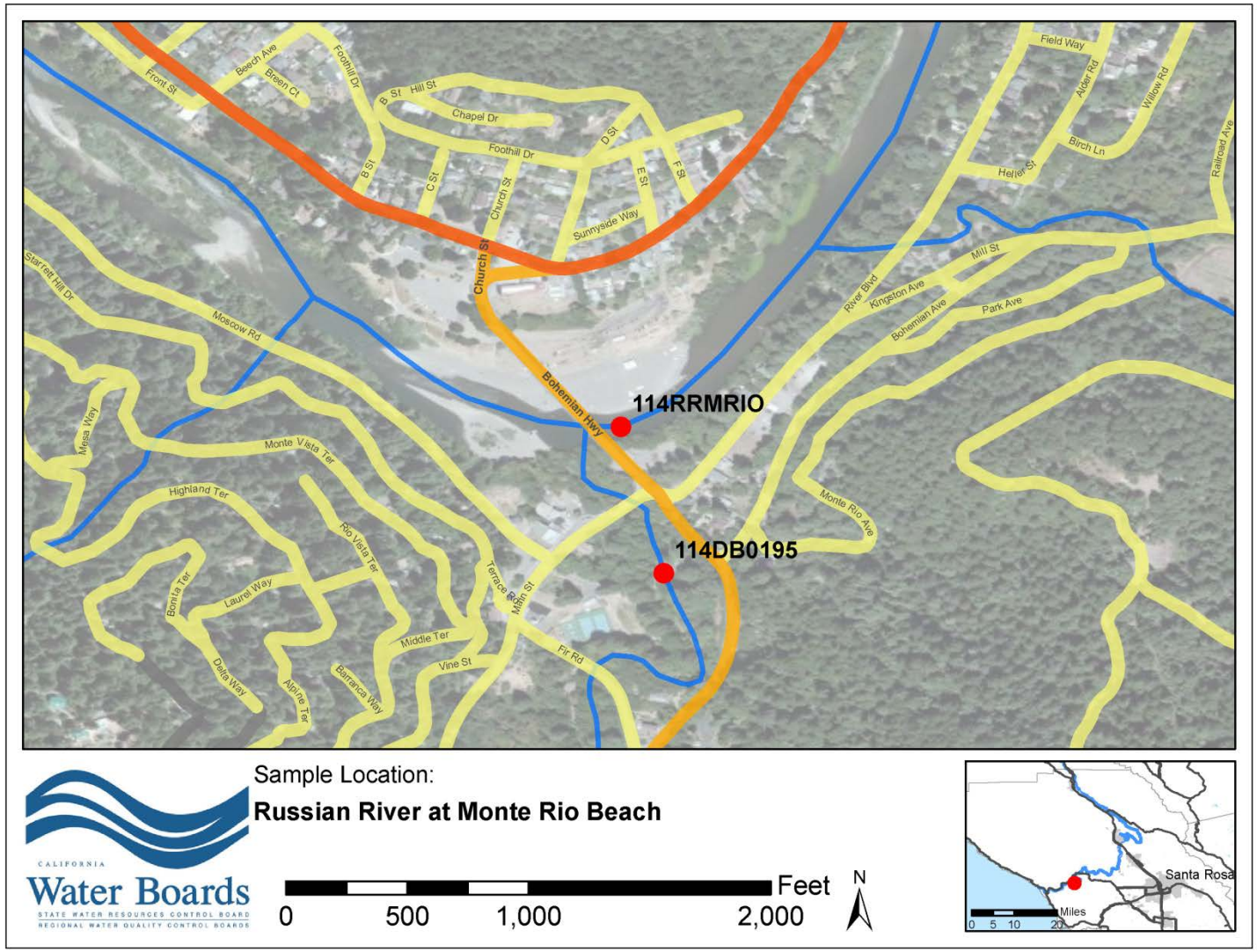


Figure 8: Russian River at Commisky Station Road.

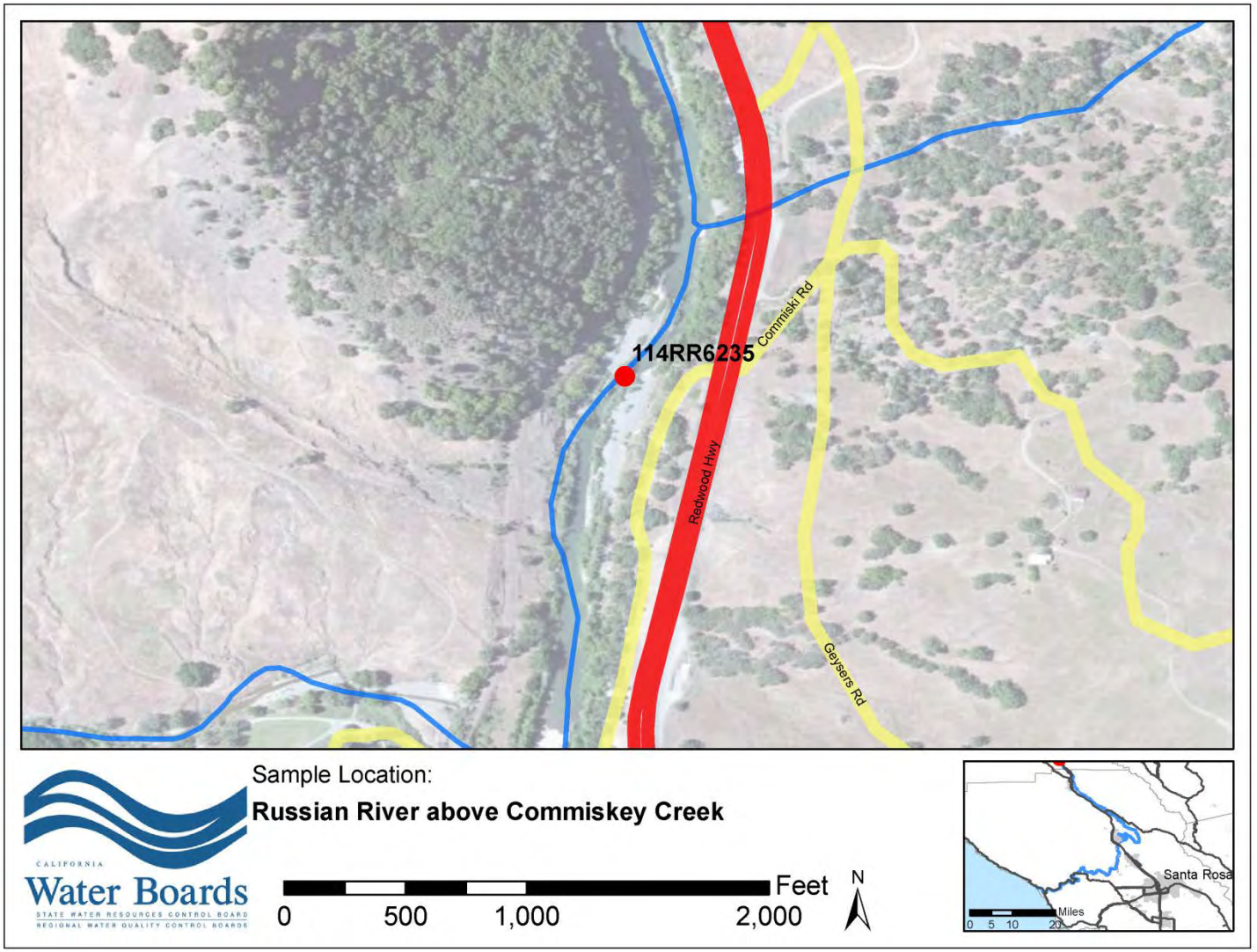


Figure 9: Russian River at Cloverdale River Park.

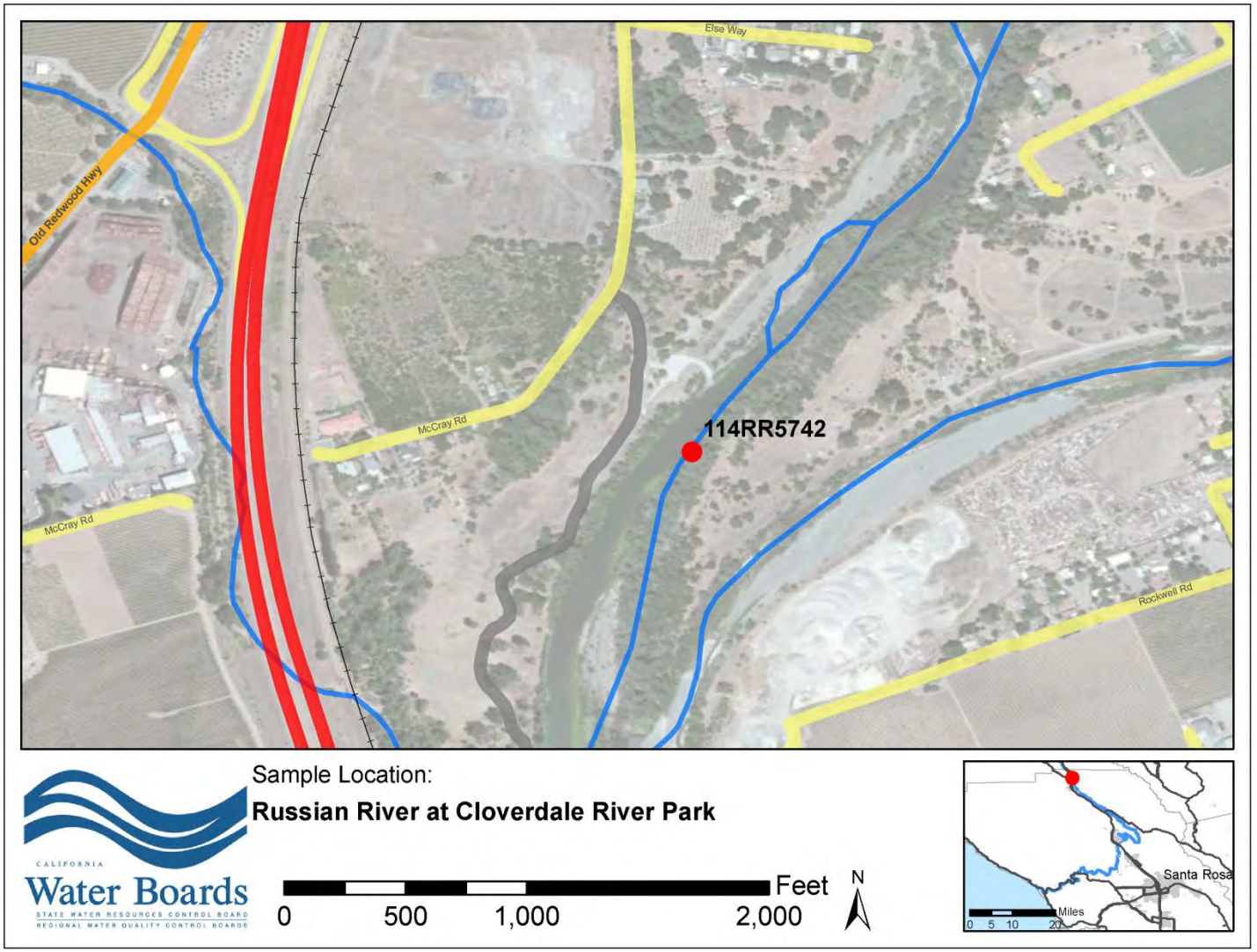


Figure 10: Russian River at Highway 128.

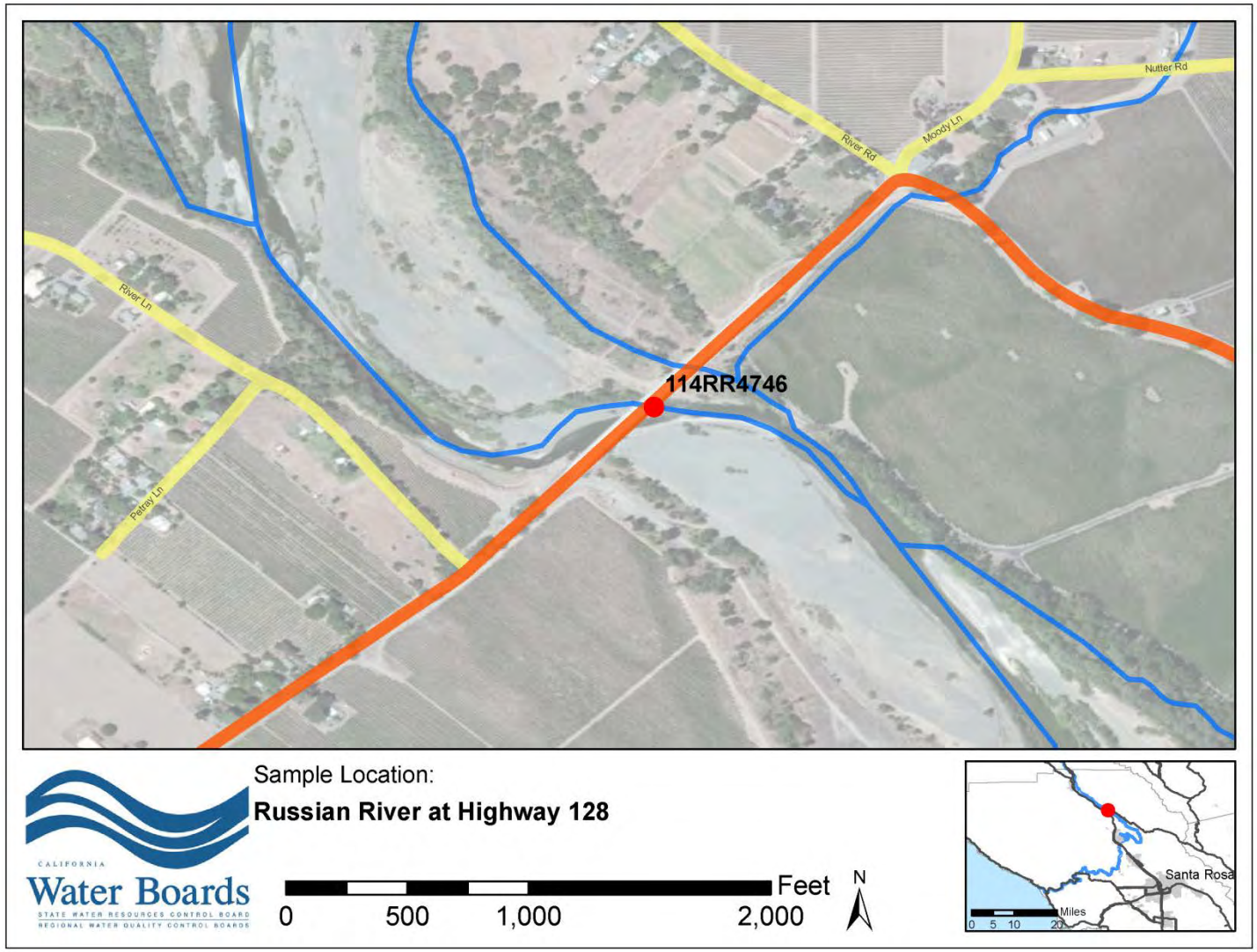


Figure 11: Dutch Bill Creek at Fir Road.

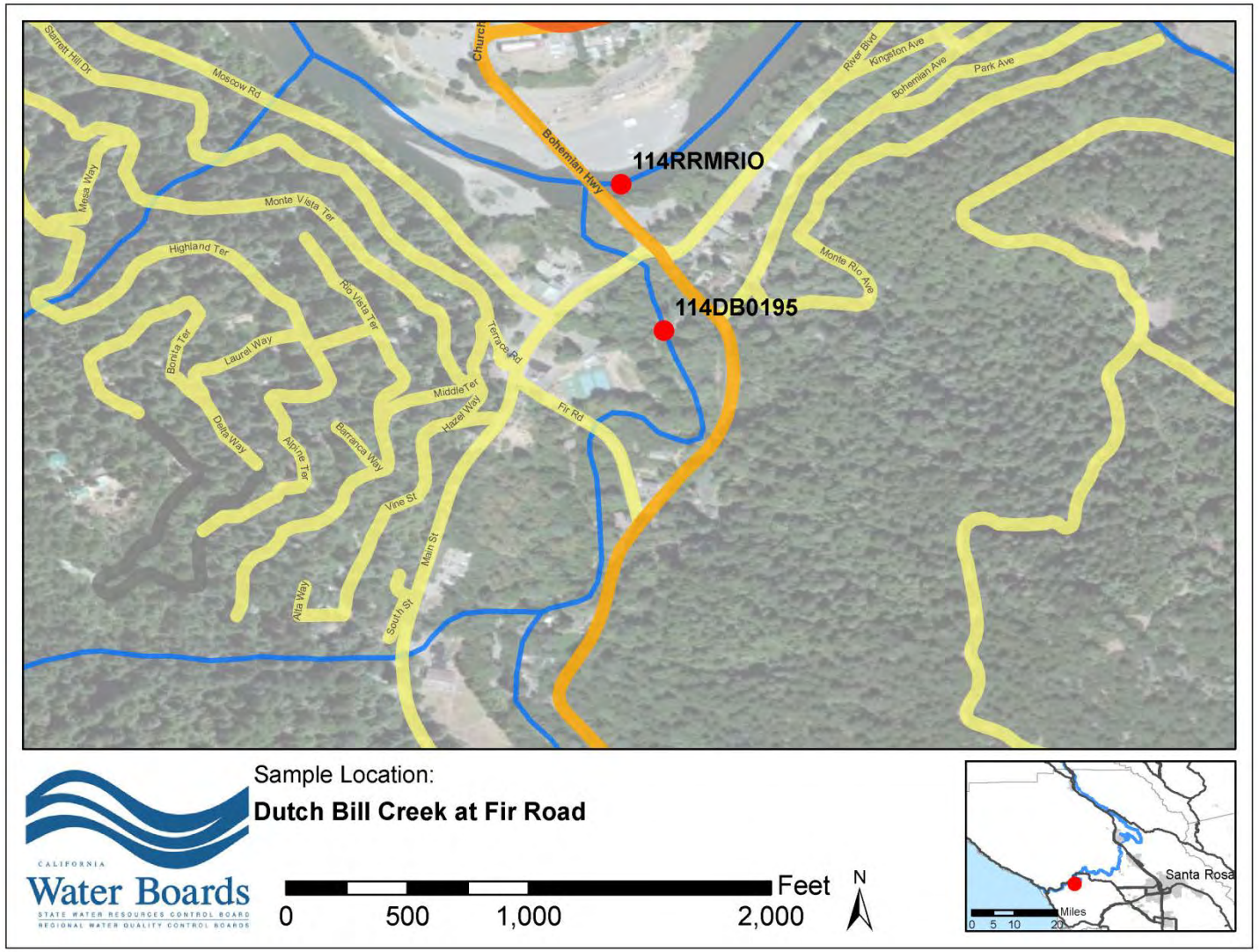


Figure 12: Russian River at Jenner Boat Ramp.

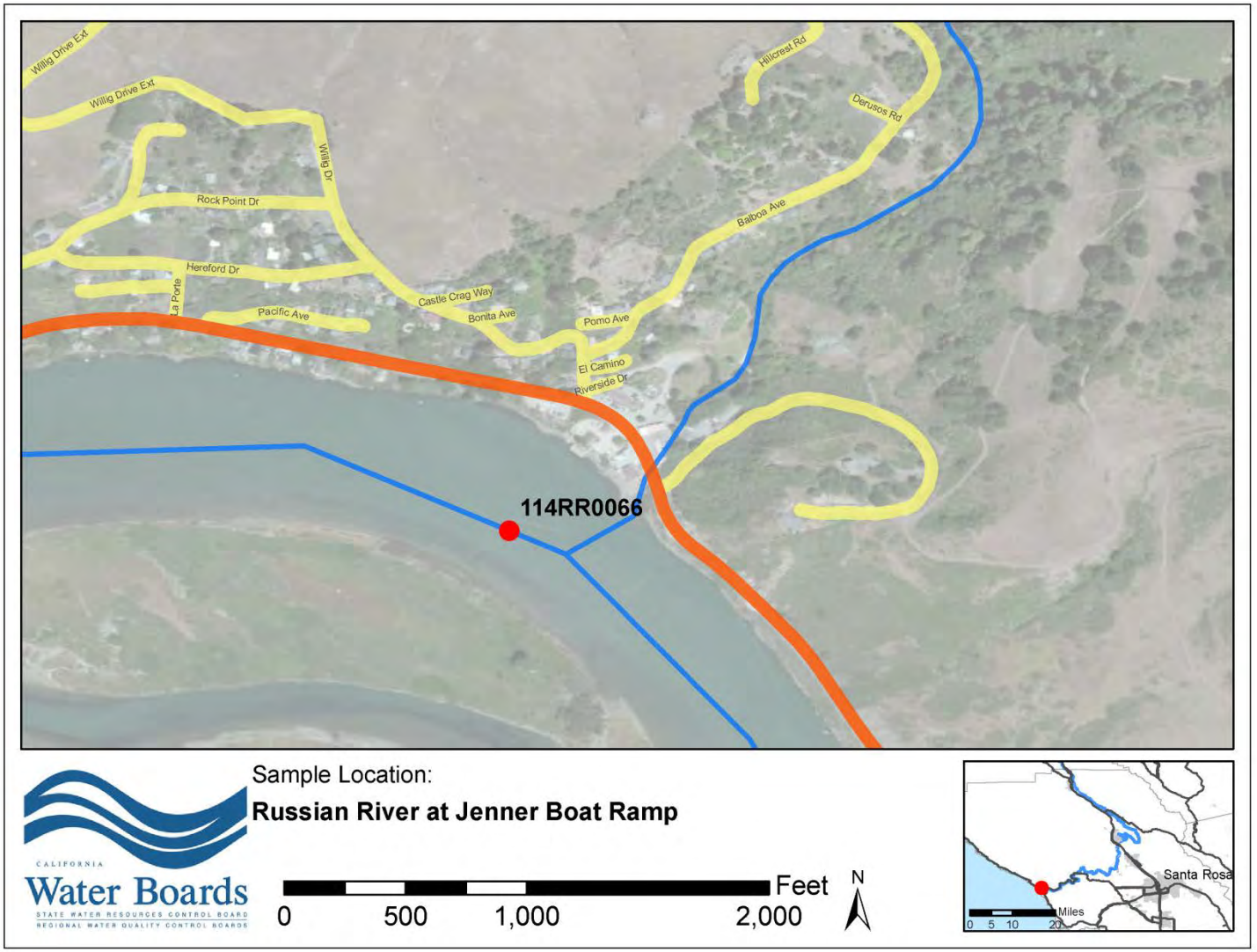


Figure 13: Santa Rosa Creek at Los Alamos Road.

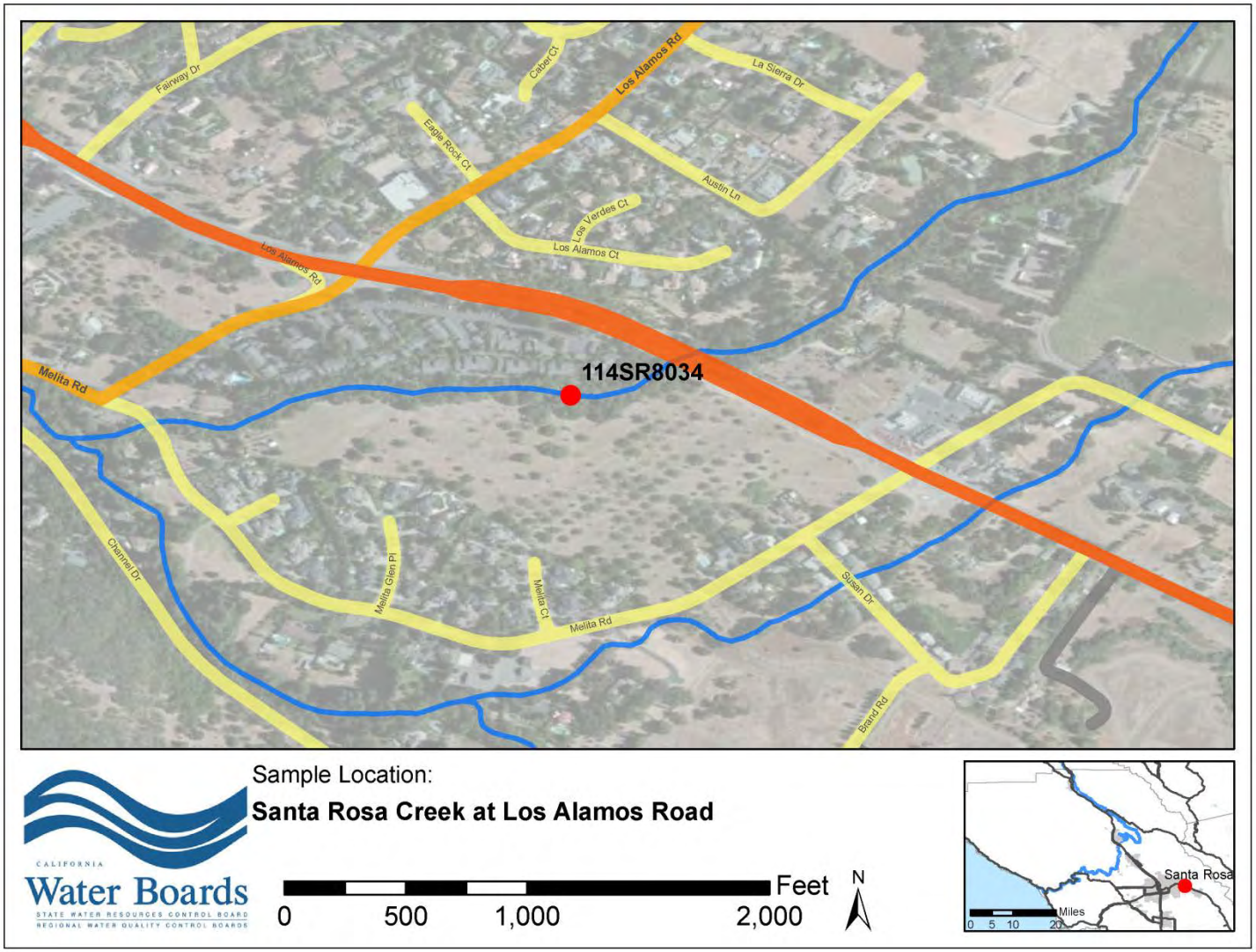


Figure 14: Santa Rosa Creek at Prince Memorial Greenway.

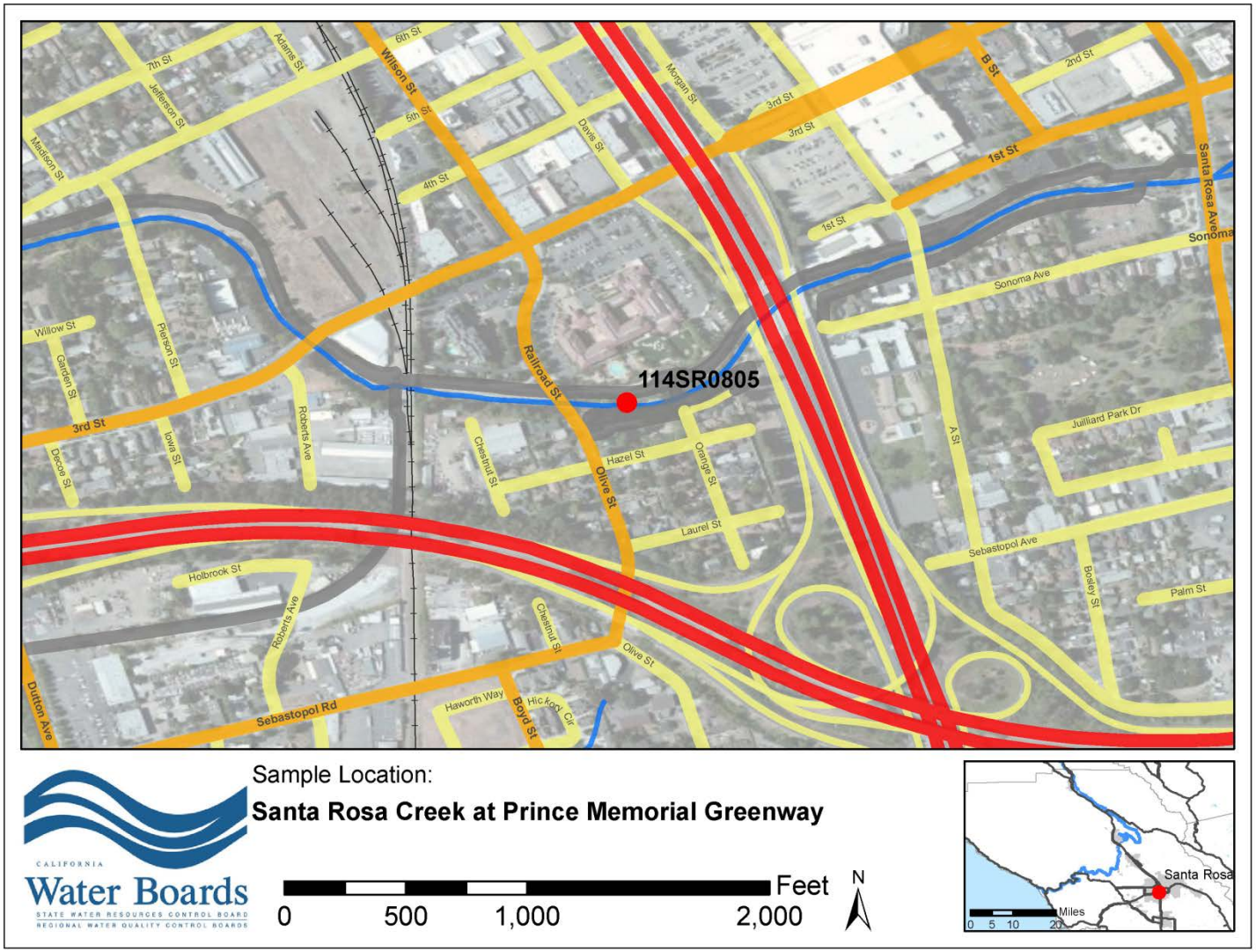


Figure 15: Laguna de Santa Rosa at Sebastopol Community Center.

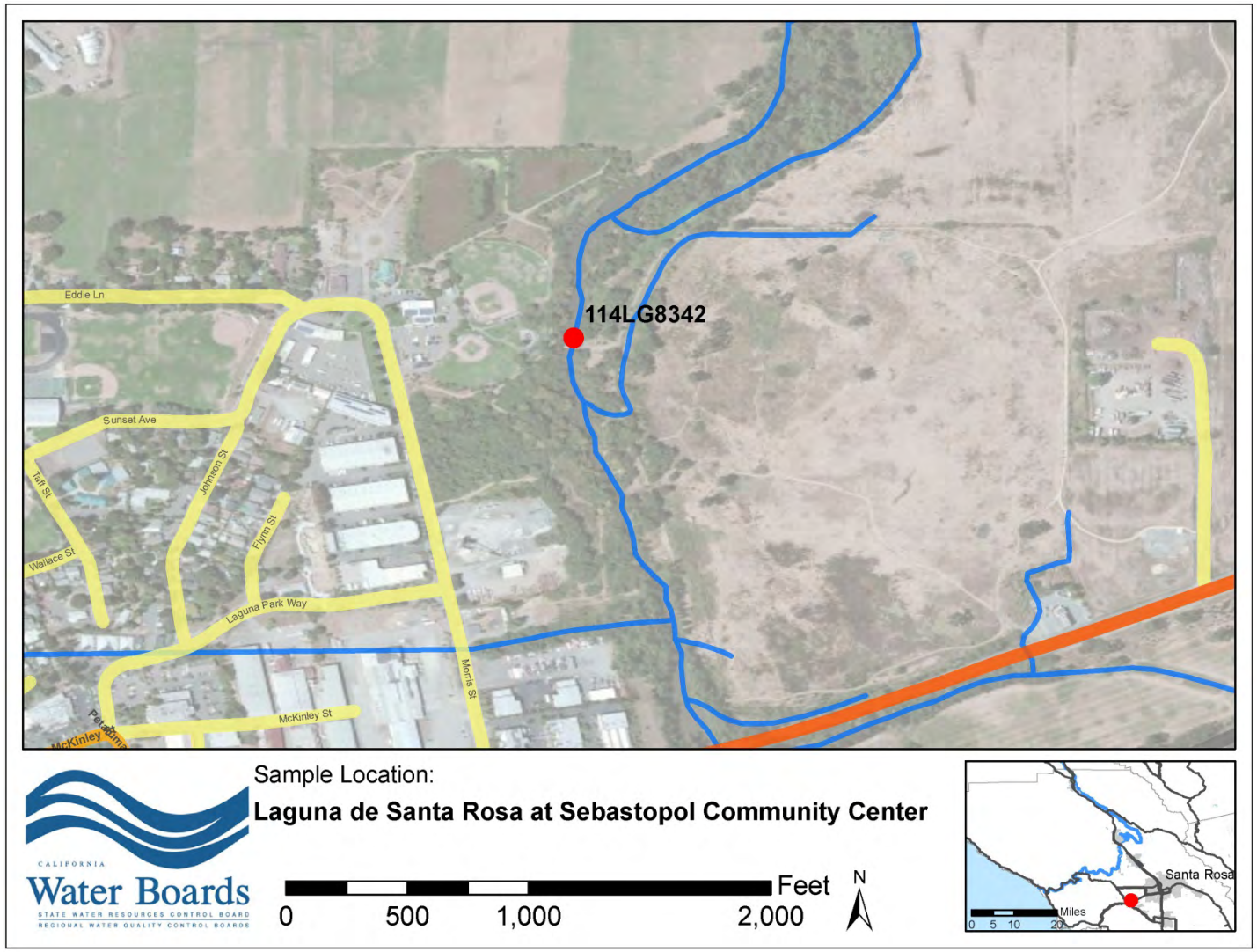


Figure 16: Green Valley Creek at Martinelli Road.

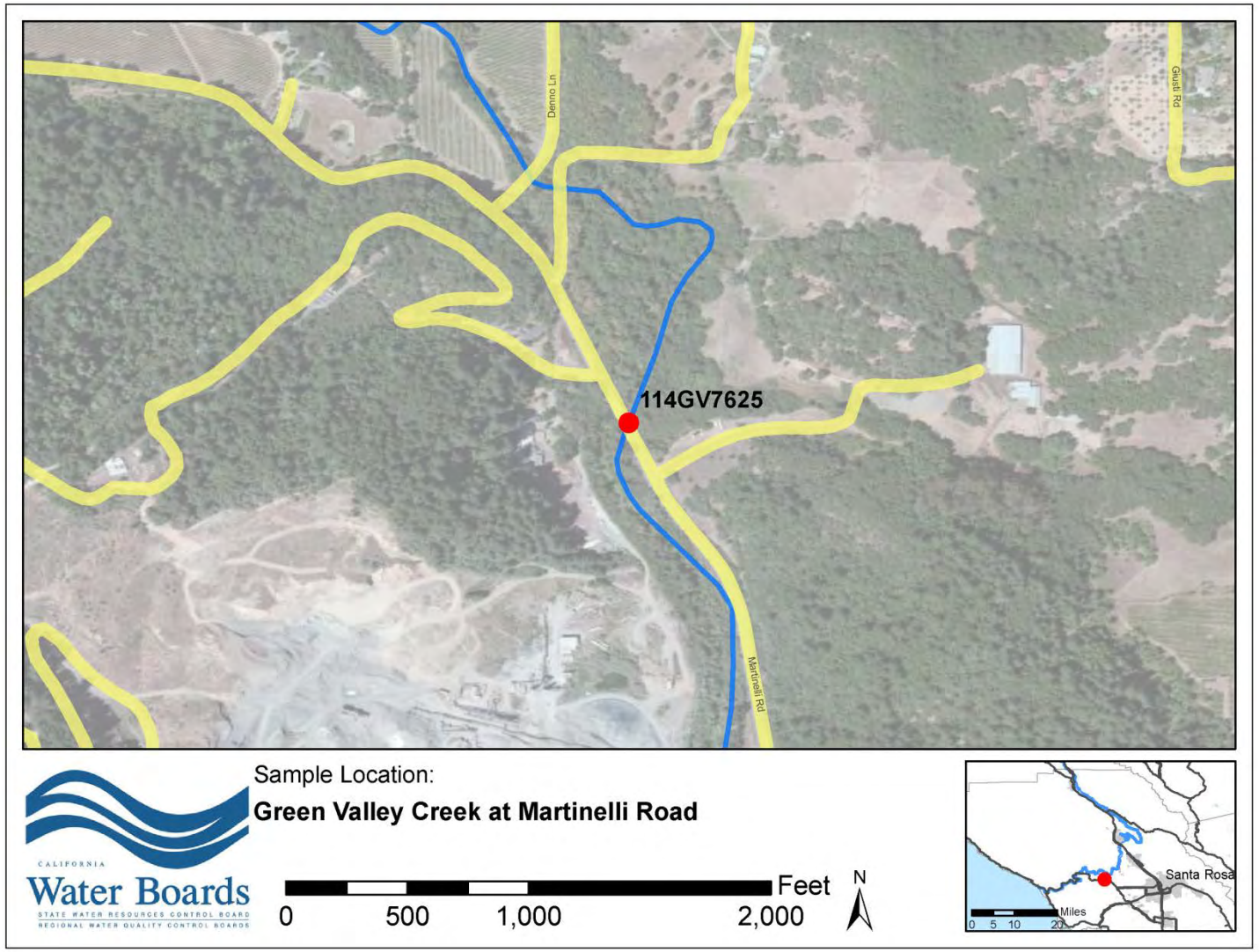


Figure 17: Piner Creek at Fulton Road.

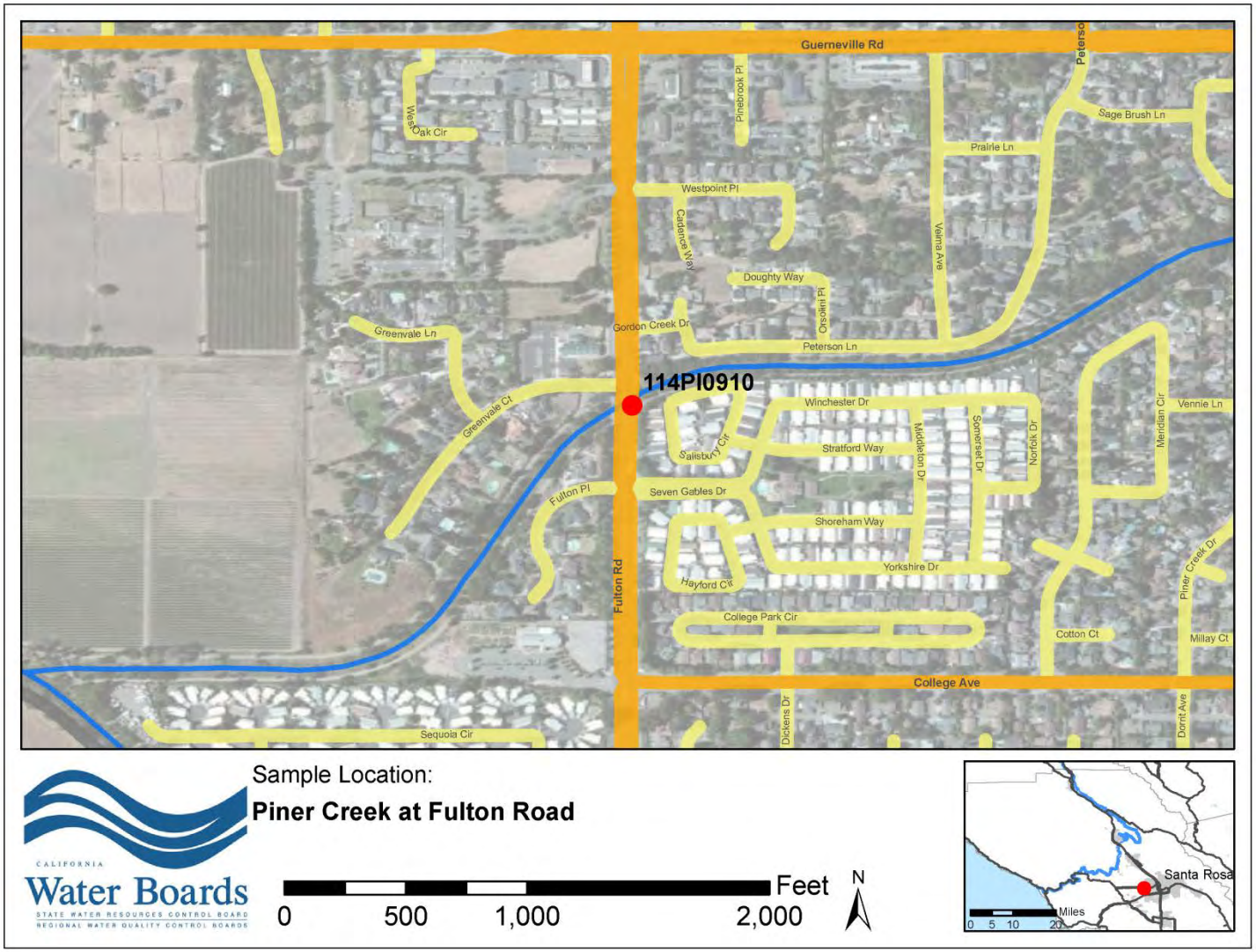


Figure 18: Copeland Creek at Commerce Drive.

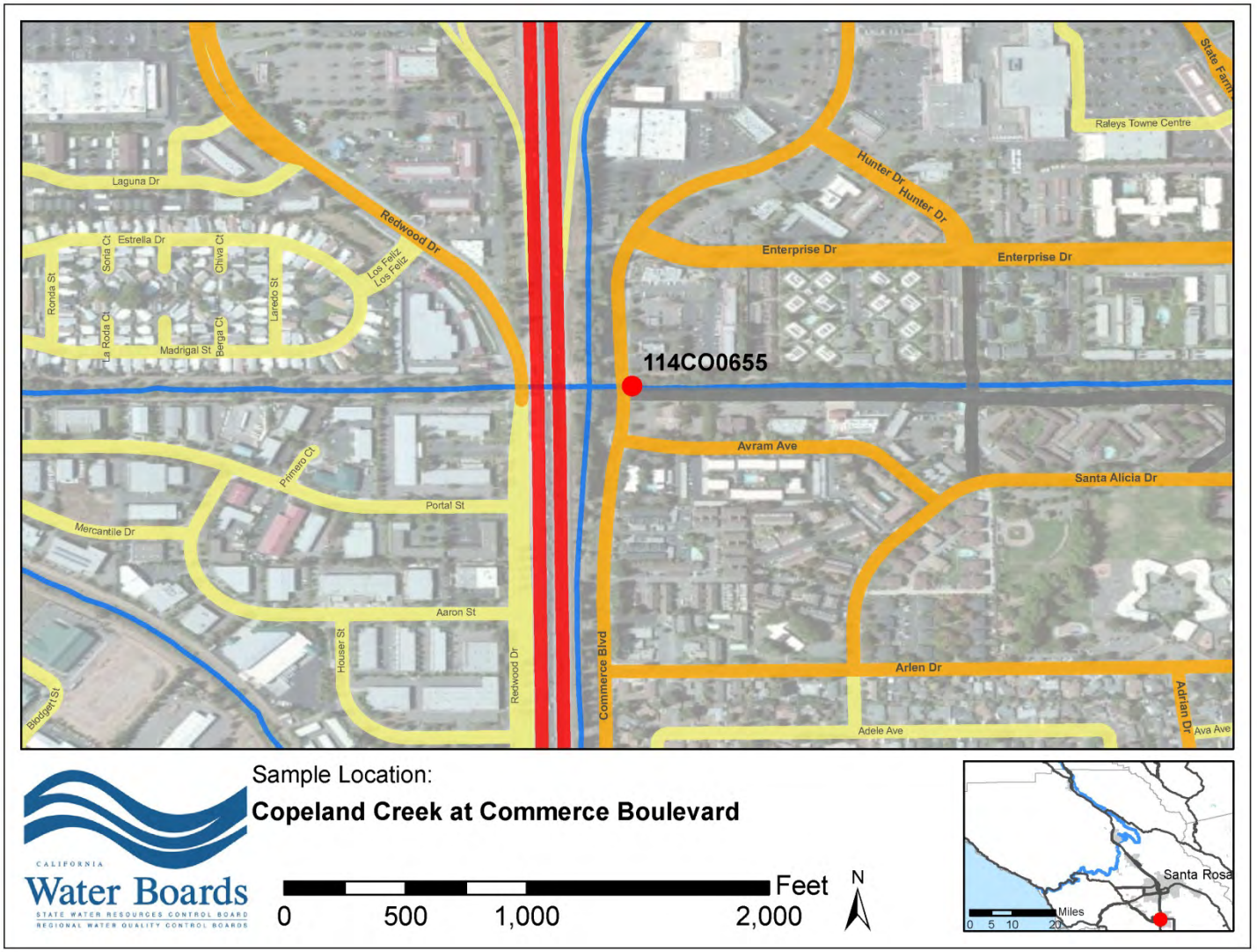


Figure 19: Foss Creek at Vine Street.

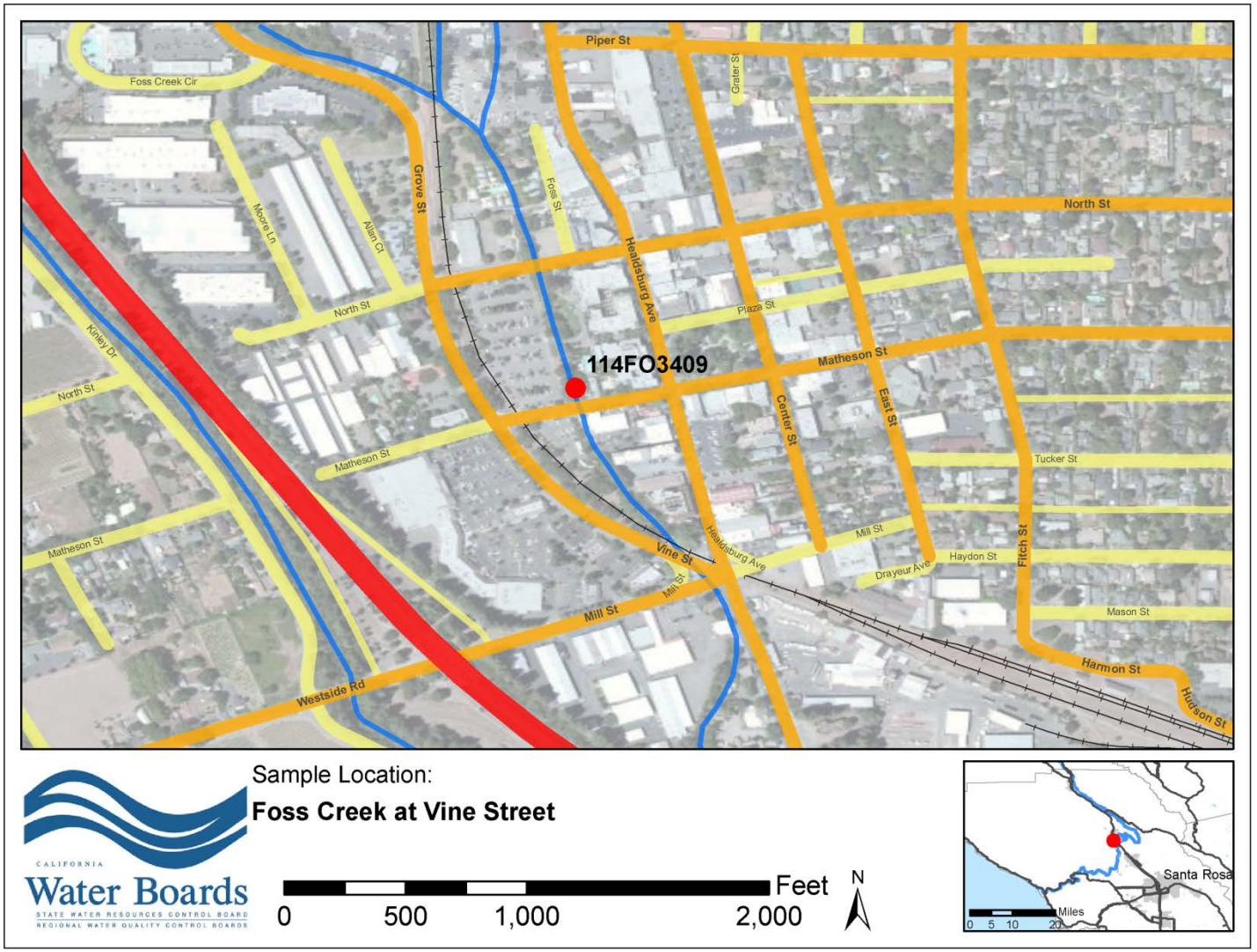


Figure 20: Unnamed Tributary at Sanford Road.

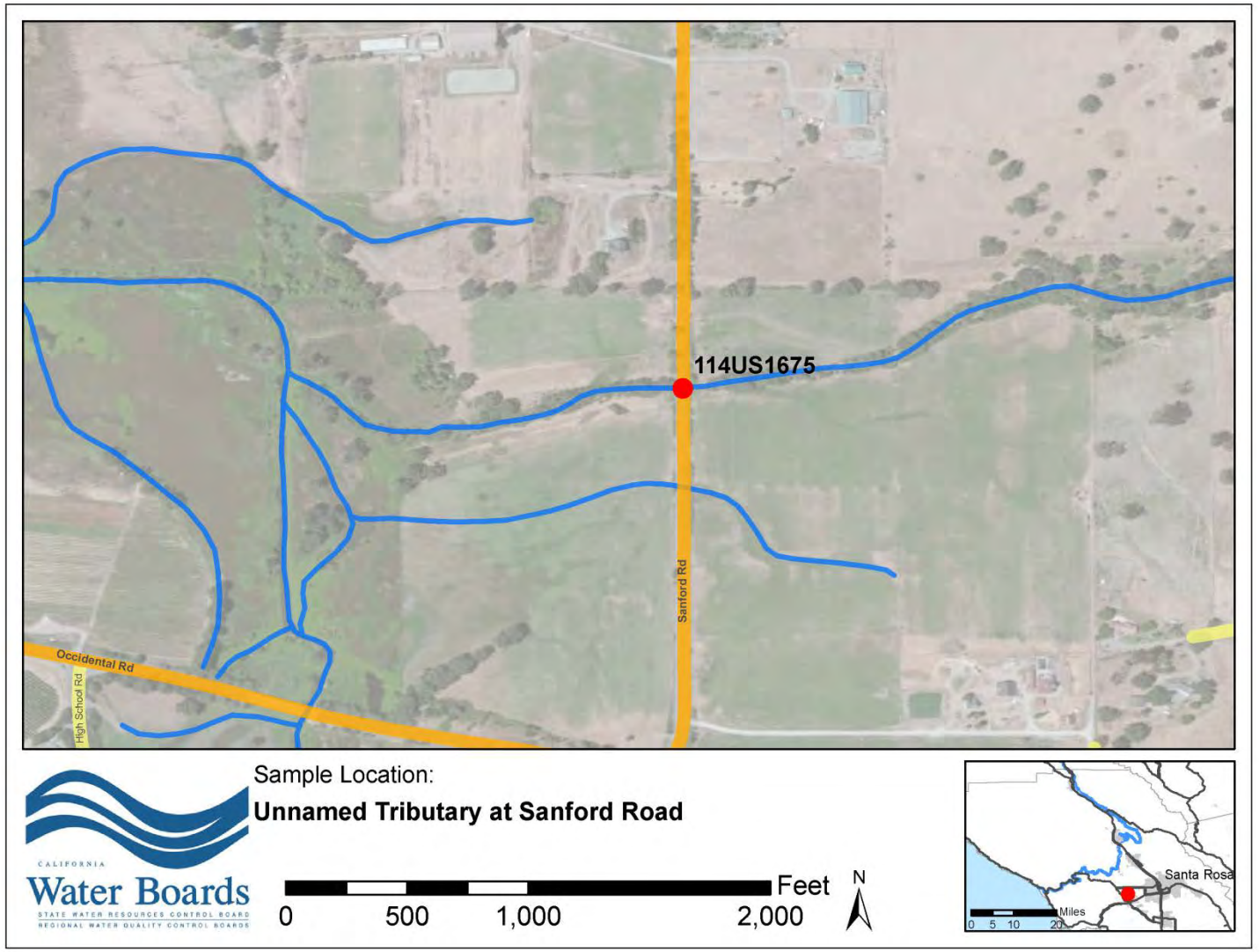


Figure 21: Unnamed Tributary at Old Redwood Highway.

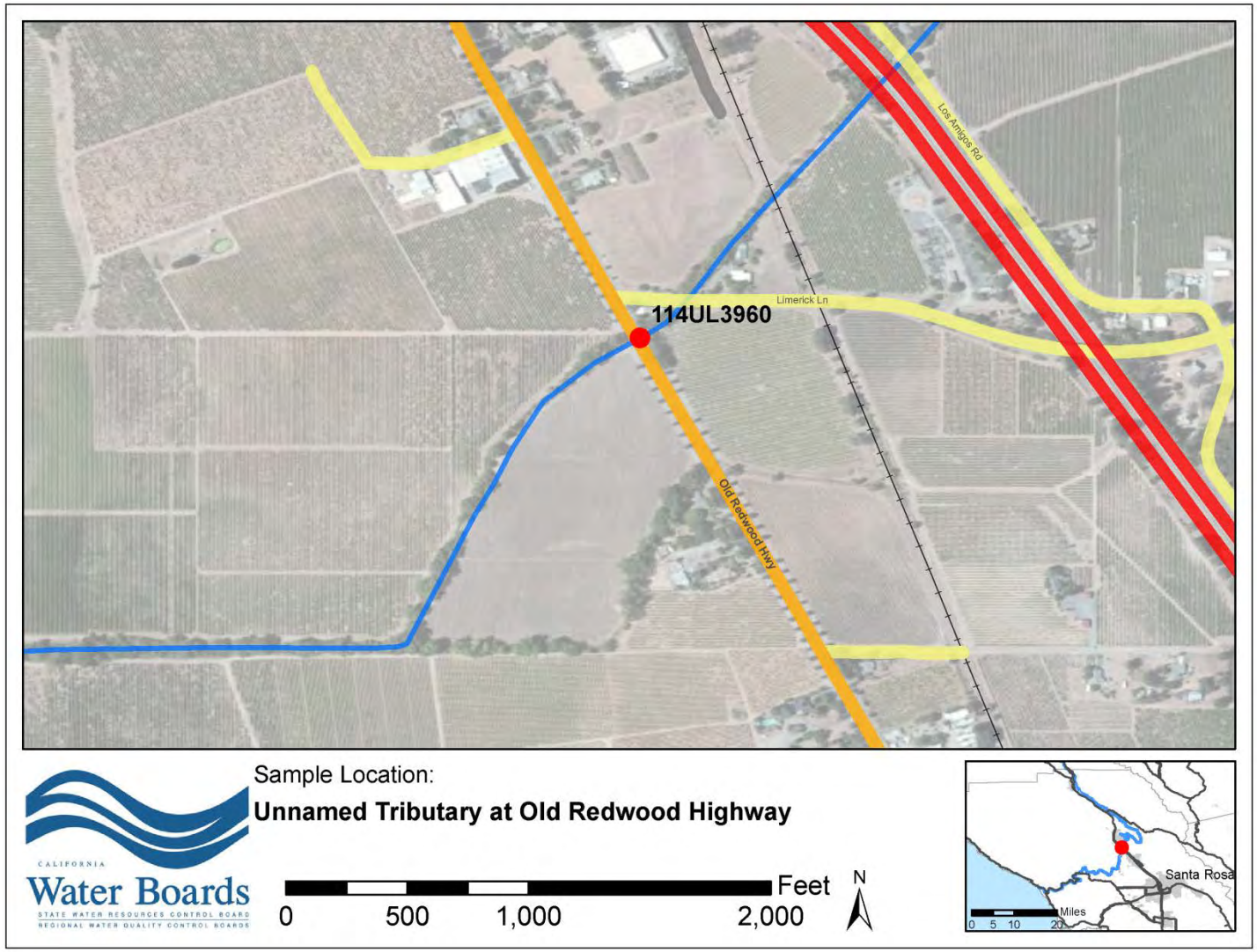


Figure 22: Unnamed Tributary at Daywalt Road.

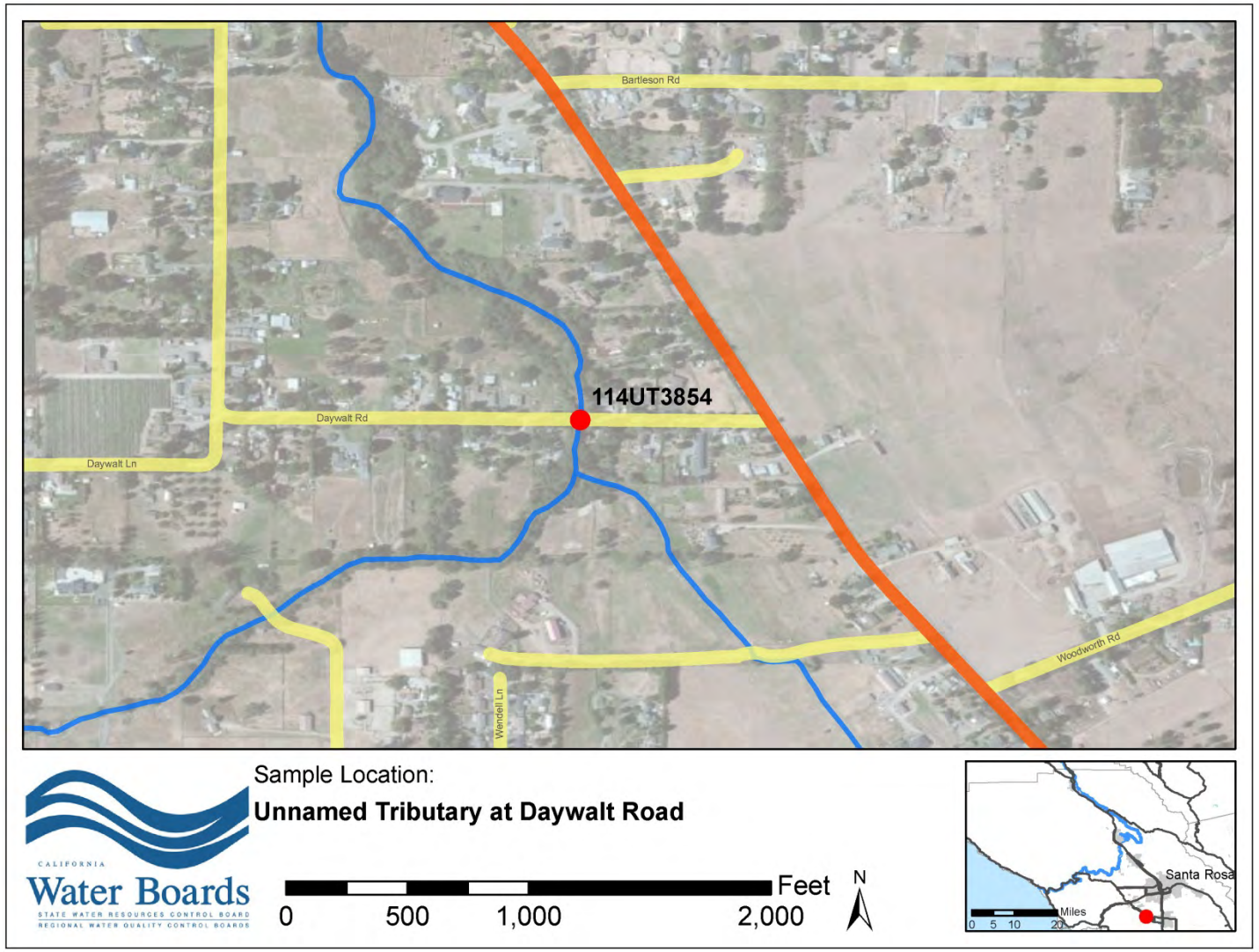


Figure 23: Unnamed Tributary at River Road.

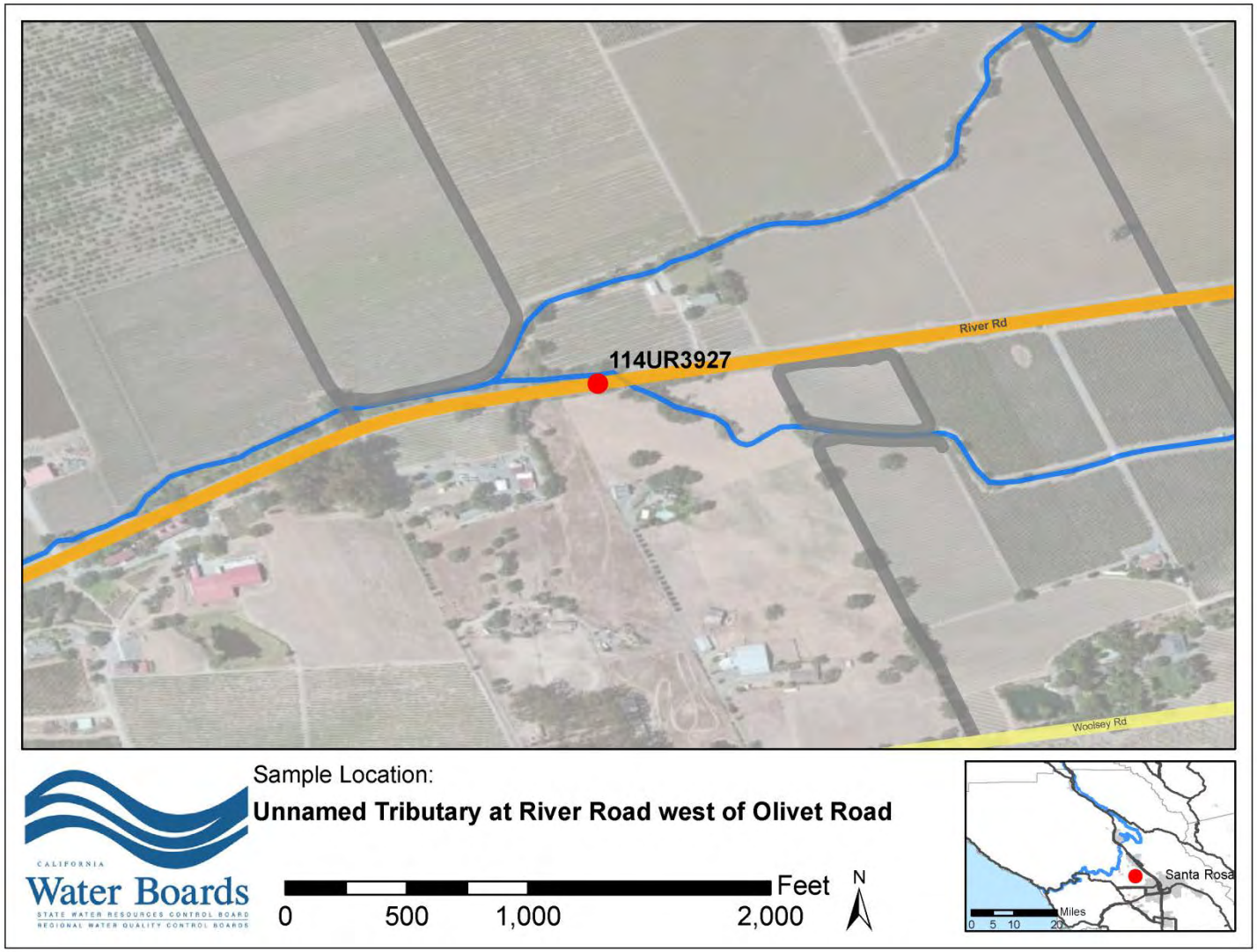


Figure 24: Abramson Creek at Willowside Road Levy.

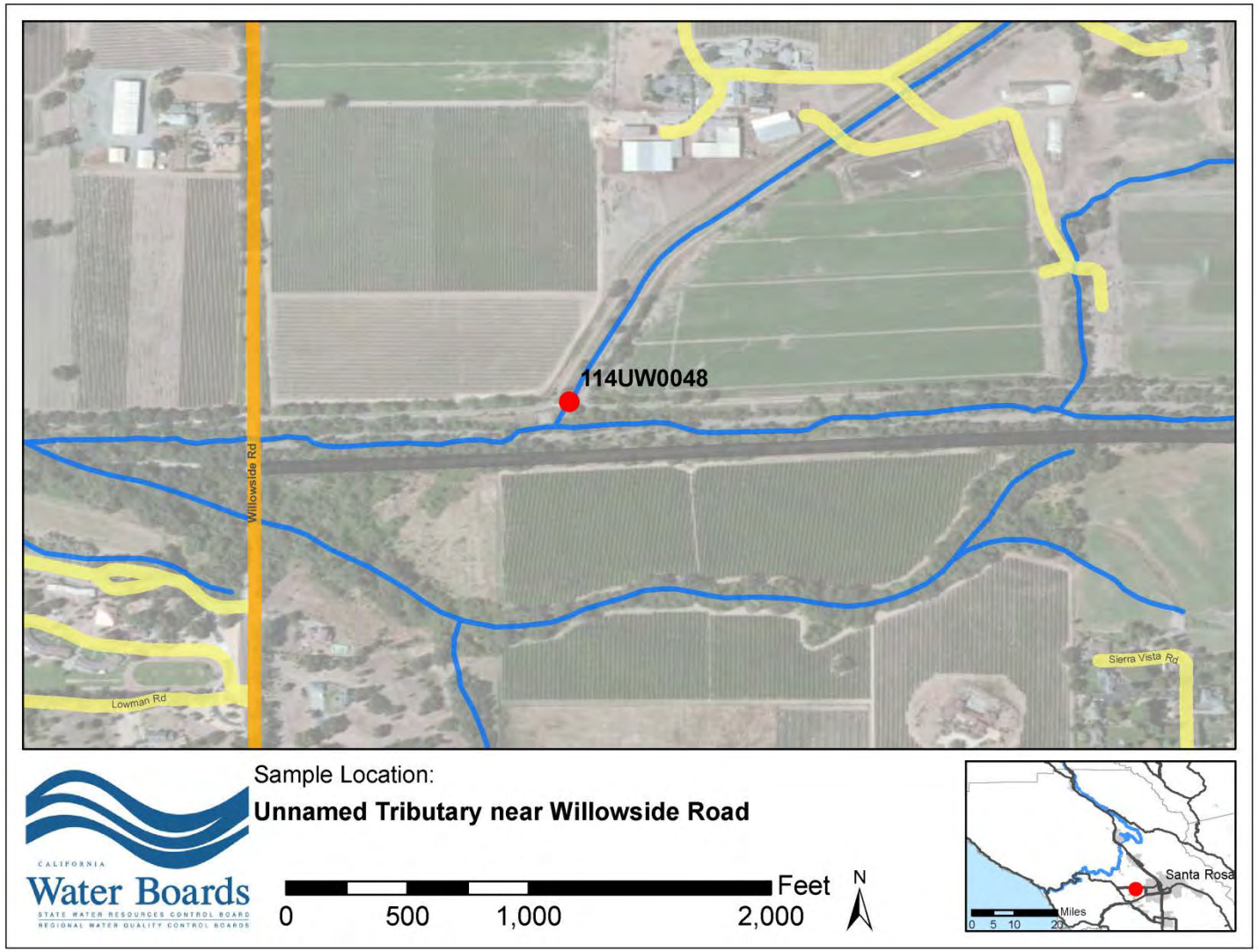


Figure 25: Unnamed Tributary at Lambert Bridge Road.

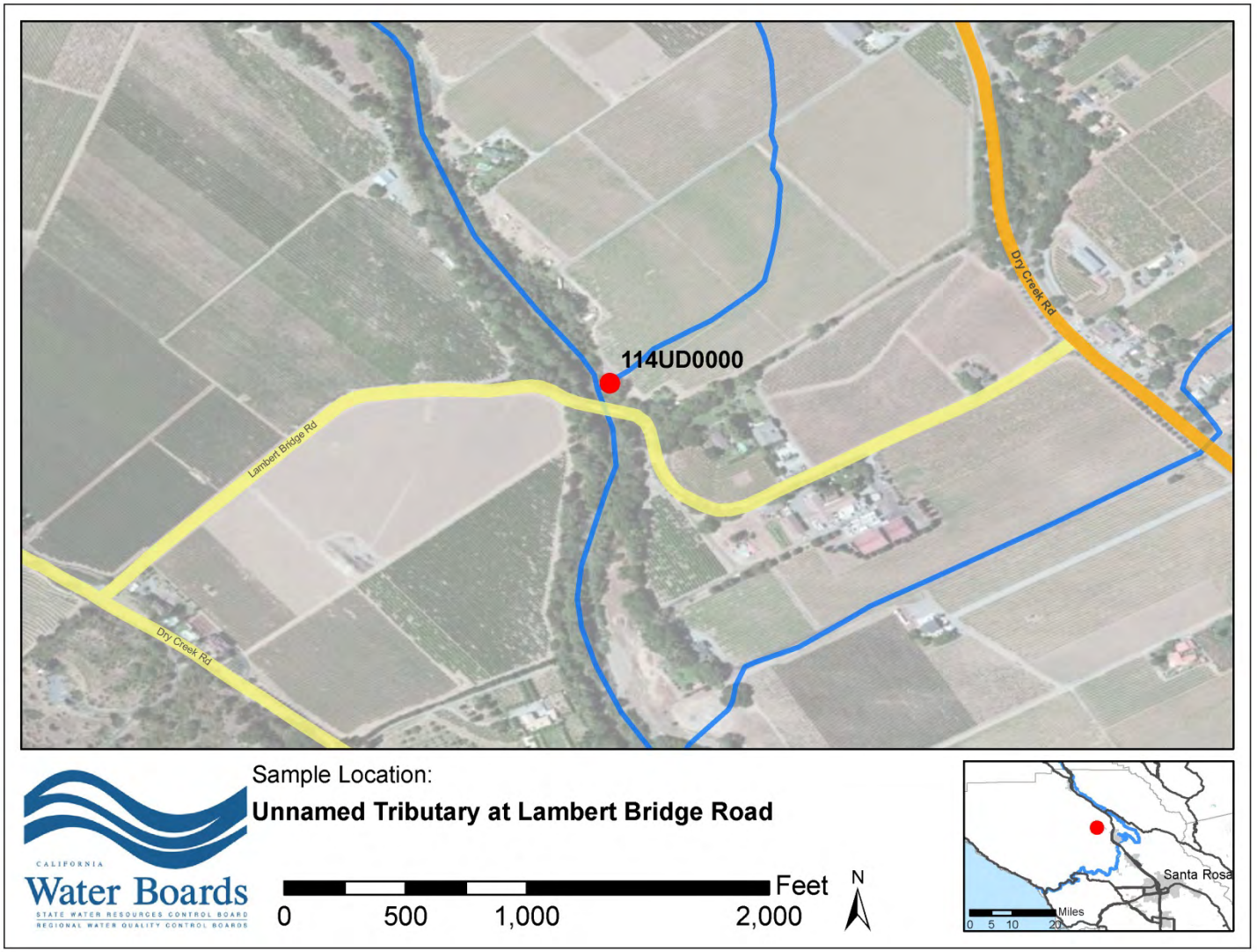


Figure 26: Crane Creek at Snyder Lane.

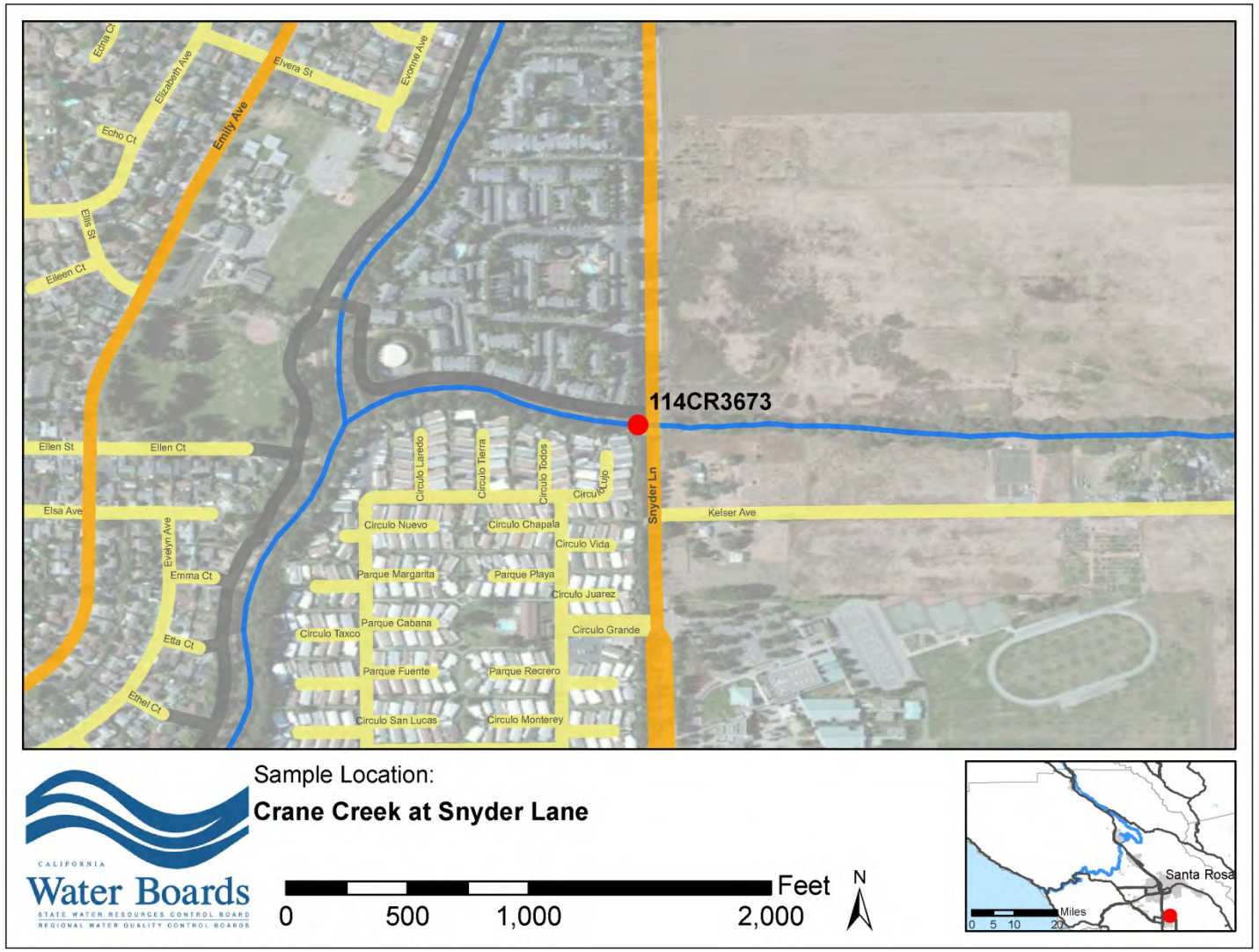


Figure 27: Gossage Creek at Highway 116.

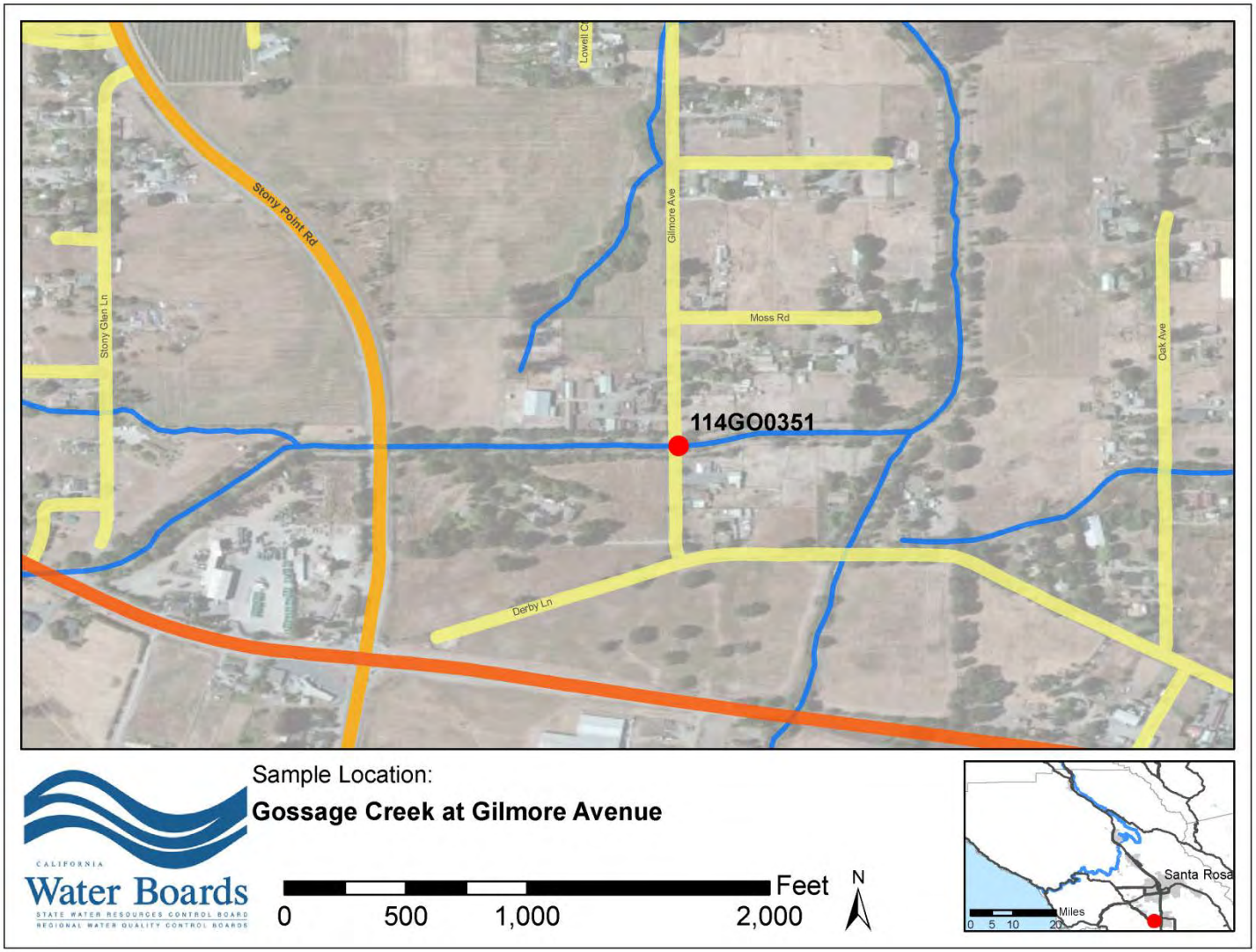


Figure 28: Blucher Creek at Lone Pine Road.

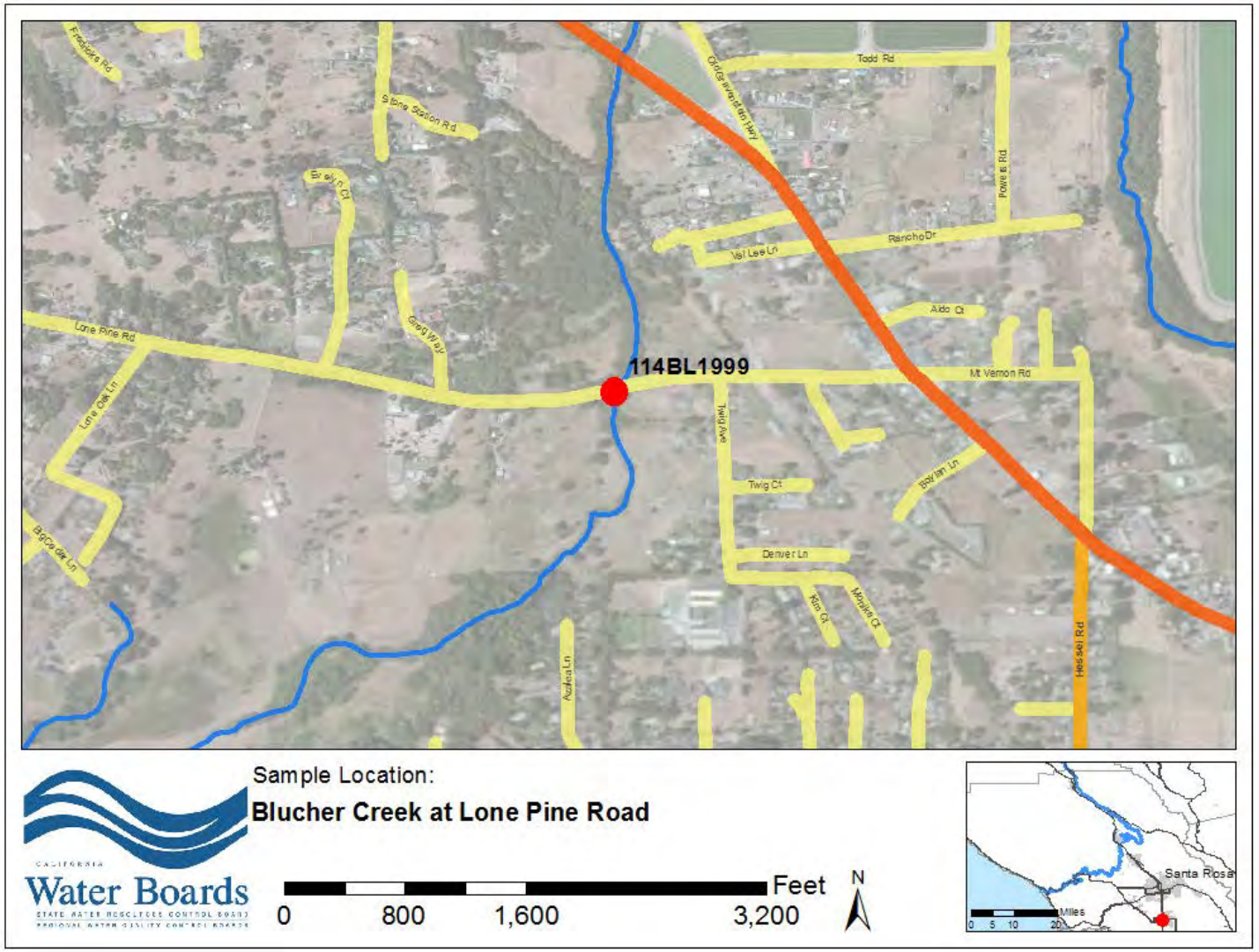


Figure 29: Palmer Creek at Palmer Creek Road.

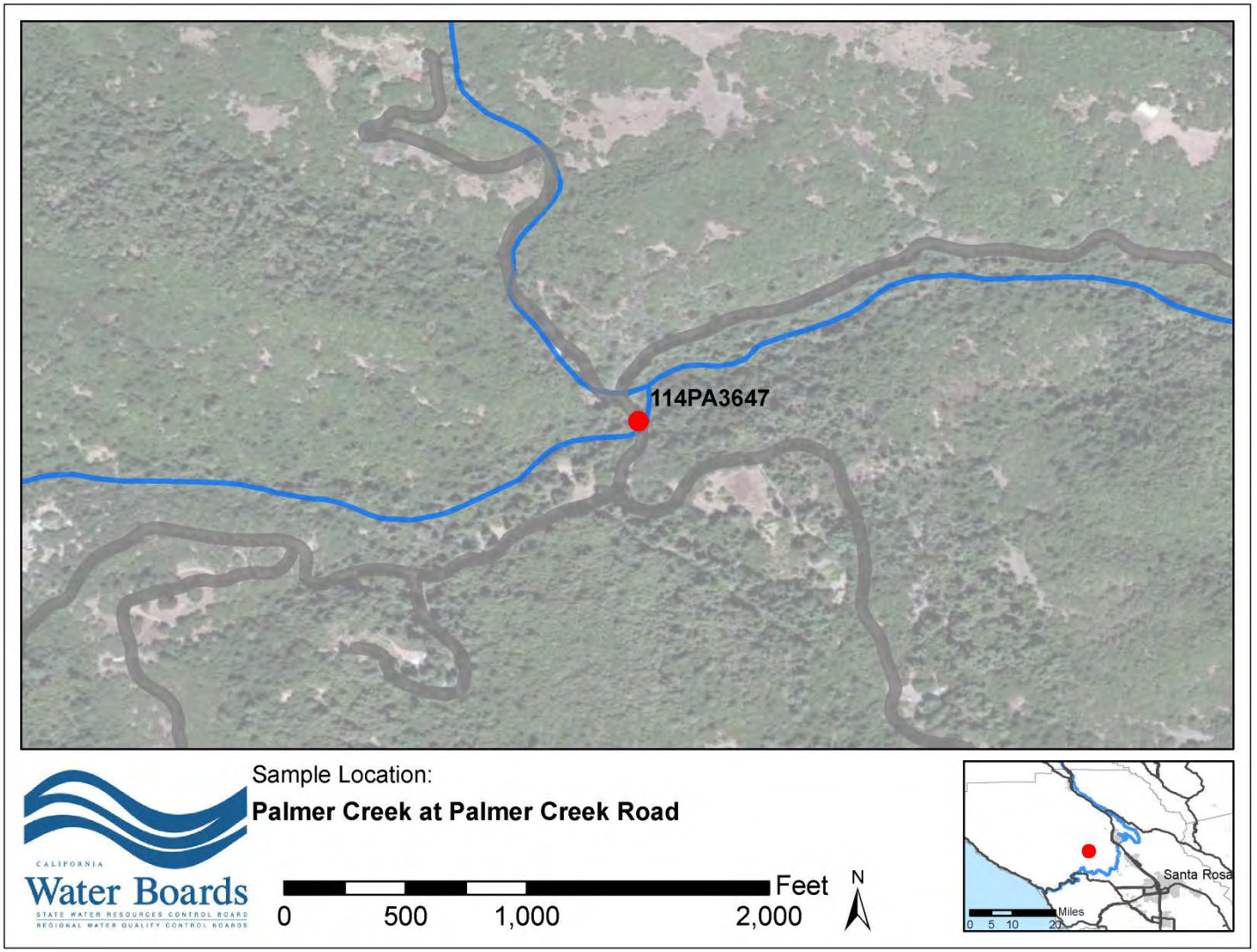


Figure 30: Unnamed Tributary to Russian River at Mays and Neely Roads.

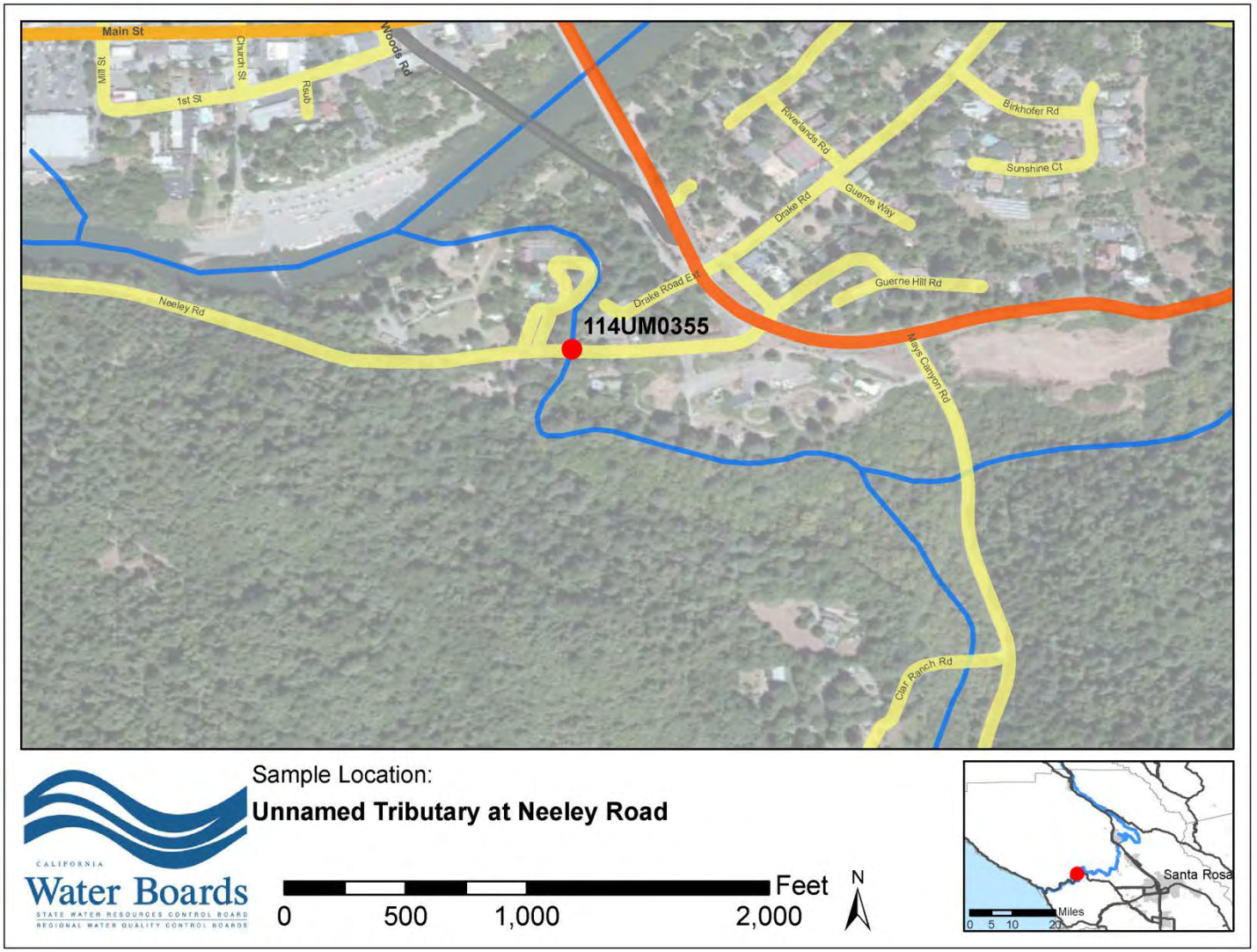
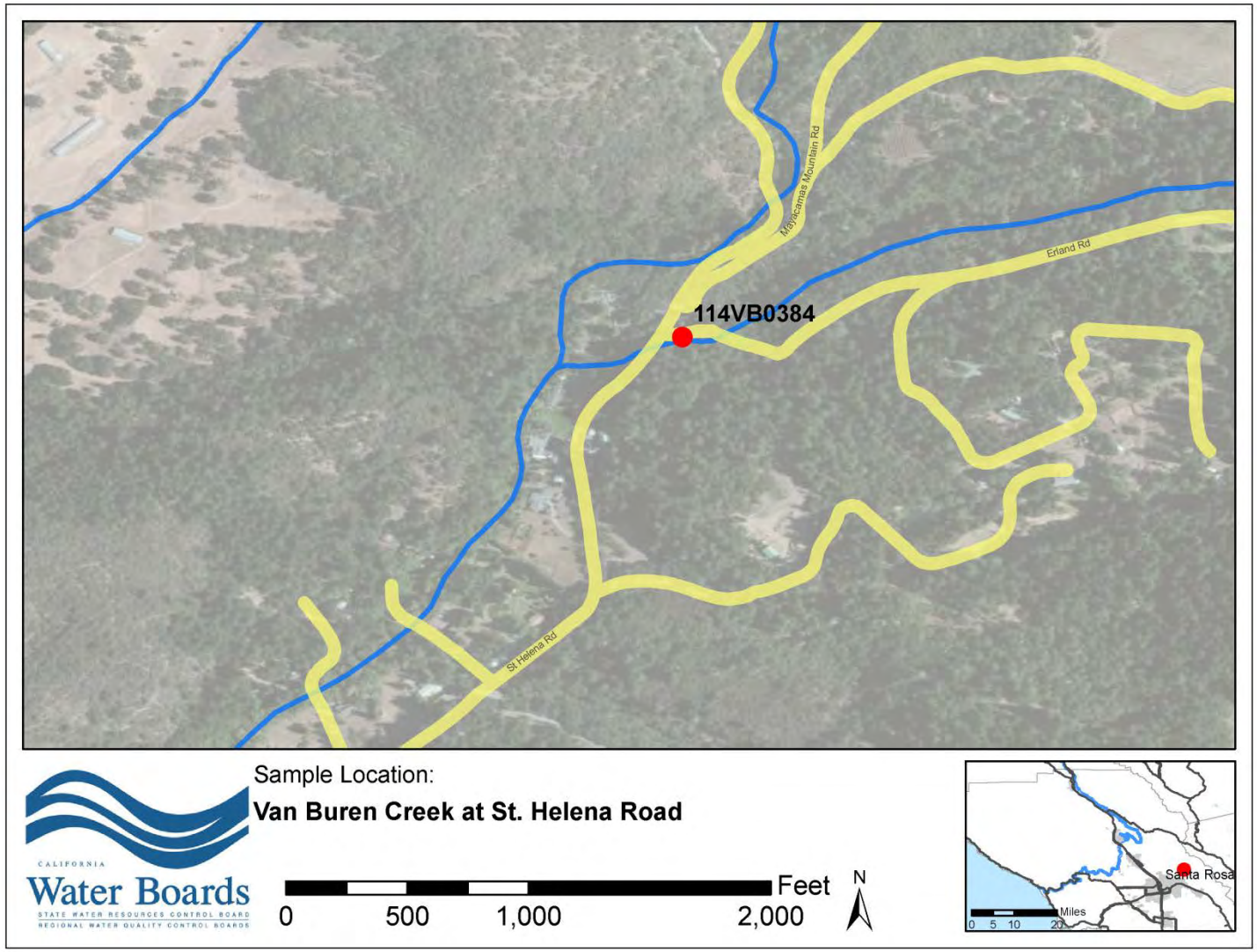


Figure 31: Van Buren Creek at St. Helena Road.



Appendix 6:

Section A-1
Approval Sheet Signatures

Appendix 7:

Region 1 Microbiology Laboratory

Quality Assurance Plan

Regional Water Quality Control Board

North Coast Region

Region 1 Microbiology Laboratory

Quality Assurance Plan

Version 1.0

Originated by:

Carrieann Lopez
Rich Fadness

North Coast Regional Water Quality Control Board

(April 2, 2011)

Group A: Plan Management

A1: Title and Approval Sheet

Document Title Quality Assurance Plan

Lead Organization Regional Water Quality Control Board – North Coast Region
Surface Water Ambient Monitoring Program
5550 Skylane Blvd - Suite A
Santa Rosa CA 95403

Primary Contact Rich Fadness
Regional Water Quality Control Board – North Coast Region
Regional Surface Water Ambient Monitoring Program Coordinator
Phone Number: 707-576-6718
Email Address: RFadness@waterboards.ca.gov

Effective Date April 2, 2011

Approvals

See Appendix 6: Section A-1 Approval Sheet Signatures. Originals are kept on file by the Regional Water Quality Control Board - North Coast Region (Region 1) Microbiology Laboratory Director.

Executive Officer:
Catherine Kuhlman, Regional Water Quality Control Board – North Coast Region

See Appendix G : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Co-Laboratory Directors:

Charles Reed, Regional Water Quality Control Board – North Coast Region

See Appendix G : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Caryn Woodhouse, Regional Water Quality Control Board – North Coast Region

See Appendix G : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Quality Assurance Officer:
Rich Fadness, Regional Water Quality Control Board – North Coast Region

See Appendix G : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Principal Analyst:
Melinda Pope, Regional Water Quality Control Board – North Coast Region

See Appendix G : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

A2: Table of Contents

Group A: Quality Assurance Plan Management

Group B: Data Generation and Acquisition

Group C: Assessment and Oversight

Group D: Data Validation and Usability

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A3: Distribution List

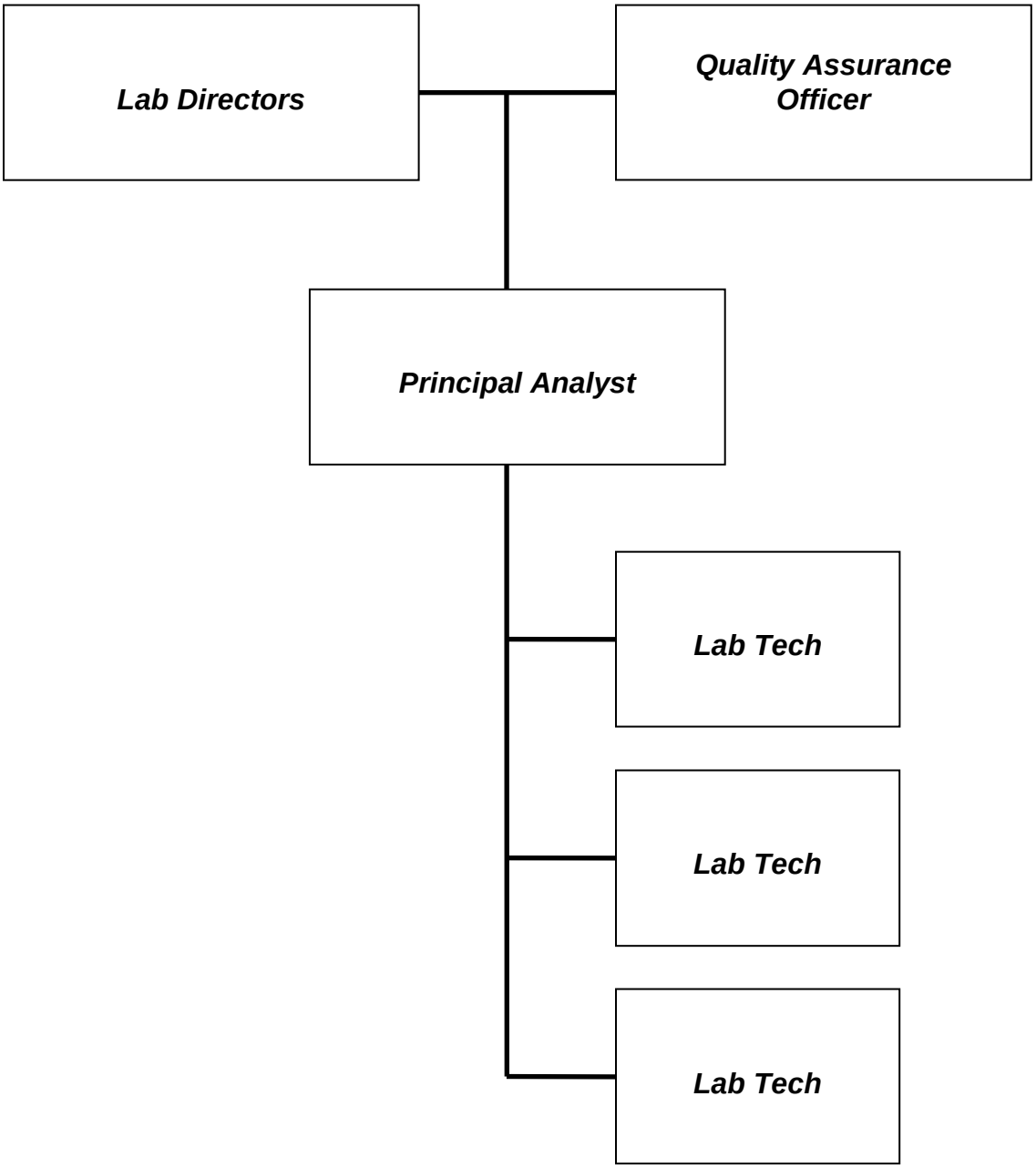
This Quality Assurance Plan (QAP) will be officially distributed to the Regional Water Quality Control Board – North Coast Region (NCRWQCB) Co-Laboratory Directors, Quality Assurance Officer and Principal Analyst with additional distribution to the Surface Water Ambient Monitoring (SWAMP) QA Team, and Environmental Laboratory Accreditation Program (ELAP).

Table 1: QAP Distribution List Primary Contact Information

Contact Information	Organization's Mailing Address
Co-Laboratory Director Main Contact: Charles Reed Phone: 707-576-2752 Email: CReed@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Co-Laboratory Director Main Contact: Caryn Woodhouse Phone: 707-576-2701 Email: CWoodhouse@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Principal Analyst Main Contact: Melinda Pope Phone: 707-576-6732 Email: MPope@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
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

A4: Organization and Responsibilities

Figure 1: Organizational Chart of the Region 1 Microbiology Laboratory



Laboratory Directors

The Laboratory Directors are responsible for the daily activities and functions of the lab. They are responsible management and supervision of the laboratory and its personnel. Their duties include, but are not limited to:

- Set up goals for the laboratory.
- Set up laboratory policies, objectives, principles, & general procedures.
- Manage the on-going requirements of the QA/QC activities.
- Conduct routine audits to ensure compliance of the QAP.
- Ensure data reported meets required QA .
- Review and approve the final data reports.
- Coordinate laboratory accreditation efforts.
- Receive samples, and ensure sample integrity.
- Recruit appropriate laboratory personnel and student assistants.
- Oversee training of laboratory personnel.
- Ensure all laboratory personnel perform analyses in strict accordance with the SOPs.
- Maintain laboratory inventory and Material Safety Data Sheets (MSDS).
- Manage laboratory receiving.
- Procure office and laboratory supplies and equipment.
- Ensure proper disposal of samples and spent reagents after completion of analyses.
- Ensure timely analysis of samples.

They are primarily responsible to ensure that all employees have received the necessary level of training to make them capable of properly executing their duties

Quality Assurance Officer

The QA Officer, who reports directly to the Laboratory Director, is responsible for implementing the QA program for the Region 1 Microbiology Laboratory by assuring the production of accurate, valid, and reliable data by continuously monitoring the implementation of the laboratory quality assurance program. To preserve impartiality in data and system reviews and to avoid conflicts of interest, the QA Officer is not involved in routine analysis and production of lab data. These responsibilities include, but are not limited to:

- Prepare and revise the QAP manual.
- Assist in the development of new, or revisions to existing SOPs.
- Maintain and update all SOPs.
- Develop standard operating procedures and quality assurance plans and assure that they are sound, correct, and meet regulatory requirements.
- Monitor the QAP to ensure complete compliance with its objectives.
- Conduct routine system and performance audits to identify potential problems and to ensure compliance with the SOPs.
- Coordinate external QA/QC audits and corrective actions in response to deficiencies identified during laboratory audits.

- Establish QC procedures and provide internal control samples.
- Perform statistical analyses of QC data and establish databases that accurately reflect the performance of the laboratory.
- Assure that subcontracted laboratories are providing qualified data with acceptable quality control.

Principal Analyst

The Principal Analyst is ultimately responsible for the reliability of all lab operations and analytical data. These responsibilities include, but are not limited to:

- Maintain a working knowledge of the Region 1 Microbiology Laboratory QAP.
- Ensure that all data is generated in compliance with the QAP.
- Coordinate with Laboratory Director concerning laboratory issues.
- Assist Lab Director in training of laboratory personnel.
- Oversee that proper laboratory policies and procedures are being followed.
- Coordinate work schedules, procurement requests, and other necessities.
- Receive samples, and ensure sample integrity.
- Perform work in strict accordance with the SOPs.
- Ensure that all related documentation is complete and accurate.
- Perform data entry into written laboratory records.
- Prepare preliminary and final lab reports.
- Maintain calibration procedures and their frequencies.
- Maintain and troubleshoot laboratory instruments.
- Update instrument calibration and maintenance logs.
- Report any laboratory issues to the Lab Directors.

Laboratory Technician

The Laboratory Technician is responsible in performing routine analysis of environmental samples. These responsibilities include, but are not limited to:

- Maintain a working knowledge of the Region 1 Microbiology Laboratory QAP.
- Receive samples, and ensure sample integrity.
- Perform work in strict accordance with the SOPs.
- Ensure that all related documentation is complete and accurate.
- Perform data entry into written laboratory records.
- Perform calibration procedures and their frequencies.
- Maintain and troubleshoot laboratory instruments.
- Maintain instrument calibration and maintenance logs.
- Report any laboratory issues to the Principal Analyst.

A5: Introduction

The Regional Water Quality Control Board – North Coast Region (Region 1) Microbiology Laboratory is an environmental analytical laboratory located in Northern California, in the City of Santa Rosa. The primary role of the laboratory is to perform bacterial water analyses for Regional Board regulatory, monitoring, surveillance, enforcement, and planning programs.

The State Water Resources Control Board (SWRCB) Quality Assurance (QA) Program Plan requires each regional board to have a written QA plan that describes standard laboratory operating procedures, internal quality control checks, routine procedures used to assess data, precision, accuracy and completeness, and an outline of QA mechanisms that are used to ensure the reliability of data.

The Environmental Laboratory Accreditation Program (ELAP) of the California Department of Health Services (DHS) requires the Region 1 Microbiology Laboratory to develop and implement a Quality Assurance Plan (QAP) in order to document the QA procedures that have been implemented by Region 1 Microbiology Laboratory staff.

Purpose

This quality assurance plan (QAP) identifies the quality assurance (QA) for the Region 1 Microbiology Laboratory. Its primary purpose is to:

- Ensure that Region 1 Microbiology Laboratory activities adhere to the QA requirements of the Environmental Laboratory Accreditation Program (ELAP).
- Serve as a guidance document for the Region 1 Microbiology Laboratory activities.

A6: QAP Objective

The objective of this QAP is to establish an effective and efficient quality management system that will ensure that the data generated by the Region 1 Microbiology Laboratory are known and documented quality. This document outlines the Quality Assurance procedures implemented by Region 1 Microbiology Laboratory personnel to facilitate scientifically valid and legally defensible data that can be used confidently within its own programs, as well as other agencies in their programs.

All microbiological analysis at the Region 1 Microbiology Laboratory is conducted under this QAP. It is the responsibility of Region 1 Microbiology Laboratory staff to follow the quality control practices described herein during sample transfer, storage, and analysis at all times. For each individual project, a quality assurance project plan (QAPP) describing a more specific QA issue needs to be developed.

The objective is achieved through the application of universal measurement quality objectives (see Table 2: Measurement Quality Objectives). As defined by the U.S. Environmental Protection Agency (EPA), these are acceptance criteria for the quality attributes such as precision, accuracy,

and sensitivity. Adherence to these MQOs ensures that data generated by the laboratory will be of known and documented quality.

Table 2: Measurement Quality Objectives* for Pathogens

Quality Control	Frequency of Analysis	Measurement Quality Objective
Calibration	Check temperatures in incubators twice daily with a minimum of 4 hours between each reading	Per analytical method or manufacturer's specifications
Laboratory Blank	Per batch of bottles or reagents	No growth
Reference Material	Per 20 samples or per analytical batch, whichever is more frequent	80-120% recovery
Positive Control	Per 20 samples or per analytical batch, whichever is more frequent	80-120% recovery
Negative Control	Per 20 samples or per analytical batch, whichever is more frequent	No growth
Laboratory Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	RPD<25% (n/a if native concentration of either sample<RL)
Field Quality Control	Frequency of Analysis	Measurement Quality Objective
Field Duplicate	5% of total project sample count (coliforms: one per 25 tube dilution tests)	RPD2<25% (n/a if native concentration of either sample<RL1; coliforms: within 95% confidence interval as defined by IDEXX Laboratories)
Field Blank, Travel Blank, Equipment Blank	Per event	Blanks<RL1 for target analyte

*Unless method specifies more stringent requirements
[1]RL – Reporting Limit
[2]RPD – Relative Percent Difference

Activities of QAP

In order to accomplish the QAP objective, the following activities are incorporated:

- Ensure that sample integrity is maintained.
- Maintain data integrity, validity, and usability.
- Document all aspects of the measurement and analytical process in order to provide data that is technically sound and legally defensible.
- Ensure that the precision and accuracy of the data are known and acceptable based on currently available methodologies.
- Ensure that analytical measurement systems are maintained in an acceptable state of stability and reproducibility.
- Detect problems through data assessment and establish corrective action procedures to keep the analytical process reliable.
- Continue to fulfill the requirements of the California Environmental Laboratory Accreditation Program, which includes participation in proficiency testing, internal and external audits, and other quality evaluation procedures.

Basic Elements of QAP

The QA plan consists of the following three basic elements:

- Prevention
  - Prevention requires an orderly program of planning and positive actions before or during analyses to ensure that analytical systems are functioning properly.
- Assessment
  - Assessment is a form of control that includes periodic checks on performance to determine precision and accuracy.
- Correction
  - Correction is an action taken to determine causes of quality defects and to restore proper functioning of the analytical system.

QAP Approval

The Region 1 Microbiology Laboratory Quality Assurance Officer is responsible for preparing the QAP. After review by the SWAMP Program Quality Assurance Team and approved by the Co-Laboratory Directors, the QAP is incorporated as a laboratory control document and is distributed to appropriate laboratory personnel. The QAP has approval signatures of the Executive Officer, Co-Laboratory Directors, Principal Analyst, and the Quality Assurance Officer. The QAP will be reviewed annually and revisions are made to ensure its effectiveness. A document name, version number, revision date, and page number are shown on the cover page as well as on each page.

QAP Updates and Distribution

All originals of the first and subsequent amended QAP will be held at the Region 1 Microbiology Laboratory by the Laboratory Director. Updates to this QAP will be distributed to the appropriate personnel and previous versions will be discarded. The Laboratory Director under the direction, supervision, and review of the QA Officer, will be responsible for distributing an updated version of the QAP. Copies of the QAP will be distributed to all parties involved directly or by mail. Any future amended QAP will be held and distributed in the same fashion.

A7: Quality Objectives and Criteria for Measurement Data

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). See Table 3: Measurement Quality Objectives for a list of MQOs for parameters analyzed by Region 1 Microbiology Laboratory.

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.

Table 3: Data Quality Objectives for Laboratory Measurements

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Calibration	Check temperatures in incubators twice daily with a minimum of 4 hours between each reading	Per analytical method or manufacturer's specifications
Laboratory Blank	Per batch	No Growth
Positive Control Samples	Per culture medium or reagent lot	80-120% recovery
Negative Control Samples	Per culture medium or reagent lot	No growth
Laboratory Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	RPD[2]<25% (n/a if native concentration of either sample<RL[1])
Field Quality Control	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count (coliforms: one per 25 tube dilution tests)	RPD[2]<25% (n/a if native concentration of either sample<RL[1]; coliforms: within 95% confidence interval as defined by IDEXX Laboratories)
Field Blank, Travel Blank, Equipment Blank	Per event	Blanks<RL[1] for target analyte

*Unless method specifies more stringent requirements
[1]RL – Reporting Limit
[2]RPD – Relative Percent Difference

Accuracy

Accuracy is the measurement of a sample of known concentration and comparing the known value against the measured value. Accuracy for bacteria will be determined by analyzing both positive and negative control samples. A positive control is similar to a standard. Similarly, negative controls will be used to assess cross-contamination and the sterility of reagents and equipment.

Precision

Precision is defined as the measure of agreement among repeated measurements of the same parameter in the same sample under the same analytical condition, calculated as either the range or as the standard deviation.

- Laboratory split samples provide information regarding the precision of the laboratory procedures.
- Precision will be determined by having the same analyst complete the procedure for duplicate field and laboratory split samples.

- Precision of chemistry laboratory measurements will be measured by comparison of the sample to a laboratory matrix spike / matrix spike duplicate (MS/MSD).
- Precision will be measured by the degree of agreement between the sample and MS/MSD results.
- Only samples with a ±25% relative percent difference (RPD) will be valid.

Completeness

Completeness is the number of analyses generating useable data for each analysis divided by the number of samples collected for that analysis.

Bias

Bias is the systematic or persistent distortion of a measurement process that causes errors in one direction. Bias of laboratory measurements will be assessed by comparison of the sample to a laboratory MS/MSD. Spike concentrations are sufficient to eliminate the bias that would be created by the undetectable quantity of the parameter being determined.

Representativeness

Representativeness is the degree to which measurements accurately represent the true environmental condition. 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. Bias or lack of representativeness can occur if samples are not preserved, stored, or analyzed appropriately, causing conditions in the sample to change. Any deviations will be documented in the analytical reports.

A8: Special Training and Certification

Personnel are responsible for complying with QA/QC requirements that pertain to their organizational/technical function. To ensure that all personnel involved in analytical activities are able to carry out their duties, they are required to undergo a training program. Training is administered by the Laboratory Directors. The program is presented to all staff and must be completed prior to assumption of assigned duties. It includes; review of the job description, overview of the QA program, overview of the safety program, and initial on-the-job training.

Each technical staff member must adequately demonstrate a specific knowledge of their particular function and a general knowledge of laboratory operations, laboratory safety, test methods, QA/QC procedures, and records management.

Laboratory Personnel Training Records

Region 1 Microbiology Laboratory office maintains training records for each employee. The records include the analyst's name, the method(s) and date(s) for which the analyst has completed training, the person(s) (supervisor) certifying completion of the training session, the date(s)

recertification is needed and the date(s) recertification was/were completed (if appropriate). Only analysts who have completed training and demonstrated proficiency may conduct analytical methods independently. An analyst in training must be directly supervised by an analyst who has completed training. The training record may also include additional educational courses, professional seminars attended, in house training courses, etc.

- The Region 1 Microbiology Laboratory QA Officer is responsible to ensure training has been completed.
- Training records for the Region 1 Microbiology Laboratory staff are maintained at the Region 1 Microbiology Laboratory office.
- All training records will be made available for review during audits.

A9: Documents and Records

Documents and records generated from the Region 1 Microbiology Laboratory will be organized and stored in compliance with this QAP. This will allow for future retrieval, and to specify the location and holding times of all records.

QAP and SOP Updates and Distribution

All originals of the first and subsequent amended QAPs and SOPs will be held at the Region 1 Microbiology Laboratory office by the Laboratory Director. Updates to the QAP and SOP will be distributed to the appropriate personnel and previous versions will be discarded. The Region 1 Microbiology Laboratory Director under the direction, supervision, and review of the QA Officer, will be responsible for distributing an updated version of the QAP and SOP. Copies of the QAP and SOP will be distributed to all parties involved directly or by mail. Any future amended QAPs and SOPs will be held and distributed in the same fashion.

Record Keeping

The Region 1 Microbiology Laboratory Director will document and track all on-site aspects of sample receipt and storage, analyses, and reporting.

The following logs will be maintained by laboratory personnel:

- **Sample COC /Sample Processing Worksheets**
  The COC/sample processing worksheets will be kept in a 3 ring binder housed in Region 1 Microbiology Laboratory. The COC/worksheets contain date of receipt, number of samples, laboratory sample number, analyses to be completed, time processed samples go into incubator, results, pertinent notes pertaining to the entire process from receipt to results, and analyst's initials.

- **Laboratory QC Binder**
  The Laboratory QC Binder contains all information regarding laboratory QC procedures and checks on equipment, analyst comparison, and materials; materials and supplies receipt, material lot numbers and material QC check information. It will also contain

preventive or corrective maintenance, equipment failure, calibration and all communication regarding pertinent information of each piece of equipment specifically.
Each instrument and activity will have a designated tab within the binder.

- **Routine Equipment Log Sheets**
  Incubators – 2 times daily at least 4 hours between.
  Refrigerators – 1 time daily

Data Management

- **On-Site Data Management**
  The Region 1 Microbiology Laboratory Director will maintain a centralized EXCEL database of information collected and analytical results for microbiological samples on the Region 1 network. Electronic data will be copied to CD media for backup storage in public files at the Region 1 office.
- **Off-Site Data Management**
  Data reviewed and approved on-site by the Region 1 Microbiology Laboratory Director will be entered into the SWAMP Information Management System (IMS) by Region 1 Microbiology Laboratory staff. Verified and validated data is stored in the SWAMP Information Management System (IMS), which includes both a temporary and permanent side. Data on the temporary side remains inaccessible via the web but is accessible to State Water Resources Control Board (State Board) and Regional Board staff. Compilation and interpretation of this temporary data is made possible through Microsoft Access features, as well as specialized tools developed by the SWAMP Data Management Team. Data on the permanent side of the IMS will be accessible to the public through a web interface. (see Appendix I Online Resources)

Document Changes

Significant changes to documents (SOPs, QAP, and Laboratory Safety Manual LSM) shall be reviewed and approved by the Co-Laboratory Directors and the QA Officer.

The Co-Laboratory Directors and the QA Officer shall have access to pertinent background information upon which to base their review and approval.

Significant changes include, but are not limited to: change to calibration protocol, deviations from referenced methods, changes to sample processing protocols or a change to quality control acceptance limits or policy.

Final of Records and Documents

The original data sheets and reports produced are accumulated into project-specific files and maintained at the Region 1 Microbiology Laboratory office for a minimum of five years.

Group B: Data Generation and Acquisition

B1: Sampling Methods

Sample Collection

Special consideration is given to the procurement, storage, and transportation of samples to be analyzed. Procedures ensure that the analyte(s) originally present in the sample matrix has not undergone degradation or concentration, and that contaminants which might interfere with the analysis have not been added.

The generation of quality data begins with the collection of the samples and therefore the integrity of the sample collection process is of concern to the laboratory. Samples must be collected in such a way that contamination by foreign materials is not introduced into the sample and no material of interest escapes from the sample prior to analysis. It is the responsibility of the sampling crews to ensure the proper collection and delivery of their sample as outlined in the project-specific QAPPs and SOPs. Region 1 Microbiology Laboratory will provide whatever support possible to assist the sampling crews in this endeavor, such as providing proper supplies and instructions.

B2: Sample Handling and Custody

Water samples will be labeled with the project name, run number, site location, date and time of collection, and lab analyses to be conducted. Samples will then be stored and transported on ice, maintaining a maximum temperature of 10°C, until processed. Samples will be delivered, under a chain-of-custody (COC) (see Appendix D Region 1 Microbiology Laboratory Chain of Custody (COC)/ Bacteria Worksheet), to the appropriate laboratory, and analyses will be initiated within specified holding times (see Table 4: Sample Volume/Container, Initial Preservation, and Holding Time)

The samples will be kept on ice from the time of sample collection until delivery to the laboratory. Exposure to sunlight is avoided, as ultraviolet rays can be detrimental to bacteria, resulting in unreliable analytical results. Samples are, therefore, placed in a cooler with a closed lid immediately following collection. Each field sample is uniquely identified with a sample label written or printed in indelible ink. Sample containers are identified with the project title, appropriate identification number, the date and time of sample collection, and preservation method.

The principal documents used to identify samples and to document possession will be COC records, field logbooks, and field tracking forms. COC procedures will be used for samples throughout the collection, transport, and analytical process.

COC procedures will be initiated during sample collection. A COC record will be provided with each sample or group of samples. Each person who will have custody of the samples will sign the form and ensure the samples will not be left unattended unless properly secured. Completed COC

forms will be placed in a plastic envelope and kept inside the cooler containing the samples. Once delivered to the laboratory, the COC form will be signed by the person receiving the samples. The temperature will be measured and condition of the samples will be noted and recorded by the receiver. COC records will be included in the final data record as prepared by each of the analytical laboratories.

Table 4: Sample Volume/Container, Initial Preservation, and Holding Time

Analyte	Units	Recommended Container	Recommended Sample Volume	Recommended Preservation	Maximum Required Holding Time
E. Coli	MPN/100 mL	Factory-sealed, pre-sterilized, disposable Whirlpak bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 6 °C in the dark.	8 hours
Enterococcus	colonies/100 mL	Factory-sealed, pre-sterilized, disposable Whirlpak bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 6 °C in the dark.	8 hours
Total Coliform	MPN/100 mL	Factory-sealed, pre-sterilized, disposable Whirlpak bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 6 °C in the dark.	8 hours

Sample Acceptance

Prior to accepting samples, Region 1 Microbiology Laboratory requires proper, full, and complete documentation, including the sample identification, the location, date and time of collection, collector's name, preservation type, sample type and any special remarks concerning the sample; Unique identification of samples using durable labels completed in indelible ink; use of appropriate sample containers; receipt within holding times; adequate sample volume; and procedures that are used when samples show signs of damage. Data from any samples which do not meet the policy are noted on the COC defining the nature and substance of the variation.

Sample Receipt and Login

All samples submitted to Region 1 Microbiology Laboratory are delivered to the laboratory's sample receiving area and are received by the Principal Analyst or Lab Technician. Temperature of the samples are checked and recorded on the Chain of Custody (COC)/ Bacteria Worksheet. The Principal Analyst or Lab Technician compares the samples received against the Chain of Custody (COC)/ Bacteria Worksheet.

If a sample discrepancy, such as a broken or missing sample is observed at check-in by the Principal Analyst or Lab Technician, a statement to that effect is written in the remarks section of the Chain of Custody. The sampling crew must be notified and approve of any changes made to the COC.

All samples received are recorded onto Chain of Custody (COC)/ bacteria worksheet with the following information: project name, project manager, analytical parameters requested, and laboratory work order number. The laboratory work order number is a sequential number that is unique to each sample. Samples are processed through the laboratory by their unique laboratory sample numbers. Special instructions about the samples are written onto the Chain of Custody (COC)/ Bacteria Worksheet in the comments section.

Sample Tracking

Region 1 Microbiology Laboratory uniquely identifies each sample to be tested to ensure that there is no confusion regarding identity. The sample identification system includes identification for all samples, sub-samples, and subsequent extracts. A unique identification code is placed on each sample container. Each Laboratory Technician is required to maintain Chain of Custody (COC)/ Bacteria Worksheet to provide sufficient detail to enable others to reconstruct the analysis should the analyst not be available to do so. The system for tracking samples consists of the Region 1 Microbiology Laboratory Chain of Custody (COC)/ Bacteria Worksheet, Equipment Calibration/Maintenance Logbook, Materials and Supplies Logbook, and final analytical reports. This tracking system ensures that the Region 1 Microbiology Laboratory's records can be used as valid evidence should such data become the subject of litigation or any other type of review or investigation.

Sample Disposal

All samples and sample preparation products are disposed of in accordance with Federal and State laws and regulations. (see Appendix A Region 1 Microbiology Laboratory Safety Manual LSM)

B3: Analytical Methods

All methods commonly used at Region 1 Microbiology Laboratory are EPA approved. Laboratory Analytical method numbers are summarized in Table 5 Laboratory Analytical Methods. The Region 1 Microbiology Laboratory SOPs can be found in Appendix C. The SOPs describe in detail, routine analytical tasks performed at the Laboratory and typically include:

- Method title and referenced documents
- Reagents and preparation
- Method summary
- Definitions
- Health and safety warnings
- Apparatus and materials

- Calibration and standardization
- Sample preparation and preservation
- Quality control procedures
- Equations, calculations and data reduction procedures
- Data bench sheets and reporting forms
- Troubleshooting

Table 5: Laboratory Analytical Methods

Analyte	Analytical Method/ SOP	Modified for Method Yes / No	Achievable Laboratory Limits (MDL)
E. coli	Colilert® / SM 9223	No	1 MPN/100mL
Enterococcus	Enterolert®	No	1 MPN/100mL
Enterococcus	SM 9230 B	No	2 MPN/100mL

Method Review

SOPs for current methods must be reviewed annually. The most current revision of the referenced method must be reviewed at the time of the annual SOP review to assure that all method requirements and control limits are being met and any deviations from the referenced method are documented. The review/revision process must be documented.

Method Revision

The Region 1 Microbiology Laboratory retains a copy of all archived SOPs. The analyst's bench copy of an SOP is the most current version of an SOP and must be updated whenever a protocol is changed. Significant changes, including any change affecting the calibration or quality control acceptance limits must be authorized (initialed and dated) by the Laboratory Directors. The SWAMP Quality Assurance Team, Laboratory Directors and QA Officer review the SOP prior to approval by the Laboratory Directors and the QA Officer. The referenced method should be reviewed at the time of the revision to assure that all method requirements and limits are being met. It is the analyst's responsibility to assure that method specified criteria are met. The QA Officer maintains a paper copy of obsolete documents with the date revised for a minimum of 5 years. An electronic copy of all archived documents is also retained.

Initial Demonstration of Capability

For every method used in the laboratory, there must be an initial demonstration of capability prior to performing sample analysis and determining method detection limits (MDL).

Analytical batch

A batch is a group of samples containing not more than 20 samples that are similar with respect to the sampling or testing procedures being employed and are processed as a unit. Manipulation, processing, and analysis of each sample in a batch are performed simultaneously or in a continuous sequence without interruption. All samples in a batch must have the same matrix.

Batch QC Samples

For microbiology procedures, it includes a media sterility check, method blank (MB), sample duplicate, dilution water blank, and both positive and negative control cultures.

Method Blank

A method blank is an analyte-free matrix that is carried through the complete sample preparation and analytical procedure. It is used for documenting contamination resulting from the analytical process.

- One method blank is run for each analytical batch.
- The method blank for microbial procedures should result in an absence of growth or colonies.
- Corrective actions must be taken if the MB does not meet the acceptance criteria, which include locating, and reducing the source of the contamination and re-extracting and reanalyzing any samples associated with the contaminated MB.
- Sample results are not corrected for blank contamination unless required by the specific method.

Laboratory Reagent Blank

A laboratory reagent blank (LRB) will be run every time a new lot of reagents is received.

Sources of contamination are determined and interference eliminated prior to sample processing.

B4: Quality Control

Internal Laboratory Quality Control

The Region 1 Microbiology Laboratory employs the use of internal quality control samples to assess the validity of the analytical results of all samples. Internal QC is a way for Region 1 Microbiology Laboratory to assess the analytical measurement system and check whether it is in control. The analytical procedures will dictate the internal QC applied. For internal QC, the following types of QC samples can be applied to each analytical batch or routine operation:

- **Laboratory Blanks**
  Laboratory blanks are prepared using laboratory reagent water and are treated exactly as a sample, including exposure to all glassware, equipment, solvents and reagent used with other samples. The laboratory blank is used to determine if the method analytes or other interferences are present in the lab environment, reagents, or equipment. If contaminants are present that interfere with the determination of any analyte, detection limits must be elevated or affected compounds must be qualified accordingly. If a blank is contaminated, then the source of the contamination must be identified and eliminated.
- **Laboratory Duplicate Sample**
  A laboratory duplicate sample is prepared with every sample batch to assess precision for each matrix type. The precision of the duplicate sample is reported as relative percent difference (RPD). RPD acceptance criteria are method specific. Duplicate samples that fall outside of the acceptance criteria must be reanalyzed or qualified if required.

External Quality Control

In addition to internal laboratory quality control samples, there are also field and proficiency quality control samples that are evaluated. External quality control samples are generally specified in project monitoring plans for the purpose of assessing sample contamination or laboratory proficiency. External QC samples include:

- **Field Duplicate Samples**
  Two separate samples collected at the same time and from the same site under identical conditions and treated exactly the same through all field and laboratory procedures. Duplicate analysis gives a measure of the variability in the sample collection, shipment, storage, and analysis.
- **Field Blank Samples**
  A reagent water blank prepared at the sample site by filling empty sample containers with analyte-free water, adding preservatives (if applicable) and then taking the samples to the lab for analysis. The samples are treated as regular samples including exposure to the sample site conditions, storage, preservation and lab procedures. It is used to determine if method analytes and/or other contaminants are present in the field sampling environment.
- **Equipment Blank Sample**
  A blank sample used to monitor the effectiveness of the cleaning procedures used on field sampling equipment. Equipment blanks are prepared by taking a quantity of analyte-free water to the sample site, rinse the piece of equipment with the analyte-free water directly into the sample container, add preservatives (if applicable) and then treat as a regular sample. At least one equipment blank is prepared for each type of analyte group collected with each item of equipment.

Proficiency Evaluation (PE)

A fortified sample used to evaluate the performance of the laboratory. PE samples consist of solutions of known concentrations of target analytes sent to the laboratory to be analyzed as an unknown it is also referred to as a “blind” sample, or certified reference material (CRM). Based on

either statistically derived or legislatively assigned acceptance criteria, the results are graded as "acceptable" or "non-acceptable". Participation in PE sample studies provides a means by which the laboratory can discover analytical problems and improve performance. Samples may be used to evaluate an analyst.

Dilution of Samples

Final reported results must be corrected for dilution carried out during the process of analysis. In order to evaluate the QC analyses associated with an analytical batch, corresponding batch QC samples must be analyzed at the same dilution factor. For example, the results used to calculate the results of matrix spikes must be derived from results for the native sample, matrix spike, and matrix spike duplicate analyzed at the same dilution. Results derived from samples analyzed at different dilution factors must not be used to calculate QC results.

Laboratory Corrective Action

The Region 1 Microbiology Laboratory has a Corrective Action Program that ensures the proper documentation and dispositions of conditions requiring corrective action. The system also ensures that the proper corrective action is implemented to prevent recurrence of the condition.

The Corrective Action Program applies to all situations that impact data quality. Any QC sample result outside of acceptance limits requires corrective action. Once the problem has been identified and addressed, corrective action may include the reanalysis of samples, or appropriately qualifying the results. These situations may include, but are not limited to, quality control criteria being exceeded, statistically out-of-control events, deviations from normally expected results, suspect data, deviations from the standard operating procedure, and special sample handling requirements. The procedure consists of documenting the condition requiring corrective and implementing corrective condition.

When a condition requiring corrective action arises, a Corrective Action Report is initiated. The initiator describes the condition requiring corrective action. An investigation, if necessary, is conducted to determine the cause of the condition. A corrective action is recommended based on the results of the investigation.

The Corrective Action Report is reviewed by the Laboratory Directors and Region 1 Quality Assurance Officer who either approve the recommended corrective action or indicate the appropriate corrective action.

The Region 1 Quality Assurance Officer has the responsibility of following up and making sure that the corrective action is implemented. Implementation of the corrective action is documented by the Corrective Action Report being signed and dated by the person who implemented the corrective action, along with the Laboratory Directors and Region 1 Quality Assurance Officer.

Table 6: Laboratory Corrective Action

Laboratory Quality Control	Corrective Action
Laboratory Blank	The sample analysis must be halted, the source of the contamination investigated, the samples along with a new laboratory blank prepared and/or re-extracted, and the sample batch and fresh laboratory blank reanalyzed. If reanalysis is not possible due to sample volume, flag associated samples as estimated.
Certified Reference Material (CRM)	If deemed appropriate, affected samples and associated quality control may be reanalyzed following instrument recalibration.
Matrix Spike	The spiking level should be approximately 2-5 times the ambient concentration of the spiked sample. Appropriately spiked results should be compared to the matrix spike duplicate to investigate matrix interference. If matrix interference is suspected, the matrix spike result must be qualified.
Matrix Spike Duplicate	The spiking level should be approximately 2-5 times the ambient concentration of the spiked sample. Appropriately spiked results should be compared to the matrix spike duplicate to investigate matrix interference. If matrix interference is suspected and reference material recoveries are acceptable, the matrix spike duplicate result must be qualified.
Laboratory Duplicate	For duplicates with a heterogeneous matrix or ambient levels below the reporting limit, failed results may be qualified. Other failures should be reanalyzed as sample volume allows.

B5: Instrument/Equipment Testing, Inspection, and Maintenance

The Laboratory Directors and Principal Analyst are ultimately responsible for ensuring equipment is properly tested, inspected and maintained. Laboratory Technicians may be delegated the responsibilities of carrying out these tasks.

Electronic laboratory equipment usually has recommended maintenance prescribed by the manufacturer. These instructions will be followed as a minimum requirement. Due to the cost of some laboratory equipment, back up capability may not be possible. But all commonly replaced parts will have spares available for rapid maintenance of failed equipment.

A separate log book will be maintained for each piece of equipment. All preventive or corrective maintenance will be recorded. The total history of maintenance performed will be available for inspection during audits.

If deficiencies are found, the necessary maintenance will be performed and then the equipment will be re-calibrated and re-inspected. A pre- and post-calibration will be run to determine if the problem has been fixed. If this does not correct the problem, then the equipment will be taken out of use and sent to the manufacturer for servicing. Deficiencies that cannot be immediately corrected will be annotated on the field or lab worksheets, as applicable, and noted in the instruments specific maintenance logbook.

Table 7: Testing, Inspection, Maintenance of Analytical Instruments

Equipment / Instrument	Maintenance / Testing / Inspection Activity	Responsible Person	Frequency	Reference
Water Purification System	Water QC Check Cleaning /Inspection	Lab Director / Principal Analyst	Daily Monthly	Laboratory QC Binder
Autoclave	Temperature Check Cleaning/Inspection/Sterilization Confirmation	Lab Director / Principal Analyst	Per Cycle Monthly	Laboratory QC Binder
Incubators	Temperature Check Cleaning/Inspection	Lab Director / Principal Analyst	2x Daily Monthly	Laboratory QC Binder
Tray Sealer	Cleaning /Inspection	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder
UV Lamp	Inspection	Lab Director / Principal Analyst	Per Use	Laboratory QC Binder
Pipettes	Cleaning/Inspection	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder
Refrigerator	Temperature Check Cleaning/Inspection	Lab Director / Principal Analyst	1x Daily Monthly	Laboratory QC Binder
Thermometers	Temperature Check	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder

B6: Instrument/Equipment Calibration and Frequency

All equipment and instruments are operated and calibrated according to the manufacturer's recommendations. Operation and calibration are performed by personnel properly trained in these procedures. Documentation of all calibration information is recorded in the appropriate logs. If equipment is not meeting calibration criteria according to the manufacturer's recommendations, it is the responsibility of the Laboratory Technicians to notify the Laboratory Directors and the Principal Analyst who will be responsible for addressing the problem. This may include repair or replacement of equipment. All corrective actions are documented in the appropriate log.

Table 8: Instrument/Equipment Calibration and Frequency

Equipment / Instrument	Calibration Description and Criteria	Responsible Person	Frequency	Reference
Water Purification System	As described in manual	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder
Autoclave	As described in manual	Lab Director / Principal Analyst	Annually	Laboratory QC Binder
Incubators	±0.5°C	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder
Tray Sealer	Tray Wells completely isolated	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder
UV Lamp	Positive IDEXX Comparator readings	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder
Pipettes	As described in manual	Lab Director / Principal Analyst	Monthly	Laboratory QC Binder
Refrigerator	1 - 4 °C	Lab Director / Principal Analyst	Annually	Laboratory QC Binder
Thermometers	National Institute of Standards and Technology (NIST) certified	Lab Director / Principal Analyst	Annually	Laboratory QC Binder

B7: Inspection/Acceptance of Supplies and Consumables

The procurement of supplies, equipment, and services must be controlled to ensure that specifications are met for the high quality and reliability required for laboratory function. The Laboratory Directors and Principal Analyst are each responsible for inspection and acceptance of supplies and consumables used by their respective portions of this study. The actual inspection may be delegated to lab staff.

Records are kept for all standards, including the manufacturer/vendor, the manufacturer's Certificate of Analysis or purity (if supplied), the date of receipt, recommended storage conditions, and an expiration date after which the material is not used unless it is verified. After being verified and logged into the appropriate logbooks, the original containers provided by the vendor are labeled with an expiration date and in-house identification number.

Upon receipt and prior to use, all reagents and supplies 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.

Commercially prepared media for microbiological analyses are used within the manufacturer's designated shelf life. All manufacturer-supplied specifications, which may include shelf life, storage conditions, sterility, performance checks, and date, are to be followed.

Table 9: Inspection/Acceptance Testing Requirements for Supplies and Consumables

Supplies / Consumables	Inspection / Testing Specifications	Acceptance Criteria	Frequency	Responsible Individual
IDEXX Colilert® Media Lot	Media properly reacts to known organisms when tested using the IDEXX Quanti-cult	Organism (T. coli) (E. coli) E. coli (+) (+) K. pneumoniae (+) (-) P. aeruginosa (-) (-) Within expiration date	When new media lots are received and prior to use	Laboratory Director / Principal Analyst
IDEXX Enterolert® Media Lot	Media properly reacts to known organisms when tested	Positive - Enterococcus faecium or Enterococcus faecalis Negative - Serratia marcescens or E. coli	When new media lots are received and prior to use	Laboratory Director / Principal Analyst
IDEXX Colilert/Enterolert Sample Vessels	Sterile Vessels	Vessels are sealed and accompanied with certification of sterilization	Upon arrival and prior to use	Laboratory Director / Principal Analyst
IDEXX Quanti-Tray/2000	Sterile Quanti-Tray/2000	Quanti-Tray/2000 are packaged in sealed bags and accompanied with certification of sterilization	Upon arrival and prior to use	Laboratory Director / Principal Analyst

B8: Data Management

Data Management On-Site

The Region 1 Microbiology Laboratory Directors will maintain a centralized EXCEL database of information collected and analytical results for microbiological samples on the Region 1 network. Electronic data will be copied to CD media for backup storage in public files at the Region 1 office.

Data Management Off-Site

Data reviewed and approved on-site by the Region 1 Microbiology Laboratory Directors will be entered into the SWAMP Information Management System (IMS) by Region 1 Microbiology Laboratory staff. Verified and validated data is stored in the SWAMP Information Management System (IMS), which includes both a temporary and permanent side. Data on the temporary side remains inaccessible via the web but is accessible to State Water Resources Control Board (State Board) and Regional Board staff. Compilation and interpretation of this temporary data is made possible through Microsoft Access features, as well as specialized tools developed by the SWAMP Data Management Team. Data on the permanent side of the IMS will be accessible to the public through a web interface. (see Appendix I Online Resources)

Group C: Assessments and Oversight

C1: Assessments and Response Actions

Internal Audits

Internal audits are performed to verify that laboratory operations continue to comply with the requirements of the quality system. Such audits are performed by the QA Officer. Where the audit findings cast doubt on the correctness or validity of the laboratory's results, an immediate corrective action is initiated.

The internal audits include an examination of laboratory documentation on sample receiving, sample log-in, sample storage, chain-of-custody procedures, sample preparation and analysis, instrument operating records, etc. A standardized checklist system is employed to ensure that specific items are consistently reviewed for compliance. Copies of the final internal audit report, along with an associated corrective action reports, are printed and given to all laboratory personnel, including the Lab Directors.

Internal Audits are a tool to:

- verify analyst compliance with the laboratories quality assurance policies
- address any ongoing quality issues
- highlight technical, equipment or management support needed

Internal audits fall into two general categories, performance and system audits. In both, the appropriate implementation of corrective action is assured to effect permanent solutions to problems that are detected.

Performance Audits

A performance audit verifies the laboratory’s ability to correctly identify and quantify substances in samples. It involves the analysis of a sample or reference material and comparing the results with results of other laboratories. Performance audits include those required for certification and those routinely conducted on a voluntary basis as an internal check on performance to demonstrate competency.

System Audits

A system audit includes examining all aspects of the laboratory. System audits include assessments about, but not necessarily limited to, the following:

- Personnel – Education, training and experience.
- Physical aspects of the laboratory – Examine cleanliness, orderly, waste disposal operations.
- Standard Operating Procedures (SOPs) – Assess whether current and complete.

- Equipment/Instruments used in the laboratory - Check if equipment/instruments are clean, well maintained and regularly inspected as evidenced by equipment/instrument log books. Check that calibration is done correctly, properly documented, and whether documentation is done in blue, waterproof ink.
- Test substances, Reagents, and Samples – Check notebooks, or logbooks to see if substances are properly identified, look at container labels for proper identification (identify material, concentration, composition, storage requirements, expiration date, and initials of person making up material) for sample.
- Chain of Custody – Look at procedures, documentation, and records management.
- Inspect records and raw data, including notebooks, computer printouts, worksheets, chain of custody record sheets, protocol, and SOPs to confirm work was carried out according to requirements and in accordance with the prescribed methods.
- Random check of analytical values reported against numbers in the worksheets and electronic database.
- Review laboratory records to verify that sample holding times were met, calibration checks adequate, equipment monitoring records are performed as required (e.g., temperature records), and sample preservation records are maintained and correct.
- Corrective action documentation complete and current.

All internal audits will be made by the Region 1 Microbiology Laboratory QA Officer. Region 1 Microbiology Laboratory staff will conduct periodic and annual reviews of Analytical procedures. Internal audits will be observed practices against those found in the Region 1 Microbiology Laboratory SOPs.

If an audit discovers any discrepancy, the Region 1 Microbiology Laboratory QA Officer will discuss the observed discrepancy with the appropriate person responsible for the activity. The discussion will begin with whether the information analyzed 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 Region 1 Microbiology Laboratory QA Officer has the power to halt all analytical work if the deviation(s) noted are considered detrimental to data quality.

Copies must be maintained in a file of all internal audits, training, QA audit reports completed, as well as documentation of any deficiencies and corrective actions necessary to remedy such deficiencies. When requested, these records must be accessible.

External Audits

External audits are driven by laws and/or regulations and administered through governmental agencies to ensure the laboratory's ability to meet minimum standards when reporting analytical data that is required under mandated monitoring programs. These audits assess performance evaluations and on-site systems inspections. External audits are conducted by Environmental Laboratory Accreditation Program (ELAP) on a scheduled basis. The audits serve to assess the laboratory's overall status for maintaining its certification. A list of tests the Region 1 Microbiology Laboratory is approved for under ELAP is presented in (Appendix F Laboratory

Certification). Failure to meet the minimum requirements can result in a downgrading or loss of the laboratory's certification. The audit records and resulting corrective action reports are administered by the Region 1 Microbiology Laboratory QA Officer and maintained in laboratory files.

Corrective Action

Corrective action investigates suspect procedures and/or data. Corrective action is intended to prevent the recurrence of similar problems and to promote continuous improvement in the quality of service through training and education. Some type of corrective action is required whenever any of the following conditions exist:

- Suspicious results discovered during the testing or analytical operations.
- Suspicious results discovered during the data validating procedures.
- Suspicious results discovered during the internal report review procedures.
- Suspicious results discovered in independent audits.
- Suspicious results from equipment or instrument failure.

The analyst at the bench has the primary responsibility for ensuring the quality and acceptability of test results. One of the most effective means of error prevention is to respond immediately to suspicious data or equipment malfunctions from the bench. Taking proper corrective action at this point can reduce or prevent producing erroneous or poor quality data that may have serious consequences in how the information is expected to be used. Specific control procedures, calibration checks, control charts, operational check lists, etc., are in place to detect instances in which corrective action might be necessary.

Administration of Corrective Actions

The Region 1 Microbiology Laboratory Directors, Principal Analyst and QA Officer will formally initiate the investigation of the problem. The goal of the investigation is to determine the cause and develop a strategy to correct the problem and keep it from recurring. All may oversee the implementation for the solution to the problem, verify the correction, and document the effectiveness. The Region 1 Microbiology Laboratory QA Officer receives the final report and reviews it for approval. If applicable, the QA Officer will notify the respective Project Coordinator regarding the outcome of the investigation and provide corrections or other options, if available.

A corrective action report will include details of the investigation including:

- Relevant dates.
- Name(s) of the analyst(s) involved.
- Identification of affected samples.
- Status of equipment.
- Procedures used.
- Statement of problem, cause(s), corrective action(s) taken, and verification of corrective action.

- Complete report initialed and dated by the Region 1 Microbiology Laboratory Directors, Principal Analyst and QA Officer.

Regardless of who initiates the corrective action report, the QA Officer is responsible for the maintenance of the corrective action reports.

C2: Reports to Management

The Region 1 Microbiology Laboratory Directors will review all draft laboratory reports to ensure the accuracy of analysis, data analysis and data interpretation.

The QA Officer will provide the Laboratory Director with the following reports of information:

- Quality assurance reports
- Laboratory QA Plan Updates (annual and periodic)
- Internal system and performance audit reports
- Corrective action reports issued as a result of system or performance audit QA Irregularities/deficiencies
- QA goals and objectives for the upcoming year

The original data sheets and reports produced are accumulated into project-specific files and maintained at the Region 1 office for a minimum of five years.

Group D: Data Validation and Usability

D1: Data Review, Verification, and Validation

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

- **Responsibility for Data Review**
  It is the responsibility of the Principal Analyst to assemble a data package containing all relevant raw data needed for data review and validation for each batch of samples processed. All corrections must be properly initialed, dated and the reason for the revision documented. The Region 1 Microbiology Laboratory Directors and QA Officer then 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.
- **Checking for Typical Errors**
  In-house examination of the data produced from sample analysis 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 MQO**
  Data generated by analytical activities will be reviewed against measurement quality objectives (MQO). This will ensure that the data will be of acceptable quality.
- **Checking Against QA/QC**
  Data will be checked against QA/QC requirements developed and documented in this QAP. Checks will include evaluation of laboratory duplicate results, laboratory blank data pertinent to each method and analytical data set.
- **Data Verification**
  Data verification is the process of evaluating the completeness, correctness, and conformance / compliance of a specific data set against the method, procedural, or contractual specifications.
  The Region 1 Microbiology Laboratory Directors and QA Officer are responsible for data verification.
- **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. The Region 1 Microbiology Laboratory Directors and QA Officer are responsible for validation of data.

Data Separation

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

- Data that meets all acceptance requirements
- Data that has been determined to be unacceptable for use
- Data that may be conditionally used and that is flagged

Data Entry and Storage

Verified and validated data will be entered into the SWAMP Information Management System (IMS) by Region 1 Microbiology Laboratory staff. The verified and validated data is stored in the SWAMP Information Management System (IMS), which includes both a temporary and permanent side. Data on the temporary side remains inaccessible via the web but is accessible to State Water Resources Control Board (State Board) and Regional Water Quality Control Board staff. Compilation and interpretation of this temporary data is made possible through Microsoft Access features, as well as specialized tools developed by the SWAMP Data Management Team. Data on the permanent side of the IMS will be accessible to the public through a web interface. (see Appendix I Online Resources)

D2: Verification and Validation Methods

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

- All sample analysis 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.
- For data in the SWAMP IMS, Region 1 Microbiology Laboratory Directors and QA Officer will perform an independent recheck of at least 10% of these records as the validation methodology.

If there are any data quality problems we will try to identify whether the problem is a result of project design issues, sampling issues, analytical methodology issues, or QA issues. 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 appropriate people involved will be assembled to discuss and try to resolve the issue(s) as a group. The Region 1 Microbiology Laboratory Director and QA Officer have the final authority to resolve any issues that may be identified during the verification and validation process.

Data Validation

The Region 1 Microbiology Laboratory Directors are responsible for confirming that submitted data meets the criteria specified in the SWAMP QAPrP. After data is loaded into the temporary side of the IMS, The DMT again reviews it against SWAMP criteria associated with the following:

- Completeness
- Holding times
- Matrix spike/matrix spike duplicate (MS/MSD)
- Laboratory duplicates
- Surrogates
- Certified reference material (CRM)
- Laboratory control sample (LCS)
- Method blanks
- Field QC samples
- Reporting limit (RL)

Appendix A:

Region 1 Microbiology Laboratory

Laboratory Safety Manual

Regional Water Quality Control Board

North Coast Region

Region 1 Microbiology Laboratory

Laboratory Safety Manual

Version 1.0

Originated by:

Carrieann Lopez

North Coast Regional Water Quality Control Board

(April 2, 2011)

A1: Title and Approval Sheet

Document Title Laboratory Safety Manual

Lead Organization Regional Water Quality Control Board – North Coast Region
Surface Water Ambient Monitoring Program
5550 Skylane Blvd - Suite A
Santa Rosa CA 95403

Primary Contact Rich Fadness
Regional Water Quality Control Board – North Coast Region
Regional Surface Water Ambient Monitoring Program Coordinator
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Effective Date April 2, 2011

Approvals

Originals are kept on file by the Regional Water Quality Control Board - North Coast Region (Region 1) Microbiology Laboratory Director.

Executive Officer:
Catherine Kuhlman, Regional Water Quality Control Board – North Coast Region

See Appendix A : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Co-Laboratory Directors:

Charles Reed, Regional Water Quality Control Board – North Coast Region

See Appendix A : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Caryn Woodhouse, Regional Water Quality Control Board – North Coast Region

See Appendix A : Section A-1 Approval Sheet Signatures _____ _____
Signature Date

Principal Analyst:
Melinda Pope, Regional Water Quality Control Board – North Coast Region

See Appendix A : Section A-1 Approval Sheet Signatures
Signature Date

Safety Officer:
Rich Fadness, Regional Water Quality Control Board – North Coast Region

See Appendix A : Section A-1 Approval Sheet Signatures
Signature Date

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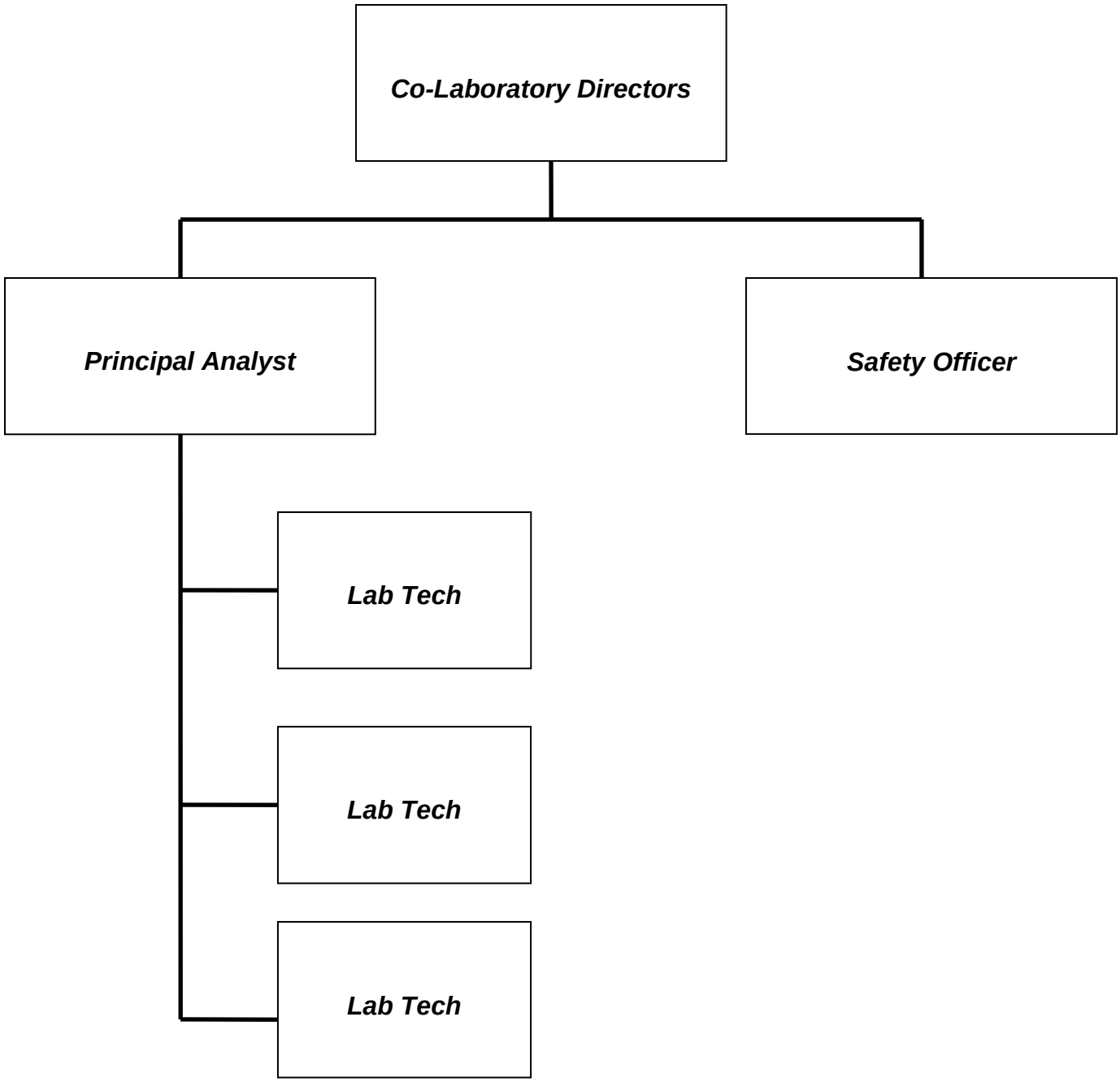
A3. Organization and Responsibilities

Figure 1: Organizational Chart of the Region 1 Microbiology Laboratory



Laboratory Director

The Region 1 Microbiology Laboratory Director is ultimately responsible for the safety of all laboratory personnel. Responsibilities include, but are not limited to:

- Set up safety goals for the laboratory.
- Set up laboratory safety policies, objectives, guidelines, & general procedures.
- Review and approve the Laboratory Safety Manual (LSM).
- Manage the on-going requirements of the LSM activities.
- Implement new and revised LSM procedures to improve safe working conditions.
- Ensure that the Region 1 Microbiology Laboratory QA Officer conducts routine audits to ensure compliance of the QAP, SOP and LSM.
- Oversee the development of standard safety procedures and assure that they are sound, correct, and meet regulatory requirements.
- Review and approve modifications of safety procedures.
- Oversee proper training for laboratory personnel, and confirm personnel qualifications for working in the laboratory.
- Monitor Federal legislation that may be imposed on the laboratory in the general areas of health and safety.
- Oversee the development and implementation of an emergency response plan.

Safety Officer

The Region 1 Microbiology Laboratory Safety Officer reports directly to the Laboratory Director and is responsible for the development of safety procedures of all laboratory personnel. Responsibilities include, but are not limited to:

- Set up safety goals for the laboratory.
- Set up laboratory safety policies, objectives, guidelines, & general procedures.
- Review and update the Laboratory Safety Manual (LSM).
- Develop new and revised LSM procedures to improve safe working conditions.
- Conducts routine audits to ensure compliance of the QAP, SOP and LSM.
- Develop standard safety procedures and assure that they are sound, correct, and meet regulatory requirements.
- Update and modify safety procedures.
- Develop and implement the emergency response plan.

Principal Analyst

The Region 1 Microbiology Laboratory Principal Analyst reports directly to the Laboratory Director and is responsible for the daily operation and management of laboratory safety. The responsibilities of this position include:

- Assist Laboratory Director in establishing safety goals for the laboratory.
- Conduct routine audits to ensure compliance of the LSM program.

- Ensure that all laboratory personnel have read and understand the LSM, and that all personnel are adequately trained and qualified to perform laboratory duties.
- Regularly inspect all laboratory safety equipment for proper function and expiration dates.
- Assist the Safety Officer in modifying laboratory procedures as necessary, with the approval of the Region 1 Microbiology Laboratory Directors.
- Ensure proper disposal of samples, standards, and reagents after completion of analyses.
- Maintain complete records of laboratory incidents, inspections, training, and all other relevant laboratory safety issues.
- Regularly document laboratory performance, all regulatory requirements not being met, and status of the LSM.
- Coordinate emergency response and maintain effective relations with external emergency services.

Laboratory Technician

The Region 1 Microbiology Laboratory Technicians are responsible for reviewing and adhering to general safety guidelines while working in the laboratory. This includes:

- Maintaining a working knowledge of the Region 1 Microbiology Laboratory LSM.
- Performing work in strict accordance with LSM procedures and guidelines.
- Reporting all incidents and hazards to the Region 1 Microbiology Laboratory Director or Principal Analyst immediately.
- Knowing the locations of all first-aid kits, fire extinguishers, spill kits, fire exits, Material Safety Data Sheets (MSDS), and other relevant safety devices.
- Only operating laboratory equipment for which the individual is qualified and has been trained to use.

A4. Regulations, Guidelines, and Permit Requirements

The following agencies regulate laboratory activities and provide guidance and direction concerning the use of chemicals in the Region 1 Microbiology Laboratory.

The **Occupational Safety and Health Administration (OSHA)** develops and enforces regulations based on federal statutes. OSHA regulates health and safety in the workplace, establishes chemical exposure limits, and sets minimum standards for work place health and safety (www.osha.gov).

The **Environmental Protection Agency (EPA)** develops and enforces environmental regulations to protect human health and the environment. The EPA regulates hazardous waste, machinery emissions, waste water, storm water, and other hazardous materials in an effort to reduce their impact on the environment (www.epa.gov).

The **National Institute for Occupational Safety and Health (NIOSH)** is a research division of the Centers for Disease Control and Prevention created by the Occupational Safety and Health Act

of 1970. NIOSH conducts research, makes recommendations for the prevention of work related illness, and publishes sources of chemical toxicity information (www.cdc.gov/niosh).

The **National Fire Protection Association (NFPA)** provides codes and standards for fire safety, chemical storage, egress, and laboratory engineering controls for laboratories using chemicals (www.nfpa.org).

The **United States Department of Transportation (DOT)** regulates packaging, shipping, and documentation of hazardous materials during transportation and distribution including shipping and receiving (www.dot.gov).

A5. Introduction

The Regional Water Quality Control Board – North Coast Region (Region 1) Microbiology Laboratory is an environmental analytical laboratory located in Northern California, in the City of Santa Rosa. The Laboratory is located at 5550 Skylane Blvd Suite A Santa Rosa CA 95403-1072, housed in a single room within the Region 1 office building. The primary role of the laboratory is to perform bacterial water analyses for Regional Board regulatory, monitoring, surveillance, enforcement, and planning programs.

B1. Laboratory Safety Manual (LSM)

A thorough safety manual is essential for the safety and well-being of all persons working in and around the laboratory. This LSM covers the general safety policy of the Region 1 Microbiology Laboratory, which is committed to safe working conditions and practices. These policies are based on the national consensus standard and guidelines for general lab safety, as well as OSHA, EPA, NIOSH, NFPA, and DOT standards.

Objective

The objective of this LSM is to establish an effective and efficient safety management system that will ensure the health and safety of Region 1 Microbiology Laboratory staff and property, and reduce the likelihood of hazards and incidents associated with laboratory functions.

Approval

The Region 1 Microbiology Laboratory Safety Officer is responsible for preparing the LSM. After review and approval by the Co-Laboratory Directors, the QAP is incorporated as a laboratory control document and is distributed to appropriate laboratory personnel. The LSM has approval signatures of the Executive Officer, Co-Laboratory Directors, Principal Analyst, and the Safety Officer. The LSM will be reviewed annually and revisions are made to ensure its effectiveness. A document name, version number, revision date, and page number are shown on the cover page as well as on each page.

Updates and Distribution

All originals of the first and subsequent amended LSM will be held at the Region 1 Microbiology Laboratory by the Laboratory Director. Updates to this LSM will be distributed to the appropriate personnel and previous versions will be discarded. The Laboratory Director under the direction, supervision, and review of the Safety Officer, will be responsible for distributing an updated version of the LSM. Any future amended LSM will be held and distributed in the same fashion.

Activities of the LSM

In order to accomplish the LSM objective, the following activities are incorporated:

- Ensure and document that all laboratory personnel have read and thoroughly understand the LSM.
- Maintain safety integrity by regularly monitoring and confirming that all staff are adhering to laboratory safety guidelines.
- Document all activities and potential hazards associated with laboratory safety.
- Immediately address concerns raised by staff concerning laboratory safety issues.
- Ensure that staff have all necessary resources to maintain the safety standards set forth in the LSM.

- Ensure that personal safety systems are maintained in working condition such as fume hoods, spill kits, first aid kits, fire extinguishers, emergency eyewash and shower stations, personal protective equipment, etc.
- Ensure that staff is trained and knowledgeable in the use of safety systems.

Basic Elements of LSM

The LSM consists of the following three basic elements:

- **Prevention**
  Prevention requires a critical evaluation and assessment of potential hazards and safety concerns within the laboratory environment.

- **Assessment**
  Assessment is a form of control that includes periodic checks on performance to determine effectiveness and protection level.

- **Correction**
  Correction is an action taken to remedy an existing or potential safety hazard, and to reduce the probability of reoccurrence.

B2. Assessment and Management of Risk

Accurate assessment of the risks associated with laboratory operations and the implementation of measures to effectively manage those risks are critical components of laboratory safety. Risk assessment focuses primarily on the prevention of laboratory-associated exposure to physical, chemical, and biological hazards. Risk management is the application of appropriate administrative, engineering, and physical controls to reduce the potential for accidental exposure or release to the environment. The assessment and management of risk is an ongoing process and must be continually evaluated to reflect changes in the quantity or type of hazardous substances present in the laboratory, types of procedures to be performed, and current regulations and recommendations from government agencies regarding safe laboratory practices.

Risk Assessment

The Region 1 Microbiology Laboratory Directors perform risk assessments that consider the types of hazards present in the laboratory, the risk of exposure to laboratory personnel, and the type of work to be performed. Prudent planning is a critical component of risk assessment. The following factors should be considered when determining the risk associated with a particular project or procedure:

- Hazards associated with the procedure.
- Potential for a harmful personal exposure to occur.
- Potential for release of a hazardous substance to the environment.
- Level of training and experience of personnel.

- Use and condition of laboratory equipment.
- Availability of safety equipment such as chemical fume hoods and/or biosafety cabinets.
- Appropriate Personal Protective Equipment.
- Type and volume of hazardous substances used and waste generated.
- Proper storage.
- Potential for production of harmful byproducts.
- Appropriate response procedures in the event of an emergency.

Exposure to hazardous substances can occur through inhalation, ingestion, contact with or absorption through skin or mucous membranes, or through injury. When evaluating laboratory procedures, the Region 1 Microbiology Laboratory Directors shall consider likely routes of exposure to hazardous substances used in the laboratory, safety precautions and equipment (such as Personal Protective Equipment and chemical fume hoods) that can be utilized to minimize the risk of exposure, and exposure response procedures to be implemented in the event of an exposure.

Risk of injury due to physical hazards (e.g., thermal, electrical, mechanical) shall also be evaluated. Attention will be given to the location of physical hazards and the availability of proper safeguards. In addition, good housekeeping practices and routine equipment maintenance shall be implemented to prevent injuries resulting from trip hazards, frayed wires, malfunctioning equipment, or damaged instruments.

Risk Management

Risk management involves the use of measures designed to reduce potential exposure of laboratory personnel, the community, and the environment to hazards present in the laboratory. A comprehensive risk management program includes administrative, engineering, and physical controls that reduce the duration, frequency, and severity of exposure to laboratory hazards. Administrative controls include written safety procedures and practices, training, documentation, access restrictions, and proper signage and labeling. Engineering controls include facility features such as laboratory design, ventilation systems, storage areas, and safety equipment. Physical controls are provided by Personal Protective Equipment and good chemical hygiene practices.

B3. Administrative Controls

Administrative controls are precautionary measures implemented to reduce the risk of accidents in the laboratory through training, signage and labeling, record keeping, and surveillance.

Training

Laboratory personnel, students, support services staff, and visitors entering laboratories or laboratory support rooms are required to receive safety training commensurate with their level of participation in laboratory activities and the duties they are to perform. The Region 1 Microbiology Laboratory Director offers training in chemical safety and biological safety as well as specialized training for specific laboratory procedures and hazards. Other types of training may be required (e.g., Autoclave Equipment Training, Fire Extinguisher Training, and first aid).

Chemical Safety Training

All personnel working in laboratories or facilities where chemicals are used or stored must receive chemical safety training before starting work in the laboratory. Training familiarizes individuals with common chemical hazards, and safe practices for acquisition, use, storage, and disposal of chemicals.

Laboratory Security and Access

The Region 1 Microbiology Laboratory contains hazardous substances that can pose a serious danger to public health if handled by untrained personnel or removed from the laboratory. In addition, laboratories contain expensive instruments and equipment that must be protected from unauthorized use, vandalism, and theft. Therefore, it is imperative that appropriate security precautions are implemented to prevent unauthorized individuals from gaining access to laboratory materials and equipment. The following security procedures must be followed:

- Identify potential security risks in the laboratory (e.g., laboratory doors left open, doors left unlocked when the laboratory is unattended, or unsecured hazardous substance storage areas).
- Develop and implement laboratory security procedures to prevent unauthorized entry to the laboratory and access to hazardous substances.
- Develop and implement laboratory access restrictions to protect the health and safety of individuals entering the laboratory.
- Train laboratory personnel to implement security procedures.
- Keep doors closed at all times and locked when no authorized personnel are present.
- Do not leave hazardous substances unattended or unsecured at any time.
- Restrict access to freezers, refrigerators, storage cabinets, and other equipment where hazardous substances are stored.
- Limit laboratory access to approved laboratory personnel who are properly trained with regard to the hazards present in the laboratory and the type of work they will perform.
- Escort visitors to and from the laboratory.

Signs and Labels

Signs and labels are used to clearly identify specific laboratory hazards, safety equipment, emergency supplies, critical information, and designated areas within the laboratory. The following signage requirements apply to the Region 1 Microbiology Laboratory.

Emergency Contact Information

The Region 1 Microbiology Laboratory posts emergency contact information near each laboratory entrance sign, exit and above first aid kits. The following information should be provided: office phone number of the Region 1 Microbiology Laboratory Director responsible for the laboratory, after hours contact information, and Fire Department.

Figure 2: NFPA Safety Diamond

Legend:

Blue (Health)	**Red** (Flammability)	**Yellow** (Reactivity)	**White** (Specific Hazard)
4 –Deadly	4 –Very flammable	4 –May detonate	OXY-Oxidizer
3 –Extreme danger	3 –Readily ignitable	3 –Shock and heat may detonate	ACID-Acid
2 –Hazardous	2 –Ignited with heat	2 –Violent chemical change	ALK-Alkali
1 -Slightly hazardous	1 –Combustible	1 -Unstable if heated	COR-Corrosive
0 -Normal materials	0 -Will not burn	0 –Stable	Water reactive

Labeling Equipment

The following items must be identified with labels or signage:

- Safety Equipment
- Emergency shower
- Eye wash station
- First aid supplies
- Fire extinguishers
- Spill supplies
- Designated areas for work
- Chemical storage areas

Laboratory Equipment

Broken equipment that is not operational must be taken out of service and labeled to prevent further use by laboratory personnel. Notify the Region 1 Microbiology Laboratory Director and Safety Officer immediately of broken or malfunctioning safety equipment (i.e., fume hoods, cabinets, emergency shower, etc.).

Additional Signage

All laboratory equipment (e.g., refrigerators, freezers, centrifuges, and incubators) and waste disposal containers in which biohazardous material are used or stored must be labeled to indicate

the type of hazard present. For biohazardous materials, the label must contain the universal symbol for biohazard (Figure 3) and the word "Biohazard". Labels should be affixed to the container or as close as possible to the container using string, wire, adhesive, or any other method that prevents their loss or unintentional removal.

Figure 3: Universal Symbol for Biohazard

Required Safety Records

The Region 1 Microbiology Laboratory Director maintains records regarding laboratory safety and compliance. Records are kept at the Region 1 Microbiology Laboratory office where they are available to laboratory personnel and inspectors. To facilitate record keeping, wall bins are in the laboratory where safety manuals, the MSDS Binder, and safety and compliance records may be kept.

MSDS (Material Safety Data Sheet)

Location of MSDS Sheets

MSDS sheets are kept for every hazardous material kept inside the Region 1 Microbiology Laboratory. These sheets are bound in a marked, yellow notebook, and located in the Region 1 Microbiology Laboratory. Personnel should take the time to read over the MSDS sheets to become familiar with the hazards associated with laboratory chemicals and reagents.

Using MSDS Sheets

The MSDS for each hazardous material in the laboratory details the use, handling, storage, and hazard of the material.

- **Chemical Identification**
  Identifies the material and lists the name of the chemical and the manufacturer's name and address. This section may also list an emergency phone number.

- **Hazardous Ingredients**
  Describes the hazard associated with the material. It also lists the Permissible Exposure Limit (PEL), which is the maximum amount of safe exposure to the material for a human being. These safe exposure limits are usually figured for average exposures over a typical work shift.

- **Physical Data**
  This section describes the chemicals appearance, odor, and other characteristics.

- **Fire and Explosion Data**
  Details at what temperature the chemical ignites; also called the "flashpoint". If a chemical ignites below 100°F, it is considered to be flammable. A chemical is considered combustible if it ignites at 100°F or above. Also lists what material/method will put out the fire safely- such as water spray, foam, or other type of fire extinguisher.

- **Health Hazards**
  This section lists symptoms of overexposure, such as a skin rash, burns, headache, nausea, or dizziness. It may also list any medical conditions that can be aggravated by exposure to the chemical.

- **Reactivity Data**
  Describes incompatibility with other materials that may cause the chemical to burn, explode, or release dangerous gases. Instabilities of the material are also listed, such as exposure to heat or direct sunlight, which may result in a dangerous reaction such as an explosion.

Laboratory Inspections

The Region 1 Microbiology Laboratory is periodically inspected by federal, state, and local agencies. These regulatory agencies may visit the Region 1 Microbiology Laboratory at any time, with or without prior notification, to assess safety and compliance. During these visits, inspectors may ask to examine laboratories, question laboratory personnel, and examine laboratory records. The Region 1 Microbiology Laboratory Directors and Safety Officer routinely inspect the laboratory. Inspections are performed in accordance with government regulations and are used to address safety issues identified in the laboratory. Inspections also serve to prepare the laboratory for inspections from outside agencies.

The Region 1 Microbiology Laboratory Directors and Safety Officer inspects the laboratory access and security; housekeeping; signage and labeling; safety equipment; spill supplies; operation and certification of chemical fume hoods, cabinets, and glove boxes; chemical segregation and storage; waste handling procedures; and laboratory records.

Following an inspection, any violations of safety must be dealt with immediately.

The Region 1 Microbiology Laboratory Director and Safety Officer has the authority to close the laboratory or discontinue certain activities when there is an immediate or imminent threat to human health, property, or the environment.

B4. Engineering Controls

Engineering controls are facility features and equipment intended to reduce the likelihood or severity of an exposure. This includes laboratory design, safety equipment, and safety guards on laboratory equipment.

Laboratory Design

Appropriate design and traffic flow is critical to the development of a safe work environment for laboratory personnel.

(Ventilation, Indoor Air Quality, Heating, and Cooling) (currently undefined).

Fire Safety

The Region 1 Microbiology Laboratory should meet the requirements of the National Fire Protection Association (NFPA) NFPA-45 Standard on Fire Protection for Laboratories Using Chemicals.

- Passageways and aisles must be a minimum of 36 inches wide and must remain unobstructed.
- The location of emergency exits for the laboratory must be clearly marked.
- Emergency exits must be clearly marked.
- Doors must remain unobstructed.

Lighting

Region 1 Microbiology Laboratory is equipped with adequate glare-free lighting.

Region 1 Microbiology Laboratory is equipped flashlight to provide sufficient lighting to assist personnel in evacuating the laboratory.

Floors

Region 1 Microbiology Laboratory follows the recommendations for laboratory flooring based on OSHA and NFPA regulations:

- Floors in laboratory are sufficiently reinforced to support the equipment present.
- Floors in laboratory are made of durable material that requires little maintenance and is resistant to chemical spills.

Laboratory Bench Tops

- Laboratory bench tops are constructed of chemically resistant and flame retardant synthetic materials.

- Bench tops are capable of supporting the weight of equipment. Sufficient space is provided for activities and equipment placement.

Sinks

Region 1 Microbiology Laboratory has a fully functional sink with a drain and pressurized water. The area in and around laboratory sinks are kept clean and uncluttered so that the sink may be used by personnel to wash their hands before exiting the laboratory.

Safety Equipment

Region 1 Microbiology Laboratory has easy access to the following safety equipment:

- Emergency showers and eye wash stations
- First aid supplies
- Spill supplies
- Fire suppression equipment

The Region 1 Microbiology Laboratory Director is responsible for providing safety equipment to laboratory personnel, routinely inspecting equipment, and repairing or replacing if necessary.

Emergency Showers and Eye Wash Stations

An emergency shower and eye wash station is available within in a ten second walk from each area where hazardous substances are used, clearly labeled, and easily accessible. All laboratory personnel must know the location of the nearest shower and eye wash stations and must be trained in their use. Emergency showers are designed to provide immediate response to chemical exposures that cover a significant part of the body. Eye wash stations are designed to provide a soft stream of aerated water to rinse the eye. Eye wash stations are capable of providing water for at least 15 minutes without interruption. Once the flow has begun, hands should be free to hold the eyelids open to better expose eyes to the rinsing action of the water.

Emergency showers and eye wash stations are installed, maintained, flushed, and tested in accordance with the American National Standards Institute (ANSI) Standard for Emergency Eye Wash and Emergency Shower Equipment (ANSI/ISEA Z358.1-2009). Both emergency showers and eye wash stations are flushed every two weeks to verify that they are operating properly and the effluent is clear.

Fire Suppression Equipment

An ABC type fire extinguisher is available near the laboratory exit. The fire extinguisher is clearly labeled and readily accessible for use and inspection. Once discharged, it must be serviced by a qualified technician or replaced.

First Aid and Spill Supplies

Region 1 Microbiology Laboratory is equipped with first aid supplies to assist laboratory personnel in responding to minor injuries and spill supplies relevant to the activities of the laboratory. These supplies are clearly marked, easily accessible, and located near the laboratory exit. All laboratory personnel must know the location of these supplies. Supplies should be routinely inspected and replaced as necessary.

C1. Personal Protective Equipment (PPE)

PPE must be provided to and worn by all Region 1 Microbiology Laboratory personnel, students, and visitors, when working in a laboratory. At a minimum, a lab coat, gloves, eye protection, and closed-toed shoes are required. In some instances, additional protection may be necessary. MSDS provide specific PPE recommendations for handling chemicals. PPE should be durable, designed to provide adequate protection, and capable of preventing exposure to hazardous substances. PPE must be removed before leaving the laboratory.

While PPE is an important component of a comprehensive laboratory safety program, it is not a replacement for good laboratory practices, administrative controls, engineering controls, and safety equipment. PPE is most effective when used in conjunction with good laboratory practices, administrative controls, engineering controls, and safety equipment. OSHA requires the use of PPE to reduce employee exposure to hazards when engineering and administrative controls are not feasible or effective in reducing these exposures to acceptable levels.

Personal Attire

Personal attire must be considered when working in the Region 1 Microbiology Laboratory, as clothing, accessories, and hair may be entangled in equipment, or accidentally spill substances unintentionally. Proper personal attire includes clothing that provides adequate coverage for the legs and close-toed footwear that provides adequate support and has suitable traction for laboratory activities. Hair should be confined or tied back. The following will **NOT** be worn in the laboratory: loose sleeves, dangling jewelry, clothing that leaves the legs exposed, or shoes with heels greater than one inch.

Eye Protection

Eye protection must be worn when working with substances or equipment that present a hazard to the eye. Eye protection must meet design requirements set forth by (ANSI Z87.1-2010) and must be appropriate for the activity being performed.

Safety glasses should fit securely and be free of smudges or scratches that may obstruct vision. Safety glasses equipped with side shields provide more complete protection than those without. Safety goggles provide an increased level of protection and should be worn when splashes may occur or glassware may explode/implode under pressure.

Contact lenses should not be worn when working in the laboratory because chemical vapors can permeate the lenses and become trapped on the surface of the eye. For individuals who wear contact lenses or glasses, safety goggles are recommended instead of safety glasses because of the additional protection goggles provide.

Face Shields

Face shields are designed to be used in combination with safety goggles to provide additional protection to the face and eyes against splashes and particulate matter. Face shields do not provide adequate protection against large projectiles or liquids, unless they are used in combination with

safety goggles. Polycarbonate face shields that offer protection against ultraviolet (UV) radiation should be worn when using instruments that produce UV light.

Safety Gloves

Safety gloves should always be worn when working with chemicals even if the chemical containers are tightly closed or the experiment being conducted is within a closed system. Gloves should be comfortable, of sufficient length to prevent exposure of the hand and wrist, and should be appropriate for the type of work to be performed. Gloves should be inspected for visible tears before use, changed when they become soiled or compromised, and discarded appropriately after use.

Safety gloves come in a variety of materials that provide different levels of protection. Laboratory personnel should use gloves that provide the highest level of protection against the substances to be used. Some individuals develop allergies to the materials used to manufacture safety gloves. If this occurs, a comparable alternative will be made available.

Lab Coats and Aprons

Lab coats should cover the entire upper body, extend to the knees, and fit comfortably without hanging too loosely from the arms. Only single use disposable lab coats or lab coats that are routinely laundered by an approved vendor may be used. Lab coats may not be laundered by laboratory personnel. Lab aprons are designed to be worn in combination with a lab coat to provide extra protection when pouring corrosive chemicals, using an acid bath, or manipulating chemicals in a manner that increases the likelihood for splashes or spills. Lab aprons should fit comfortably and extend from just below the neck to just above the tops of the feet.

D1. Chemical and Microbiological Hazards

The hazardous nature of a chemical is determined by the potential for the chemical to cause adverse health effects (toxicity) and the physical hazards inherent to the properties of the chemical (e.g., flammability, reactivity).

Chemical Toxicity

The toxicity of a chemical is the ability of that chemical to cause a reproducible dose-dependent effect on a biological system. The conditions of exposure and the susceptibility of the exposed individual influence the types of toxic effects that occur.

Conditions of Exposure

The physical and chemical properties of a chemical, route of entry, dose, and the frequency and duration of exposure are important factors to consider when assessing chemical exposure.

Physical and Chemical Properties of Chemical Substances

The physical and chemical properties of a chemical affect how it is absorbed by the body and the rate of absorption. As the ability for a toxic chemical to be absorbed increases, the potential for toxic effects to be observed also increases. After absorption, the physical and chemical properties affect whether the chemical is transported throughout the body to cause systemic effects or metabolized to a metabolite (which may be more or less toxic), and whether the chemical (or its metabolite) is stored in the body or excreted. Physical characteristics of chemical substances that affect the ability of a substance to be absorbed include the physical state of the substance (gas, liquid, or solid) and the size of solid and liquid particles. Gas particles are typically absorbed via inhalation. Liquid and solid particles may be absorbed through the skin or eyes, or ingested. In addition, very fine liquid or solid particles (dusts or aerosols) may be inhaled. The size of the particles determines the depth at which these particles deposited within the lungs: smaller particles may be deposited deep within the lungs.

Chemical properties that affect the ability of a substance to be absorbed include lipid solubility, water solubility, and the pH of the chemical. Lipid soluble substances cross biological membranes and are easily absorbed through the skin or eyes. Water soluble substances are absorbed rapidly in the lungs. Strong acids and bases typically react at the site of contact and cause localized effects as opposed to being absorbed through the skin.

Microbiological Contamination Hazard

At any given time there can be multiple infectious substances present in the Region 1 Microbiology Laboratory refrigerator:

- E. Coli
- enterobacter aerogenes
- pseudomonas aeruginosa

- enterococcus faecalis
- klebsiela pneumoniae

Route of Entry

The route of entry is the path by which a toxicant enters the body. The type of toxic effects that are observed and their time of onset are affected by the route of entry. A chemical that is ingested may cause different toxic effects than if it was absorbed through the skin. Depending on the properties of the chemical, upon ingestion it may undergo metabolism or be absorbed into the bloodstream through the lining of the gastrointestinal tract. If absorbed through the skin, the chemical may remain locally in the tissues surrounding the point of contact or enter the bloodstream and be circulated throughout the body.

The route of entry may also determine whether local or systemic effects are observed. Irritation, a local effect, is observed at the site of contact. Systemic effects are delayed because they occur on target organs that may be located far from the site of contact. For systemic effects to occur, the toxicant must be transported through the bloodstream from the site of entry to the target organ.

All Region 1 Microbiology Laboratory personnel working with chemicals and pathogens must be aware of possible routes of entry and should implement procedures and practices that reduce their risk of exposure.

Skin and Eye Contact

A common way for chemicals to enter the body is through direct contact with the skin or eyes. Skin contact with a chemical may result in a local reaction, such as a burn or rash, or the chemical may be absorbed into the bloodstream and cause systemic effects at distal sites in the body.

Inhalation

Inhalation is the most common route of entry for chemical vapors, particulates, and aerosols. The term aerosol refers to liquid and solid particles suspended in a gaseous medium. Aerosols can contain droplets of hazardous chemicals, dust, fumes, biological materials, or other hazardous substances, and can remain suspended in the air for long periods of time. Small aerosol particles, if inhaled, may penetrate deep within the respiratory tract. The following activities can produce aerosols: centrifugation, homogenization (e.g., use of a blender, grinder, or mortar and pestle), mixing, vortexing or stirring, use of a separatory funnel, and pipetting. Inhaled substances may cause localized effects on the lungs or be absorbed into the bloodstream, causing systemic effects.

Ingestion

Ingestion is another possible route of entry. Although direct ingestion of a laboratory chemical or pathogen is unlikely, an individual may ingest contaminated food or beverages, touch the mouth with contaminated fingers, or swallow inhaled particles which have been cleared from the respiratory system. Direct ingestion may occur as a result of the outdated and dangerous practice of mouth pipetting. The risk of ingesting hazardous chemicals may be reduced by not eating,

drinking, smoking, applying cosmetics, or storing food in the laboratory, and by washing hands thoroughly after working with chemicals, even when gloves were worn.

Percutaneous Exposure

Percutaneous exposure may result from a needle stick; puncture with a contaminated sharp object, or through wounds, abrasions, and eczema. In the case of percutaneous exposure, the chemical may enter directly into the bloodstream and cause both local and systemic effects.

Dose

Dose is the amount of a toxic substance that is absorbed by an individual. Dose is reported in milligrams (mg) of toxicant per kilograms (kg) of body weight (mg/kg) for acute exposures and in mg/kg per day for repeat-dose exposures.

All chemicals have the potential to cause toxic effects. The dose to which an individual is exposed over time determines whether toxic effects occur and the severity of the effects. For a chemical to have a toxic effect, it must first come in contact with or be absorbed by the body. Metabolism, storage, and excretion may protect an individual from experiencing adverse effects at lower doses.

Frequency and Duration of Exposure

Frequency and duration of exposure affect the types of adverse effects experienced. An acute exposure is characterized by a single exposure to a relatively high dose of a hazardous chemical with a short duration of exposure. Immediate or delayed effects of acute exposure are more severe but may be reversible if exposure ceases. Repeat exposure to a hazardous substance, even at doses below which acute effects are observed, may result in long term adverse health effects due to bioaccumulation of the toxic chemical. Bioaccumulation occurs when an individual is exposed to a toxic chemical and then exposed again before recovering from the previous exposure by means of metabolism or excretion.

Individual Susceptibility to Hazardous Chemicals

Individual susceptibilities play a significant role in the effects observed as a result of exposure to hazardous chemicals. Most chemicals have an odor that is perceptible at a certain concentration, referred to as the odor threshold. However, there is considerable individual variability in the perception of odor. Laboratory personnel allergic to a sensitizing agent or allergen may experience adverse effects while those who are not allergic may not experience adverse effects at all. In addition, the health of an employee or simultaneous exposure to other hazardous substances may exacerbate the effect.

All Region 1 Microbiology Laboratory personnel should be familiar with the health hazards associated with toxic chemicals they use.

Toxic Effects Due to Chemical Exposure

During the course of their work, laboratory personnel may be exposed to small doses of chemicals that do not have the potency to generate an immediate effect on the senses. Over time, this exposure may cause discomfort and the development of exposure symptoms. All laboratory personnel should be able to recognize the following signs of a chemical exposure:

- Headaches
- Difficulty breathing or shortness of breath
- Increased mucous production
- Irritation or watering of the eyes
- Irritation of the nose or throat
- Confusion, dizziness, drowsiness, or loss of consciousness
- An unfamiliar chemical odor
- Irritation, rash, or discoloration of the skin
- Unusual muscle cramps or joint pain
- Nausea

If any of these symptoms occur, Region 1 Microbiology Laboratory personnel should notify other personnel, evacuate the laboratory, and discontinue work until proper arrangements are made to prevent exposure. Laboratory personnel should be aware of the toxicity of the chemicals used in the laboratory and precautions that should be implemented to prevent exposure. Information about the toxicity of a chemical may be found in the MSDS provided by the manufacturer. A mixture of toxic chemicals should be considered more toxic than its most toxic component.

Irritation

Irritation to toxic substances may occur as a result of dermal, ocular, or inhalation exposure. Laboratory personnel may experience localized irritation as a result of exposure and absorption of the hazardous chemical may also result in systemic health effects. Examples of irritants include ammonia, hydrochloric acid, halogens, sulfur dioxide, acetic acid, and formaldehyde.

Sensitization

Sensitization is an immune response to hazardous substances in susceptible individuals. Physiological responses to these substances vary from person to person, ranging from skin disturbances to anaphylactic shock or even death. It is possible to be allergic to a variety of substances and chemicals. The MSDS indicates whether a chemical is known to be a sensitizer.

Individuals who are sensitized to a chemical, experience a relatively normal reaction to a sensitizing agent the first time they are exposed to the agent. The initial reaction may include irritation at the site of contact if the chemical is known to be an irritant. Subsequent exposure to the sensitizing agent or to a structurally similar agent will induce an allergic response. The allergic reaction may be observed at concentrations below which prior exposure did not result in adverse effects. Subsequent exposure to the sensitizing agent (or one that is structurally similar) typically results in a progressively severe allergic response. A few examples of laboratory substances that

cause allergic reactions include metals (platinum, nickel, chromium, beryllium, cobalt), latex, and formaldehyde.

Flammable Chemicals

Liquids with a flashpoint less than 60°C (140°F) are considered flammable chemicals. The flashpoint of a chemical is the temperature at which the vapor of the chemical is capable of being ignited momentarily. Alcohols and organic solvents are the most common flammable chemicals used in the laboratory. To safely manage flammable liquids, consult the MSDS and adhere to the recommended storage and usage procedures outlined in the MSDS.

Oxidizing Chemicals

An oxidizing chemical will cause a substantial increase in the burning rate of a combustible material with which it comes in contact; undergo vigorous self-sustained decomposition when catalyzed or exposed to heat; or cause spontaneous ignition of a combustible or flammable chemical with which it comes in contact. Strong oxidizing chemicals will react with solvents, wood, and fine metal powders. Examples of strong oxidizers include: some strong acids, perchlorates, nitrates, permangenates, persulfates, and peroxides. To safely manage oxidizing chemicals, consult the MSDS.

Corrosive Chemicals

Corrosive chemicals are acids and bases that cause severe tissue damage at the site of contact. These chemicals can burn the skin, cause severe bronchial irritation, or blindness.

Strong acids are chemicals with a pH less than two (e.g., butyric acid, formic acid, glacial acetic acid, hydrochloric acid, nitric acid, sulfuric acid, perchloric acid, and phosphoric acid). Concentrated acids react violently with bases, and can react with other acid sensitive chemicals (e.g., alkali metals, hydroxides, carbonates, carbides, arsenic, cyanides, sulfides, and most metals) to produce heat or dangerous gases. Acids pose the additional hazard of being very slippery when spilled. Strong basic or caustic chemicals have a pH greater than 12.5 (e.g., sodium hydroxide, potassium hydroxide, amines, and ammonium hydroxide). Basic chemicals react dangerously with acids and oxidizing chemicals and must be segregated from these chemicals. For example, when ammonium hydroxide and sodium hypochlorite (bleach) are mixed, chlorine gas is released. To safely manage corrosive chemicals, consult the MSDS and adhere to the recommended storage and usage procedures outlined in the MSDS.

Dry Ice

Dry ice sublimates to a carbon dioxide. Carbon dioxide is a colorless odorless gas which is heavier than air and can accumulate in poorly ventilated areas. Carbon dioxide is a simple asphyxiate, a chemical that displaces oxygen and may create an oxygen-deficient atmosphere when present in high concentrations. Direct contact with dry ice can cause severe burns. Dry ice should not be stored in refrigeration units.

E1. Chemical Storage

The Region 1 Microbiology Laboratory has adequate chemical storage areas that provide sufficient and defined barriers between incompatible chemicals. Information on proper chemical storage can be found in the MSDS for each chemical.

Follow these guidelines when storing chemicals:

- Storage areas are well ventilated and located away from sunlight and ignition sources.
- Chemicals are stored in cabinets constructed of synthetic, chemically resistant materials or on metal shelving.
- Cabinets are easily accessible and clearly labeled as chemical storage areas.
- Chemicals are stored below eye level.
- Solids are stored above liquids.
- Chemicals are segregated by chemical compatibility.
- Chemical containers are clearly labeled using the complete chemical name. Chemical formulas or abbreviations are not sufficient.
- Only limited quantities of chemicals should be stored in the laboratory.
- Storage areas are inspected frequently to identify deteriorating containers and faded or missing labels.

Container Labeling

OSHA requires that each chemical container, regardless of size or use, be properly labeled with the complete chemical name (formulas, abbreviations, and sketches of the molecule are not acceptable), manufacturer information (if the chemical is in its original container), appropriate hazard information, and the date received (for ordered chemicals) or the date generated (for chemical dilutions and experimental samples). Reaction vessels, beakers, squeeze bottles, flasks, and laboratory equipment that contain chemicals must be labeled as to their contents. Non-soluble, non-erasable ink should be used to label containers, and labels should be securely attached to the side of the container. Labels affixed to container lids or stoppers are not reliable for identifying chemicals because lids may inadvertently be switched during use.

Unlabeled containers must be assumed to contain hazardous components until the contents can be identified.

Chemical Compatibility and Segregation

To prevent unwanted or dangerous chemical reactions, chemicals must be stored according to compatibility. Chemicals of the same hazard class that share the same characteristics may be stored together. Incompatible chemicals must be segregated. MSDS and container labels provide useful information regarding compatibility and storage requirements. Container labels may provide hazard symbols or list the hazards associated with the chemical (e.g., flammable, oxidizer, poison, toxic, corrosive, or reactive).

Chemical segregation can be accomplished using shelves, bins, cabinets, and other secondary containment equipment. Another way to reduce the potential for reactions between chemicals is to prevent contact by proximity. Storing solid oxidizing compounds on the opposite side of the laboratory from flammable liquids nearly eliminates the possibility of contact. Acids and bases can be separated from one another by means of a divider or wall within a corrosive cabinet.

Flammable Chemical Storage

Flammable liquids are liquids with a flashpoint less than 60°C (140°F). The guidelines below should be followed to safely store flammable chemicals:

- Flammable chemicals are stored in ventilated flammable storage cabinets.
- When refrigerating flammable chemicals, use a flammable chemicals refrigerator that meets Underwriters Laboratory, Inc. (UL) design requirements.
- Keep flammable storage areas away from electrical equipment, heat, oxidizing chemicals, and ignition sources.
- Store flammable chemicals in their original container or in a metal safety can.
- Do not keep more than three flammable storage cabinets in a laboratory, unless they are separated by 100 feet or more.
- Do not store more than 60 gallons of flammable chemicals in a given flammable cabinet.
- Do not store more than 10 gallons of flammable chemical outside of a flammable cabinet unless safety cans are used. If safety cans are used, 25 gallons of flammable chemicals may be stored outside of the flammable cabinet.

Oxidizing Chemical Storage

An oxidizing chemical is a chemical that will cause a substantial increase in the burning rate of a combustible material with which it comes in contact; undergo vigorous self-sustained decomposition when catalyzed or exposed to heat; or cause spontaneous ignition of combustible or flammable chemical with which it comes in contact. Strong oxidizing agents will react with solvents, wood, and fine metal powders. The guidelines below should be used for safely storing oxidizing chemicals:

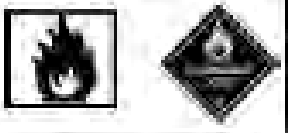
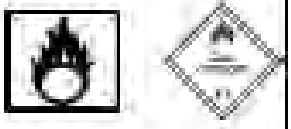
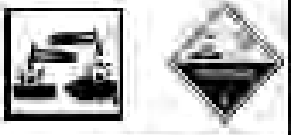
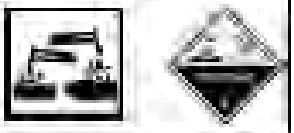
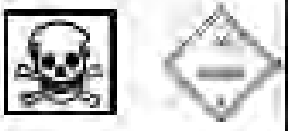
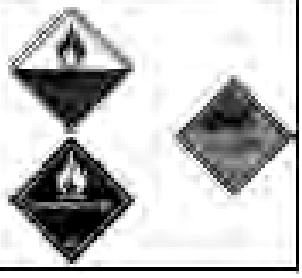
- Clearly mark the storage area where oxidizing chemicals are stored with the words: “Oxidizers”, “Oxidizing Chemicals.
- Do not store oxidizing chemicals with acids, bases, reactive chemicals, or flammable chemicals.

Corrosive Chemical Storage

Corrosive chemicals include acidic and basic chemicals. The guidelines below should be used to safely store corrosive chemicals:

- Do not store acids and bases together.
- Do not store acids with any other chemicals.

Figure 4: Chemical Hazard Classes for Chemical Storage

Chemical Hazard Class	Incompatible Material	Hazard Symbols
Flammable Materials Materials with a flashpoint less than 60° C (140° F). Examples: hexane, xylene, ether, toluene, silanes, acetone, solvents, alcohols, and ketones.	Oxidizing materials, Acids, Toxic materials, Reactive materials	
Oxidizing Materials that readily release oxygen or oxidize surrounding compounds. Examples: nitrates, nitrites, peroxides, persulfates, perchloric acid, nitric acid red, and chromic acid.	Flammable materials, Bases, Acids, Reactive materials	
Acids Materials with a pH less than 2. Examples: hydrochloric acid, nitric acid, butyric acid, formic acid, acetic acid, and phosphoric acid.	Cyanides, Bases, Oxidizing materials, Toxic materials, Reactive materials	
Bases Materials with a pH higher than 12.5. Examples: sodium hydroxide, potassium hydroxide, amines, and ammonium hydroxide solutions.	Acids, Oxidizing materials	
Toxic Materials Materials that are carcinogenic, teratogenic or pose and inhalation hazard. Examples: acrylamides, halogenated materials, ethidium bromide, phenol, chloroform, cyanides, and heavy metals.	Acids, Bases, Flammable materials	
Reactive Materials Materials that react with water/air or spontaneously combust on contact with other chemicals. Examples: metal hydrides, pyrophorics, water reactive material, borohydrides, borane complexes, anhydrides, calcium sodium, and metal powders.	Acids, Bases, Flammable materials, Oxidizing materials	

F1. Chemical Waste Management

Hazardous waste is defined by EPA as any waste material that is ignitable, corrosive, reactive, or toxic, and that "may pose a substantial or potential hazard to human health and safety and to the environment when improperly managed." This includes hazardous chemicals, biological materials, and radioactive materials.

To comply with EPA regulations, laboratory personnel must manage all chemical waste as hazardous waste according to the procedures outlined below. The Region 1 Microbiology Laboratory Director is ultimately responsible for the management of hazardous waste and must implement all relevant waste handling procedures provided in this section. Personnel who have not received this training are not authorized to handle chemical waste.

Waste Container Selection

Containers used to collect waste must be in good condition (i.e., free of cracks, punctures, or other defects), have tightly sealing lids, and be designed for the type of chemical waste generated (i.e., containers are rated to hold a specific volume and weight). If an empty chemical container is used for waste collection, the original label must be completely removed or defaced and the container must be relabeled with a hazardous waste label.

Waste Container Labeling

All chemical waste containers must have a hazardous waste label that specifies the complete chemical name and percent by volume of each constituent. If chemical waste contains biohazardous an additional label that contains the appropriate symbol must be attached to the container and the biohazardous constituents must be itemized.

Figure 5: Universal Symbol for Biohazard

Procedures for Handling Chemical Waste

The following procedures should be used for all chemical waste:

- Never dispose of hazardous waste in the laboratory sink unless authorized to do so.

- Select an appropriate container for the waste and affix a hazardous waste label to the container.
- For liquid waste, use a funnel or spigot to transfer the waste into the container and use secondary containment to catch spills.
- Do not fill waste containers to greater than 90% capacity.

Procedures for Handling Other Laboratory Waste

The following procedures are recommended for disposal of laboratory waste in the sink or domestic trash.

Sink and Domestic Trash Disposal

In limited circumstances, it is appropriate to dispose of substances in a laboratory sink or domestic laboratory trash. Chemicals may not be disposed of in the sink or domestic laboratory trash unless specific guidance and approval.

- Used PPE, paper trash, and other forms of dry laboratory trash that are not contaminated with biological materials or acutely toxic chemicals, may be discarded as domestic trash.
- Empty containers may be disposed of in the domestic laboratory trash or broken glass boxes, so long as they meet the following requirements:
- Containers must not contain any free liquid or solid residue.
- Containers must be triple rinsed prior to disposal.
- All of the manufacturer's warning, shipping, and hazard labels must be defaced, removed, or made otherwise illegible.
- Container lids and caps must be removed.
- Do not purposefully break empty glass containers.

Laboratory Glassware Disposal

Broken glass boxes available in the Region 1 Microbiology Laboratory should be used only to accumulate unwanted, defective, or broken glassware. It is inappropriate to use these containers for anything other than glass waste. Once the broken glass box is approximately 75% full, tape the seams so that the lid is secure. These boxes should be disposed of in Region 1 Microbiology Laboratory dumpsters.

G1. Laboratory Safety Procedures

The most important element of laboratory safety is adherence to good laboratory practices that reduce the risk of exposure to laboratory hazards. All Region 1 Microbiology Laboratory personnel must be trained and proficient in the practices and techniques required for work in the laboratory. The Region 1 Microbiology Laboratory Director is responsible for identifying and adopting practices and procedures designed to minimize or eliminate exposure to laboratory hazards and for training all laboratory personnel. (see Appendix A Region 1 Microbiology Laboratory Safety Manual LSM) The following general safety guidelines should be followed in the laboratory:

- Complete required safety training and specific laboratory training.
- Be familiar with the Laboratory Safety Manual, the location and use of safety equipment, MSDS, and laboratory-specific emergency procedures.
- Know the location of emergency equipment such as chemical spill supplies, emergency showers, eye wash stations, fire extinguishers, and additional laboratory specific supplies.
- Consult the MSDS of the chemicals to be used in order to determine risks associated with the chemical, appropriate PPE, and recommended safety precautions.
- Be familiar with spill response procedures for the substances being used.
- Keep the door to the laboratory closed at all times and locked when the laboratory is not in use.
- Provide warning signs to identify physical hazards (e.g., equipment that operates at extreme temperatures, exposed sharp or moving parts).
- Avoid using combustible, flammable, or reactive chemicals around ignition sources.
- Routinely inspect the laboratory for failing structures such as shelves, chemical storage units, and furniture.
- Implement good laboratory housekeeping practices and maintain a clean and tidy laboratory to prevent chemical accidents and injuries.
- Clean work surfaces regularly.
- Keep floors and access to safety equipment clean and unobstructed.
- Do not store instruments, small equipment (e.g., vacuum pumps, tabletop centrifuges, ring stands) and chemicals on the floor.
- Conduct informal inspections to identify problem areas in the laboratory and notify Region 1 Microbiology Laboratory Director of any safety issues or concerns.
- Make sure the area selected to perform procedures is equipped with the appropriate safety equipment.
- Wear appropriate PPE.
- Confine long hair, loose clothing, and jewelry.
- Do not eat, drink, use tobacco products, apply cosmetics, or store food and beverages in the laboratory.
- Follow proper procedures for labeling and storing chemicals and make sure chemical storage containers are in good condition.
- Use equipment only for its designated purpose.
- Handle glassware carefully. Shield glass apparatus that have the potential to implode or explode.

- Use mechanical pipetting devices to transfer chemicals.
- Never pipette by mouth.
- Do not smell, taste, or touch chemicals to identify, manipulate, or transfer them.
- Avoid activities that might confuse, startle, or distract other laboratory personnel.
- Experiments that require electrical devices should have controls that can automatically shut off the equipment at a determined time or cut power in the event of a spill or accident.
- Handle unknown chemicals as hazardous chemicals until they are properly identified.
- Unknown chemicals must be stored in an appropriate container and labeled as "Unknown".
- Decontaminate work surfaces, instruments, and equipment after each use and immediately after a spill according to recommended decontamination procedures.
- Follow hazardous waste disposal procedures.
- Do not discharge hazardous waste into the sewer system unless specific direction has been given.
- Wash hands after completing work and before leaving the laboratory.
- Leave lab coats and other PPE in the laboratory before exiting.
- Remain alert to unsafe conditions.
- Take steps to rectify unsafe situations and bring laboratory safety issues to the attention of the Region 1 Microbiology Laboratory Director.

Safety Procedures for Using Flammable Chemicals

In addition to the general safety guidelines listed above, the procedures below should be followed when working with flammable chemicals.

- Follow storage procedures for flammable chemicals.
- Know the location of the nearest fire extinguisher and be familiar with emergency procedures.

Safety Procedures for Chemical and Biological Decontamination

After an area has been used for chemical manipulations or is used as a designated area, it must be decontaminated before it may be used for other purposes. The selection of an appropriate decontamination method will depend upon the following:

- Physical, chemical and toxicological properties of the chemical used.
- Type of chemical or surface that is contaminated.
- Location, extent, and amount of contamination.

Follow these guidelines when decontaminating surfaces or equipment:

- Consult the MSDS regarding the physical, chemical, and toxicological properties and hazards of the chemical and for specific decontamination procedures.
- Wear appropriate PPE. At a minimum, safety glasses, gloves, and a lab coat must be worn.
- If a contaminant is volatile or otherwise reactive, neutralize the chemical prior to decontamination.

- Use an appropriate compatible cleaning solution. Most contaminated areas can be cleaned using soap and water. In place of soap and water, a 10-20% solution of ethanol may be suitable.
- Work from the outside of the contaminated area, cleaning inward using a series of concentric circles.
- Decontaminate all tools, equipment, and surfaces that come in contact with the contaminant before they are reused, repaired, or discarded.
- Decontaminate laboratory equipment according to the procedures provided above.
- Collect all contaminated chemicals (PPE, absorbent chemical, and debris) in a sealed container or bag.

Disinfection

Disinfection eliminates pathogenic microorganisms on inanimate objects. The effectiveness of a disinfection procedure is controlled significantly by a number of factors:

- Nature and number of contaminating microbes.
- Amount of organic matter present.
- Type and condition of items to be disinfected.
- Temperature.

Chemical disinfectants are used in the laboratory to treat a surface or an item before or after routine use or after a spill or other contamination.

Disinfection can be achieved with liquid germicidal agents. Because disinfectants are antimicrobial, they may, by their nature, also have toxic effects for personnel. Therefore, MSDS and other manufacturer's product information should be available and thoroughly reviewed before using these products. Appropriate PPE must be worn when using disinfectants and these compounds must be used in well-ventilated areas.

Liquid Disinfectants

Liquid disinfectants are frequently used for surface decontamination and, at sufficient concentration, to decontaminate liquid waste. All disinfectants are not equally effective in decontaminating biohazardous material. Factors such as temperature, contact time, pH, dispersion rate, penetrability and reactivity of the material at the application site must be considered when selecting the appropriate disinfectant. Hazardous properties of the disinfectant, relative to its efficacy must also be considered. Because the local sewer authority sets limitations on drain disposal of chemicals, no chemicals other than 70% alcohol (ethanol or isopropanol) and 1% sodium hypochlorite will be allowed. Disinfectant solutions that are not authorized for sewer disposal may be collected as hazardous waste.

Alcohols

The most commonly used alcohols, ethanol and isopropanol, are most effective at concentrations of 70%. Both higher and lower concentrations are less effective. Alcohols are active against

vegetative bacteria, fungi, and lipid viruses but not against spores. They are only moderately effective against non-lipid viruses. Alcohols are difficult to use as contact disinfectants because they evaporate rapidly and do not penetrate organic material well. When using alcohols, it is best to clean an object and then submerge it in alcohol for the appropriate time.

Chlorine Compounds

The most commonly used and generally effective disinfectant is sodium hypochlorite (household bleach). One percent sodium hypochlorite is an appropriate disinfectant for a wide range of bacterial (excluding bacterial spores) and viral agents. Household bleach contains 5% sodium hypochlorite and should be diluted one part bleach to four parts water before use. In waste collection flasks, bleach should be added to 20% of the final collection volume to achieve a final sodium hypochlorite concentration of 1%. Sodium hypochlorite is a strong oxidizing agent and therefore can be corrosive to metal. Additionally, the presence of high concentrations of protein can inactivate the action of chlorine products. A sealed bottle of bleach will lose about 25% of the chlorine in a year. Do not use an unopened bottle of bleach that is more than six months old. An opened bottle will lose 25% of the chlorine over 30 days. Do not use an opened bottle more than 30 days old. A freshly prepared stock solution is only effective for 24 hours.

Glutaraldehyde

Glutaraldehyde is usually supplied as a 25% solution and requires activation by the addition of an alkaline agent prior to use. The activated product may be kept for about two weeks and should be discarded when turbid. Glutaraldehyde is active against all microorganisms, but is toxic, irritating, and mutagenic and should be used only when necessary. Please follow the manufacturer's guidance when using glutaraldehyde-based products because there are many different formulations that have been designed for specific uses.

Hydrogen Peroxide

Hydrogen peroxide is usually available as a 30% solution. It may be diluted 1:5 for use as a disinfectant. It is active against a wide array of microorganisms. However, it is an oxidizing agent and should not be used on aluminum, copper, zinc, or brass.

Steam Sterilization

Moist heat sterilization (autoclaving) is used to sterilize laboratory equipment and culture media, and to decontaminate biological waste. Autoclaving uses steam under pressure (approximately 15 pounds per square inch) to achieve a chamber temperature of at least 121°C (250°F). To be effective, air in the autoclave chamber must be replaced by steam for an adequate exposure time.

- Caution should be used when using steam sterilization.
- Steam under pressure can be a scalding hazard.
- Laboratory personnel should not use autoclaves without proper training and should exercise caution when opening an autoclave.
- Allow fluids to cool prior to removal from the autoclave.

Safety Procedures for Using Sharps

Sharps are laboratory instruments or equipment capable of causing a puncture or cut, including needles, scalpels, razor blades, glass Pasteur pipettes, slides, and broken glassware. Region 1 Microbiology Laboratory personnel should be familiar with proper storage, use, and disposal of sharps. Sharps should be used only when there is not a safer alternative, and should be stored in a manner that prevents injury and should never be left unattended in a manner that could result in an accidental injury. Needles must never be recapped or reused. Sharps must be disposed of in approved sharps containers and must not be disposed of in domestic laboratory trash. Sharps that contaminated with Particularly Hazardous Substances must be handled as hazardous waste.

Safety Procedures for Laboratory Equipment

The Region 1 Microbiology Laboratory Director and Safety Officer are responsible for maintaining laboratory equipment and providing training to laboratory personnel on the correct use of equipment. A routine inspection and maintenance program that includes necessary instrument calibration, certification, and maintenance procedures should be implemented for all equipment in the laboratory to identify worn parts, frayed wires, malfunctioning instruments, faulty safe guards, and other potential hazards. Follow these equipment safety guidelines:

- Do not allow personnel to use laboratory equipment without proper training.
- Use equipment only for its intended purpose. Do not modify or adapt equipment without guidance from the equipment manufacturer.
- Use applicable safeguards when operating equipment. Do not remove or over-ride equipment safety devices.
- Inspect equipment prior to each use to identify potential safety concerns.
- Perform preventative maintenance, and maintain instrument calibration and certification as indicated by the manufacturer.
- Make sure that equipment maintenance is performed by a qualified individual.
- Properly decontaminate equipment before its removal from the laboratory (e.g., repair, laboratory relocation, surplus, or disposal).
- Verify that equipment does not contain hazardous substances such as Freon (refrigerators and cooling systems), lead (lead acid batteries), or mercury (mercury switches) before transport, removal, or disposal.
- Check electrical cords for frayed or exposed wire.
- Cover exposed mechanical devices such as belt driven vacuum pumps and moving parts on equipment.

Safety Procedures for Pressurized Systems

A system that increases or decreases ambient pressure inside of a vessel presents a pressure hazard (e.g., implosion, explosion). The guidelines below should be followed when using a pressurized system:

- Use glassware specifically designed for vacuum operations (e.g., Erlenmeyer filtration flask).
- Inspect vacuum glassware before and after each use. Discard any glass that is chipped, scratched, broken, or otherwise stressed.
- Put belt guards in place on pumps before operation.
- Always use a trap on vacuum lines to prevent liquids from being drawn into the pump, building vacuum line, or water drain.
- Replace and properly discard vacuum pump oil that is contaminated with condensate.
- Place secondary containment under equipment that has the potential to leak or break.
- Do not place a pump in an enclosed or unventilated area.

Safety Procedures for Autoclave

Autoclaves operate at high temperature and pressure. When using an autoclave, be sure to follow these safety practices:

- Before using the autoclave, check inside the autoclave for any items left by the previous user that could pose a hazard (e.g., sharps).
- Check the drain and clean the strainer before loading the autoclave.
- Load the autoclave properly as per the manufacturer's recommendations.
- Do not overfill containers when autoclaving liquids.
- Place containers and bags within an autoclave-safe tray or catch basin to provide stability and to capture overflow when autoclaving materials.
- Never place containers directly on the rack or autoclave floor.
- Make sure the door of the autoclave is fully closed (latched) and the correct cycle has been selected before starting the cycle (liquid for liquid waste, gravity for solid waste).
- Never attempt to open the door while the machine is in operation. Always check the jacket pressure gauge to make sure that it is reading 0 PSI before opening the door.
- When the cycle is complete, open the door slowly. Keep your head, face, and hands away from the opening.
- At a minimum, wear heat-resistant gloves, a lab coat, and eye protection when removing items from an autoclave.
- If the machine is not operating properly, notify the Region 1 Microbiology Laboratory Director and Safety Officer.
- Do not attempt to make repairs. This should be done only by a trained technician.
- Never autoclave items containing corrosives (including sodium hypochlorite).

Electrical Safety

The major hazards associated with electricity are electrical shock and fire. Sparks from electrical equipment can serve as an ignition source for flammable or explosive vapors. The severity and effects of an electrical shock depend on a number of factors, such as the pathway through the body, the amount of current, the length of time of the exposure, and whether the skin is wet or dry. The following practices may reduce risk of injury when working with electrical equipment:

- Only use extension cords temporarily. Extension cords must be UL or Factory Mutual (FM) approved, grounded (three-prong), and heavy duty in type.
- Replace electrical cords that have frayed or exposed wires.
- Avoid contact with energized electrical circuits.
- Disconnect the power source before servicing or repairing electrical equipment.
- If water or a chemical is spilled onto equipment, shut off power at the main switch or circuit breaker and unplug the equipment before responding to the spill.
- Only equipment with grounded (three-prong) plugs should be used. The third prong provides a path to ground that helps prevent the buildup of voltages that may result in an electrical shock or spark.
- Use circuit protection devices that are designed to automatically limit or shut off the flow of electricity in the event of a ground-fault, overload, or short circuit in the wiring system.

Determine if laboratory outlets provide adequate amperage and appropriate voltage for the electrical requirements of all equipment used. Certain pieces of equipment may require other than standard 120 volt outlets.

Motor Safety

In areas where volatile flammable chemicals are used, motor-driven electrical equipment should be equipped with non-sparking induction motors or air motors. Avoid series-wound motors, such as those generally found in vacuum pumps, rotary evaporators, stirrers, and household appliances (e.g., blenders, mixers, power drills). If it is necessary to use motorized equipment, take precautions to reduce flammable vapors. Motors pose the additional hazard of moving parts that can cause injury if they are exposed or unguarded.

H1. Laboratory Emergencies

Emergencies, by their nature, are unpredictable and unexpected events that pose a potential threat to health and safety of personnel, property, and the environment. Region 1 Microbiology Laboratory personnel should be prepared to respond to emergencies such as spills of a hazardous substance, personal exposures, injuries, fire, or equipment failures. OSHA defines a chemical emergency as "equipment failure, rupture of containers or failure of control equipment that results in an uncontrolled release of a hazardous chemical into the workplace." Examples include:

- An accidental and uncontrollable spill from a broken bottle or leaking container.
- A reaction between two incompatible reagents while in storage.
- A process or experiment begins to react unpredictably or uncontrollably.
- An exposure to hazardous substances occurs that results in injury.
- A fume hood that contains a toxic or hazardous substance fails to evacuate vapors from the hood.
- A strong odor is detected and the origin cannot be determined or the release cannot be brought under control.

Each emergency event will be unique and will require assessment to determine the appropriate response. Region 1 Microbiology Laboratory personnel are not required to respond to emergency situations. An individual who is uncomfortable responding to an emergency situation should evacuate the laboratory and request assistance. If a situation poses imminent danger to health and safety and cannot be isolated, contained, or controlled evacuate the room or building (if necessary) dial 911 from any phone. Above all else, laboratory personnel should take measures to ensure the safety of themselves and other laboratory personnel.

This section provides general information relevant to all laboratory emergencies and detailed procedures to be followed in the event of a chemical spill or exposure.

Emergency Information

The Region 1 Microbiology Laboratory clearly posts emergency contact information near each laboratory entrance sign, exit and above first aid kits. The following information should be provided: office phone number of the Region 1 Microbiology Laboratory Director responsible for the laboratory, after hours contact information, and Fire Department.

An evacuation plan is posted on the wall inside every laboratory and building exit.

Emergency Preparation

In preparing for laboratory emergencies, it is necessary to consider the type of work conducted in the laboratory and the most likely accidents that may occur. Laboratory personnel must know the appropriate emergency response procedures, the location and use of any emergency equipment, emergency contact information, and any necessary follow up procedures.

The required elements of emergency preparedness for Region 1 Microbiology Laboratory is listed below:

- Laboratory Safety Manual must provide laboratory specific emergency response information.
- MSDS for all chemicals in the laboratory must be readily available so that laboratory and emergency response personnel have immediate access to chemical specific emergency information.
- Emergency contact information must be clearly posted on the laboratory entrance sign.
- Emergency showers and eye washes must be flushed routinely so that they are operational in the event of an exposure.
- Spill supplies must be appropriately stocked and easily accessible.
- A first aid kit containing basic supplies must be stocked and easily accessible.
- Personnel should be familiar with the building evacuation plan and laboratories evacuation route.

All Region 1 Microbiology Laboratory personnel receives Fire Extinguisher training and training in first aid.

Emergency Notification

When an emergency situation arises, contact local Fire and Police by dialing 911 from any phone. Provide the following information:

- Name and telephone number of the caller.
- Location of the emergency (building name, room number, and building specific address).
- Nature of the emergency (e.g., chemical spill and chemical(s) involved, fire, injuries).
- Special considerations (e.g., the potential for explosion, acutely hazardous gases present, people trapped in rooms or building, number of people injured and type of injuries, electrical hazards, property damage and access routes to the emergency).

Evacuation Procedures

Follow these steps to evacuate a room and or building, if safe to do so:

1. Notify other laboratory personnel.
2. If conditions permit, cap and secure open vials, bottles, and other materials and turn off laboratory equipment.
3. Leave the laboratory and close the door.
4. Activate the fire alarm.
5. If safe to do so, assist anyone who may be in danger. Otherwise notify emergency response personnel once you have evacuated the building.
6. Exit the building according to the building evacuation plan in a calm manner using the closest available emergency exit.
7. Congregate at the pre-designated assembly point for the building.

Laboratory Fires

Region 1 Microbiology Laboratory personnel are not required to fight fires and should evacuate the building immediately in the event of a fire. The local Emergency Services such as the Fire Department and Police have the primary responsibility for managing emergencies and must be notified immediately of such situations by calling 911. Employees may use fire extinguishers to fight small, incipient fires (no larger than a waste basket) only if they have been trained in the proper use of a fire extinguisher and are confident in their ability to cope with the hazards of a fire. In such cases, fire-fighting efforts must be terminated when it becomes obvious that there is danger from smoke, heat, or flames. If a fire occurs in the laboratory:

1. Cap and secure items on bench tops and turn off laboratory equipment.
2. Leave the laboratory and close the door.
3. Activate the fire alarm.
4. Assist anyone who may be in danger, if you can do so without endangering yourself.
5. Exit the building according to the building evacuation plan in a calm manner.
6. Congregate at the pre-designated assembly point for the building.
7. Notify emergency response personnel that you have specific information regarding the fire.

Spills and Accident Procedures

Chemical spills require proper response procedures that take into consideration the chemicals involved, their potential toxicity or chemical hazards, routes of exposure, and the potential for releases to the environment.

Region 1 Microbiology Laboratory personnel are not required to respond to a spill. If you are uncomfortable in responding to a spill, if a spill poses imminent danger to health and safety or cannot be isolated, contained or controlled, move to a safe area and contact the Region 1 Microbiology Laboratory Director. Do not attempt to clean the spill. General spill procedure guidelines for chemical spills are below.

Spill Supplies

A spill kit is an essential safety item for all laboratories. Basic spill kit consists of:

- Absorbent material (pads, sheets, spill socks, and paper towels)
- Nitrile gloves
- Polyethylene bags
- Boundary marking tape
- Warning sign
- Spill supply inventory
- Five gallon pail with screw top lid

A PPE should also be used when responding to a spill. These items should already be available in the laboratory:

- Safety goggles

- Gloves compatible with the substances used in the laboratory
- Lab coats

Spill Response

Follow these steps when responding to a chemical spill:

1. Contact Fire Department for any spill that:
    - Poses an inhalation hazard.
    - Cannot be isolated, contained, or controlled quickly.
    - Poses imminent danger to health and safety.
    - Poses imminent danger to property or the environment.
    - You are uncomfortable responding to on your own.
2. If the spill poses an inhalation hazard, or cannot be isolated, contained, or controlled quickly, evacuate the room.
3. Signal to others to leave, close the door, and post a warning sign.
4. Remove contaminated PPE and clothing, turning exposed areas inward, and place in a polyethylene bag.
5. If a personal exposure has occurred or you experience symptoms of exposure, follow procedures outlined below in "Personal Exposure".
6. If you can safely proceed in cleaning the spill, notify Region 1 Microbiology Laboratory Director and consult the MSDS regarding the physical, chemical, and toxicological properties and hazards of the chemical to determine the appropriate response.
7. Do not attempt to clean a spill alone. Employ the assistance of other Laboratory personnel to facilitate cleanup activities.
8. Assemble spill supplies and use appropriate PPE including lab coat, gloves, and eye or face protection.
9. Take steps to limit the impact of the spill by preventing spilled substances from reaching drains and by isolating equipment and materials that may escalate the danger of the situation.
10. Contain the spill with absorbent materials.
11. Pick up any visible sharp objects with tongs and discard into a sharps container.
12. Clean the spill by working from the outer edges of the spill towards the center.
13. Clean surrounding areas (where the spill may have splashed).
14. Clean contaminated laboratory equipment as needed.
15. Place the waste generated from cleaning the spill and contaminated PPE in a polyethylene bag. Place the bag into a sturdy pail such as the one provided with the spill kit. Label the container with a Hazardous Waste label and notify the Region 1 Microbiology Laboratory Director to arrange for disposal.
16. Wash hands with soap and warm water.
17. Report all possible exposure incidents to the Region 1 Microbiology Laboratory Director.

Personal Exposure

In the event of a personal exposure, an individual's primary concern must be to minimize the degree of exposure and the possible effects. The emergency procedures employed depend on the type of hazardous substance to which the individual was exposed and the extent of exposure. Immediate emergency response procedures for inhalation, ingestion, or skin exposure incidents are provided below.

Following decontamination, laboratory personnel who have received an exposure should immediately seek medical attention at the closest medical facility.

Inhalation Exposure

Follow the steps below when there is a potential for inhalation exposure:

1. Stop breathing in order to avoid inhaling airborne substances, and quickly leave the room.
2. Signal to others to leave, close the door, and post a warning sign.
3. Leave the area immediately and seek fresh air.
4. Remove contaminated PPE and clothing, turning exposed areas inward, and place in a polyethylene bag.
5. Review the MSDS for the chemical involved to evaluate exposure data.
6. Call 911 for emergency medical assistance or seek medical attention at the closest medical facility.
7. Report all possible exposure incidents to the Region 1 Microbiology Laboratory Director.
8. The Region 1 Microbiology Laboratory Director must clear the laboratory for re-entry.

Ingestion Exposure

In the event of accidental ingestion, seek medical attention (dial 911 or the Poison Control Center at 800-962-1253). Do not induce vomiting unless directed to do so by a health care provider. Report all possible exposure incidents to the Region 1 Microbiology Laboratory Director.

Skin or Mucous Membrane Exposure

Skin or mucous membrane exposure can occur through splashes to the eye, face, exposed skin, or clothing; by touching mucous membranes with contaminated hands; or from a needle stick, puncture with a contaminated sharp object, or through wounds, abrasions, and eczema. In the event of a skin or mucous membrane exposure:

1. Remove contaminated PPE and clothing, turning exposed areas inward, and place in a polyethylene bag.
2. For mucous membrane exposure, flush the affected area with the eyewash for at least 15 minutes.
3. For skin exposure, wash affected skin with soap and cold water for at least 15 minutes. Cold water has the effect of closing the skins pores thereby slowing the rate of absorption into the body. Wash gently so as not to break the skin. For skin exposures not limited to the hands and forearms, the emergency shower should be used.

4. Call 911 for emergency medical assistance or seek medical attention at the closest medical facility listed above.
5. Report all possible exposure incidents to the Region 1 Microbiology Laboratory Director.

Allergic Reaction

Region 1 Microbiology Laboratory personnel who experience a severe allergic reaction or show symptoms of allergic reaction while working in the laboratory should leave the work area immediately and wash the infected area(s) with profuse amounts of cool water. If the reaction is severe, seek immediate medical attention at the nearest medical facility. Before returning to work, laboratory personnel who have experienced an allergic reaction to a chemical should consult with the Region 1 Microbiology Laboratory Director.

Burn Emergency

The pressurized steam and heat of the autoclave can cause scalding or burns. If you receive an injury while using an autoclave:

- Seek medical attention as soon as possible.
- Scald and burn injuries to the face, third-degree burns, or burns over large areas of the body should be treated as emergencies.
- Minor burns should be treated with first aid.
- First aid for scalding and burns include immersing the area immediately in cool water , removing clothing from the area, and keeping the area cool for at least five minutes (preferably longer).
- Any burns to the face or eyes or any burns that blister should be seen by a physician as soon as possible.
- Regardless of the degree of severity, report the injury to the Region 1 Microbiology Laboratory Director.

Equipment Failures

Equipment failures can result from power failure, defects, or malfunctions. If a piece of equipment fails while in use, take steps to contain or control possible exposures to the substances being used. It is inappropriate to continue use of hazardous substances and equipment during a power failure or equipment malfunction. In the event of a power failure, all personnel must secure the materials they are working with, turn off equipment, and leave the laboratory until power is restored.

Ventilation Failure

If laboratory building ventilation fails, all operations concerning chemicals within that laboratory or building must be discontinued. Laboratory operations may resume in the laboratory or building once ventilation has been restored and if it is confirmed that all ventilation systems are operating correctly.

Emergency Drills

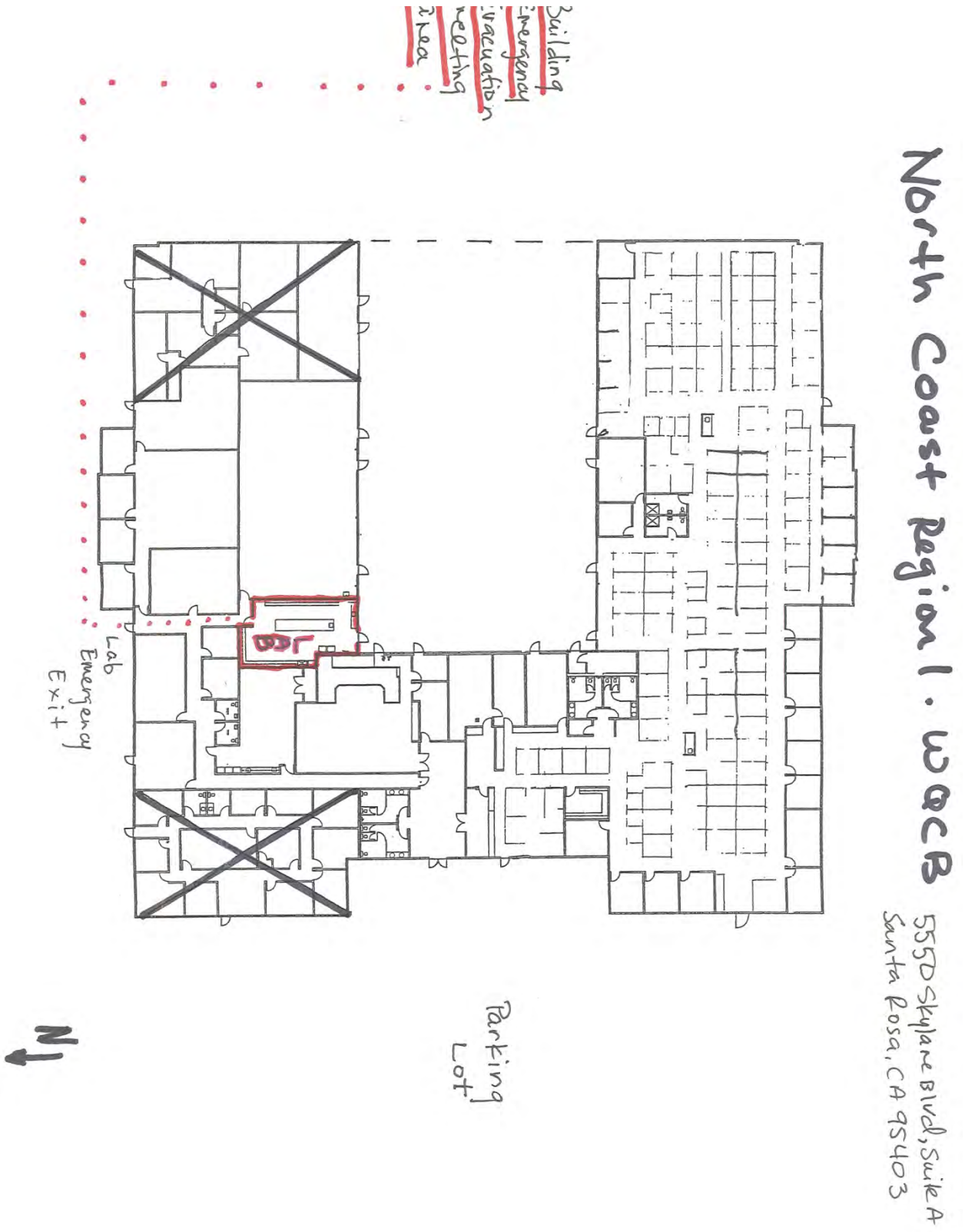
Evacuation drills should be performed annually and all laboratory personnel working in a building should be familiar with evacuation procedures for their building.

Appendix 1:

Section A-1
Approval Sheet Signatures

Appendix B:

Region 1 Microbiology Laboratory Evacuation Plan


North Coast Region • WQCB
5550 Skylane Blvd, Suite A
Santa Rosa, CA 95403
Building Emergency Evacuation Meeting Area
Lab Emergency Exit
Parking Lot
N

Appendix C:

Region 1 Microbiology Laboratory Standard Operating Procedures - Colilert®

Regional Water Quality Control Board
North Coast Region

Region 1 Microbiology Laboratory

Standard Operating Procedures - Colilert®

Coliform and E. coli Water Analysis

Version 1.0

Originated by:

Carrieann Lopez
Rich Fadness

North Coast Regional Water Quality Control Board

(March 31, 2011)

A: Title and Approval Sheet

Document Title Standard Operation Procedures - Colilert ®Coliform and E. coli Water Analysis

Lead Organization Regional Water Quality Control Board – North Coast Region
Surface Water Ambient Monitoring Program
5550 Skylane Blvd - Suite A
Santa Rosa CA 95403

Primary Contact Rich Fadness
Regional Water Quality Control Board – North Coast Region
Regional Surface Water Ambient Monitoring Program Coordinator
Phone Number: 707-576-6718
Email Address: RFadness@waterboards.ca.gov

Effective Date March 31, 2011

Approvals

Originals are kept on file by the Regional Water Quality Control Board - North Coast Region (Region 1) Microbiology Laboratory Director.

Executive Officer:
Catherine Kuhlman, Regional Water Quality Control Board – North Coast Region

See Appendix 3 : Section A Approval Sheet Signatures _____ _____
Signature Date

Co-Laboratory Directors:

Charles Reed, Regional Water Quality Control Board – North Coast Region

See Appendix 3 : Section A Approval Sheet Signatures _____ _____
Signature Date

Caryn Woodhouse, Regional Water Quality Control Board – North Coast Region

See Appendix 3 : Section A Approval Sheet Signatures _____ _____
Signature Date

Quality Assurance Officer:
Rich Fadness, Regional Water Quality Control Board – North Coast Region

See Appendix 3 : Section A Approval Sheet Signatures _____ _____
Signature Date

Principal Analyst:
Melinda Pope, Regional Water Quality Control Board – North Coast Region

See Appendix 3 : Section A Approval Sheet Signatures _____ _____
Signature Date

B: Table of Contents

List of Appendices

C: Scope and Applicability

Colilert is used for the simultaneous detection and confirmation of total coliforms and E. coli in water. They are EPA approved methods for determination of compliance with requirements of the National Primary Drinking Water Regulations for public water supplies and the Surface Water Treatment Rule promulgated under the Safe Drinking Water Act. They are also approved methods for E. coli under the CWA/NPDES regulations at 40 CFR Part 136. The tests are referred to as 'chromogenic/fluorogenic' tests, which are listed as Method 9223 in Standard Methods 20th ed. There is no EPA method number.

D: Method Summary

The Colilert test is based upon the ability of coliforms to produce the enzyme Bgalactosidase which cleaves the media substrate O-nitrophenyl-B-d-galactopyranoside (ONPG) producing a yellow color from the release of O-nitrophenyl. In addition, the enzyme B glucuronidase produced by E. coli forms a fluorescent substance when it hydrolyses 4-methylumbelliferyl-B-d-glucuronide (MUG). This combination of substrates allows detection of both total coliforms and E. coli within 24 hours. According to the manufacturer, Colilert can detect 1 CFU/100ml with as many as 2 million heterotrophic bacteria/100ml present.

E: Definitions

Coliform Bacteria

Coliform bacteria are not necessarily pathogenic but are used as indicators of fecal contamination in drinking water. Coliform bacteria may be found in both plants and animals. E. coli is enteric coliforms bacteria found in warm-blooded animals, and is therefore a more specific indicator of fecal contamination.

Colilert

Colilert is a product of IDEXX laboratories, Inc. (800-321-0207). The Colilert test is also referred to as an ONPG/MUG, MMO/MUG, or chromogenic/fluorogenic substrate test. The test is discussed in Standard Methods 9223.

MPN

Colilert can be used for enumeration of Most Probable Number (MPN) per 100 ml.

RPD

Relative Percent Difference. The RPD between duplicates is equal to 100 times the difference divided by the arithmetic mean.

F: Health and Safety / Hazardous Waste

All laboratory operations must follow health and safety requirements outlined in the current version of the Region 1 Microbiology Laboratory Safety Manual. Potential hazards specific to this SOP as well as pollution prevention and waste management requirements are described in the following sections.

Chemical Hazards

Due to the unknown and potentially hazardous characteristics of samples, all sample handling and preparation should be performed in a well-vented area.

The toxicity and carcinogenicity of each reagent used in this method may not be fully established. Each chemical should be regarded as a potential health hazard and exposure to them should be minimized by good laboratory practices. Refer to the Material Safety Data Sheets for additional information.

- Care should be taken to avoid breathing powdered microbiology media.
- Media containers and packages should be opened pointing away from the analyst.
- Samples may contain potentially pathogenic organisms.
- Gloves, lab coats and safety glasses should be worn when handling samples and equipment.
- Samples must never be pipetted by mouth.
- Laboratory equipment and benches should be cleaned daily.

Equipment and Instruments

- A 6-watt long wave ultraviolet light is used to read Enterolert. Care should be taken not to look directly at the light, and it should be pointed away from the analyst during readings.
- Follow the manufacturer's safety instructions whenever performing maintenance or troubleshooting work on equipment or instruments.
- Unplug the power supply before working on internal instrument components.
- Use of personal protective equipment may be warranted if physical or chemical hazards are present.

Waste Management

- The Region 1 Microbiology Laboratory complies with all applicable rules and regulations in the management of laboratory waste.
- Solid and hazardous wastes are disposed of in compliance with hazardous waste identification rules and land disposal restrictions.
- All analysts must collect and manage laboratory waste in a manner consistent with Region 1 Microbiology Laboratory Safety Manual.
- Contaminated media must NEVER be discarded in the trash or dumped down the drain prior to autoclaving.

- All biologically contaminated materials in the laboratory, particularly media with growth, must be autoclaved for 30 minutes prior to disposal.
- The laboratory minimizes and controls all releases from hoods and bench operations.
- If additional guidance is needed for new waste streams or changes to existing waste streams, consult with Region 1 Microbiology Laboratory Director and Safety Officer.

This procedure generates the following waste streams:

Waste Stream Description	Waste Label	Hazard Properties
Autoclaved microbiology waste	Non-Regulated Waste	Not Applicable
Laboratory Solid Waste (Non-biologically contaminated gloves, paper towels, disposable glassware, etc.)	Non-Regulated Waste	Not Applicable

G: Sample Handling and Preservation

- Samples for microbiological analysis should be collected using aseptic sampling procedures.
- If chlorinated water is to be analyzed, sterile sample bottles must contain sodium thiosulfate to neutralize any residual chlorine.
- Hold source water, stream pollution, recreational water, and wastewater samples must be below 6°C during a maximum transport time of 6 hours.
- Samplers are required to hold water samples at 6°C during a maximum transport time of 6 hours to the laboratory.

Containers and Required Sample Volume

- Samples may be collected in sterile plastic or glass bottles. The sample bottle must have at least 1 inch of headspace for mixing.

Chain-of-Custody

- Verify sample IDs, dates and times of collection against the chain-of-custody form.

Preservation Verification

- Samples should be collected, transported and shipped with ice.
- Temperatures will be recorded upon receipt using an infrared thermometer.
- If chlorinated water is to be analyzed, sterile sample bottles must contain sodium thiosulfate to neutralize any residual chlorine.

Sample Storage and Hold Times

- Refrigerate samples upon receipt in the laboratory and process within 2 hours.

H: Interferences

Colilert is primarily a water test. Its performance characteristic does not apply to samples altered by pre-enrichment or concentration. Do not use Colilert to verify presumptive coliform cultures or membrane filter colonies, because the substrate may be overloaded by a heavy inoculums of weak B-d-galactosidase-producing noncoliforms, causing false-positive results.

Colilert may give false positive ONPG and MUG reactions in the presence of Aeromonas hydrophilia and Flavobacterium. In marine or estuarine waters, Vibrio cholerae may give an ONPG-positive reaction and Providencia sp. may give a MUG-positive reaction therefore, only Colilert-18 may be used in marine and estuarine waters, and only E. coli may be reported.
The potential for false positives in surface waters may be assessed by running a dilution duplicate. False positive interference should be suspected if the dilution-corrected values are significantly lower (i.e. by more than a factor of 3) at higher dilutions.

Chlorine is toxic to microorganisms and will interfere with microbiological tests.
Samples bottles used for collection from chlorinated sources must contain sufficient sodium thiosulfate to neutralize residual chlorine.

I: Apparatus and Materials

This section describes recommended apparatus and materials to be used for the analysis. Minor deviations may be made in specific apparatus and materials provided that they are documented and equivalency is maintained.

Equipment and Instruments

- Quanti-Tray Sealer
- Incubator at 35 + 0.5°C
- 6 watt, 365 nm UV lamp
- Macro Pipette

Reagents

- Colilert dry media in Snap-Packs, stored in the dark at 4-25 °C.

Standards

- Each lot of Colilert media is checked using reference organisms.

Supplies

- Colilert Quanti-Tray and/or Quanti-Tray 2000 MPN trays.
- Colilert MPN Tables and MPN generator
- Colilert color comparator
- Sterile pipettes

J: Analytical Procedures

Instrument Operation and Calibration

- Incubators should be turned on at least 90 minutes before expected sample processing.
- The required temperature for the incubator is 35 °C + 0.5°C.
- Temperatures must be recorded twice per day of use with readings at least 4 hours apart. Temperatures are recorded from the dedicated 0.1°C increment thermometer within the incubator.
- In addition, the small 0.5°C increment thermometers, used to demonstrate temperature uniformity on the top and bottom incubator shelves of the 35°C incubator if they are in use, must read 35 $\pm$ 0.5°C.
- Sealer should be turned on at least 15 minutes before expected sample processing.

Sample Pretreatment/Preparation

- If the sample is chlorinated, the sample bottle must contain sufficient sodium thiosulfate to neutralize any residual prior to analysis.
- The IDEXX water sample bottles contain enough sodium thiosulfate to neutralize 10 ppm residual chlorine.

Sample Dilution

Surface water and wastewater samples may have to be diluted in order to give quantifiable results. In general, prior experience is necessary in order to select the appropriate dilution, since the hold time prevents reanalysis.

If prior information is unavailable then stream, lake and all marine samples should be diluted 1:10; 'first-flush' rain event samples should be diluted 1:100; and raw sewage should be diluted 1:1,000,000.

MPN Enumeration Test Procedure

Enterolert can be used for multiple tube Most Probable Number (MPN) analyses using serial dilutions as in the standard MPN test. However, it is easier and more accurate to use the Quanti-Tray 2000 for MPNs from 0 – 2400.

Sample Analysis

1. Carefully separate one Colilert Snap Pack from the strip taking care not to accidentally open adjacent pack. Tap the Snap Pack to ensure all of the powder is in the bottom part of the pack. Open one pack being careful not to touch the opening of the pack.
2. Add the reagent to the water sample in a sterile, transparent, non-fluorescent 100 ml vessel. Aseptically cap and seal the vessel. Shake until dissolved.
3. Pour the sample reagent mixture into a Quanti-Tray 2000 avoiding contact with the foil tab.

4. Seal the tray according to the instructions on the Quanti-Tray sealer.
5. Incubate for 24 hours at 35 + 0.5 °C.
6. Read the results at 24 to 28 hours.
7. Count the number of positive wells. The large well at the top of the Quanti-Tray is counted as one well.
8. Refer to the Quanti-Tray and Quanti-Tray 2000 MPN Tables to determine the Most Probable Number of total coliforms (yellow wells) and E. coli (fluorescent wells) in the sample.

The color and intensity of positive wells may vary.

Appearance	Result
Less yellow than the comparator*	Negative for total coliforms and E. coli
Yellow equal to or greater than the comparator*	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator*	Positive for E. coli

*IDEXX P/A Comparator, catalog #WP104; Quanti-Tray Comparator #WQTC, or Quanti-Tray/2000 Comparator #WQT2KC

- If no yellow color is observed, the test is negative.
- If the sample has a yellow color equal to or greater than the comparator, the presence of coliforms is confirmed. If the color is not uniform, mix by inversion and recheck.
- If the sample is yellow but lighter than the comparator, it may be incubated another 4 hours (but no more than 28 hours total) and rechecked. If the sample is coliform positive, the color will intensify. If it does not intensify, the sample is negative.
- If yellow is observed, check the vessel for fluorescence by placing a 6 watt 365 nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel.
- If fluorescence is greater or equal to the fluorescence of the comparator, the presence of E. coli is confirmed.

Procedural Notes

If an inoculated Colilert sample is inadvertently incubated more than 28 hours, the following guidelines apply:

- Lack of color is a valid negative test.
- A yellow color after 28 hours is not valid and should be repeated or verified.

Some water samples containing humic material may have an innate color. If a water sample has some background color, compare the inoculated Colilert sample to a control blank of the same water sample.
Colilert is already buffered and does not require the use of buffered water for dilutions. In order to prevent osmotic shock to bacteria from sterile deionized water, add media to the proper dilution volume before adding the sample.

Care should be taken to count only those wells that are both fluorescent and yellow. Fluorescent wells which are not also yellow are negative for E. coli.
If there is an immediate color change upon adding the media, invalidate the sample.
If the incubator will contain samples with more than one type of media the samples should be labeled with the media type to ensure that they are not misread.

Calculations

The results for presence/absence and quantification of total coliforms and E. coli are determined according to the procedures above. Results are entered onto *NCRWQCB - Region 1 Laboratory* Colilert Processing Worksheets. Positive results are recorded with a plus sign (+) and negative results with a minus sign (-).
If Colilert Quanti-trays are used, any positive wells indicate presence.
The numbers of positive wells are counted and the results are converted to the MPN for total coliforms and E. coli using the appropriate Colilert Quanti-tray matrices. The MPNs are then entered on the *NCRWQCB - Region 1 Laboratory* Colilert Processing Worksheets. MPNs below 10 are reported to one significant figure. MPNs 10 and above are reported to two significant figures.
The limit of detection for the method is 1 bacterium per 100 mL.
If the lowest dilution Quanti-tray has all wells positive, the result will be reported as >2400/100 mL times the dilution.
If a series of dilutions are run (e.g. for wastewaters) the lowest dilution producing a countable result will be reported with the following exception: if the lowest countable dilution has all large wells and 46 or more small wells positive, then the results using the next lowest dilution are be reported.

Sample Analysis

1. Carefully separate one Colilert Snap Pack from the strip taking care not to accidentally open adjacent pack. Tap the Snap Pack to ensure all of the powder is in the bottom part of the pack. Open one pack being careful not to touch the opening of the pack.
2. Add the reagent to the water sample in a sterile, transparent, non-fluorescent 100 ml vessel. Aseptically cap and seal the vessel. Shake until dissolved.
3. Pour the sample reagent mixture into a Quanti-Tray 2000 avoiding contact with the foil tab.
4. Seal the tray according to the instructions on the Quanti-Tray sealer.
5. Be sure to use the correct rubber insert in the sealer for either the Quanti-Tray 2000.

Maintenance

- The control limit for incubator temperature is 35 °C + 0.5 °C.
- When the unloaded incubator has been undisturbed for an hour or more (e.g. in the morning), the temperature reading of the calibrated internal center thermometer is typically 35 °C + 0.1 °C.
- The incubator should be recalibrated if the temperature drifts so that it is consistently 0.2 °C or more above or below 35.0°C.
- To recalibrate the incubator, press the 'Cal' button.

- Then press the up or down arrow until the digital display matches the internal temperature (e.g. 35.8 °C) and press enter.
- The digital display has now been recalibrated to match the internal temperature.
- After an hour, the display should read 41.0 and the internal thermometer should read 35 °C + 0.1 °C.
- The incubator should also be checked for several mornings to ensure that the calibration is adequate.

K: Quality Control

Demonstration of Capability

The Region 1 Microbiology Laboratory operates a formal quality control program. As it relates to this SOP, the QC program consists of a demonstration of capability, media QC, and the analysis of Method Blanks and duplicates as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated. A summary of QC criteria is provided in Appendix B.

A Demonstration of Capability must be in place prior to using an analytical procedure and repeated if there is a change in instrument type, personnel, or method. Follow procedures described in the Region 1 Microbiology Laboratory QAP for more details.

Media Quality Control

Using sterile technique, reconstitute lyophilized cultures in 99 ml bottles of pre-warmed sterile buffered water, incubate at 35 °C for 30 minutes, and mix to create a homogenous suspension. Transfer 1 mL of each control bacteria to 99 mL blanks and process as samples.

The expected results for various types of bacteria using Colilert is as follows:

Organism	Expected Result
E. coli	yellow, fluorescent
coliform, non-E. coli (e.g., Klebsiella pneumoniae)	yellow, non-fluorescent
non-coliform (e.g., Pseudomonas aeruginosa)	clear, non-fluorescent
blank	clear, non-fluorescent

If the expected results for a Colilert lot are not obtained, the lot is discarded.

Instrument Quality Control

- The incubator temperature is recorded twice daily during use.

- The Quanti-tray sealer is checked for leaks once per month when in use by closely examining the fluorogenic endpoints of a positive sample to ensure that no media leaks between wells.

Batch Quality Control

A batch consists of all the samples prepared on one day, up to 20 samples. The following Quality Control samples are analyzed per batch:

Blank:

- Sterile water blank is run with each batch.

Field Duplicate:

- For quantified samples, a field duplicate is also analyzed for each batch. One field duplicate is run for surface and wastewater samples with each day's samples or batch of 20 samples. The result for the field duplicate should be within the 95% confidence interval of its pair. The 95% confidence intervals are obtained from the Quanti-tray MPN chart or the IDEXX MPN generator for Quanti-tray 2000 (on the PC in lab). If the duplicate result does not meet this criterion, all results for the batch are flagged.
- The 95% confidence limit varies with the MPN, but is generally within a factor of 2, except for very low counts.

Lab Duplicate:

- One lab duplicate should be run for surface and wastewater samples with each day's samples or batch of 20 samples. The lab duplicate should be selected randomly or from a sample expected to be high. The results should agree within a confidence factor of 95%, as with field duplicates. To assess the potential for false positives in marine waters when the sample dilution used is less than 1:100, the lab duplicate may be run at a factor of 10 above the normal dilution. If the result for the lab duplicate is significantly lower than its less-dilute pair, this indicates that there may be false positive interference and all results should be flagged. No Quality Control comparison is made if the result for the sample is below the detection limit of the dilution duplicate.

Sample Quality Control

- Samples with inadequate volume or preservation are flagged.
- Samples which exceed hold time are flagged.
- Samples which show immediate color change upon addition of the media are invalidated.

Sample Bottle Quality Control

- Each lot of sample bottles is checked for sterility using sterile non-selective broth.
- The bottle is incubated for 24 hours and checked for growth.
- If there is growth, the lot is discarded.
- Each lot is also checked to ensure that the fill line is accurate to 100 mL + 2.5 mL.

- The bottle is filled to the line and then the water is poured into a Class A graduated cylinder. Alternately, the bottle is tared and then filled with DI water to the line and reweighed.
- The weight must be 100 + 2.5 grams.
- If the fill-line volume does not meet the specification, the lot is discarded.
- In addition, each lot of bottles containing sodium thiosulfate is checked to ensure that it is sufficient to neutralize 5 mg/L total residual chlorine for drinking water.
- A chlorine standard is used to prepare a 5 mg/L solution, and this is poured into the sample bottle to the fill line.
- The residual chlorine level is then measured. If the result is not below the quantitation limit of 0.1 mg/L total residual chlorine, the lot is discarded.

Method Performance

Method performance is demonstrated by acceptable analysis of blanks and control cultures. Method performance is demonstrated by acceptable analysis of Performance Test unknowns. If a Performance Test is unacceptable the cause will be determined and rectified as demonstrated by acceptable analysis of a new Performance Test.

L: Documentation

Standards

- The Media Lot Number is recorded.
- Reference organisms used to demonstrate the suitability of media lots or batches are traceable to ATCC or are obtained from other sources acceptable to the Environmental Laboratory Accreditation Program (ELAP).

Analytical Sequence

The analytical sequence is documented in the laboratory bench sheet prepared for each batch. Case Number, date of analysis, Media Lot Number, analyst initials, sample IDs, and dilution factors are recorded.

Data Package

The data package is produced from bench sheets and manual log records.

Maintenance Logbook

- Maintain a maintenance logbook for each instrument covered in this SOP. Whenever corrective action is taken, record the date, the problem and resolution, and documentation of return to control.

- Document all preventive or routine maintenance performed, as well as repairs or corrective or remedial actions in accordance with Region 1 Microbiology Laboratory QAP.
- Document any changes in the meter or incubator used.

M: References

American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998.

U.S. Environmental Protection Agency, Microbial Methods for Monitoring the Environment, EPA-600/8-78-017, December, 1978.

IDEXX, ® Colilert from IDEXX product instructions. Number 06-01701-03, undated.

U.S. Environmental Protection Agency, National Primary Drinking Water Regulations, 40 CFR Part 141, Analytical Methods for Regulated Drinking Water Contaminants,@ 12/5/94.

U.S. Environmental Protection Agency, Manual for the Certification of Laboratories Analyzing Drinking Water, Fifth Edition, EPA 815-R-05-004, January, 2005.

Appendix 1: Analytes and Quantitation Limits

The following table provides the target analytes list for this SOP with the CAS number and quantitation limits.

Analyte	Chemical Abstracts Registry Number (CASRN)	Water Quantitation Limit, MPN/100 mL	Soil Quantitation Limit, µg/kg
Total Coliform Bacteria	NA	1	NA
E. coli	NA	1	NA

Appendix 2: Quality Control Measures and Criteria

Standard Name	QC Limit
E. coli Total coliform	present
E. coli	present
Klebsiella pneumoniae Total coliform	present
E. coli	absent
Pseudomonas aeruginosa Total coliform	absent
E. coli	absent

Field Duplicate RPD 95%
Lab Duplicate RPD <25%
Blank <1/100mL

Incubation Temperature 41 + 0.5 °C
Incubation Period 24 - 28 hours

Appendix 3:

Section A
Approval Sheet Signatures

Appendix D:

Region 1 Microbiology Laboratory Standard Operating Procedures - Enterolert®

Regional Water Quality Control Board
North Coast Region

Region 1 Microbiology Laboratory

Standard Operating Procedures - Enterolert®

Enterococci Water Analysis

Version 1.0

Originated by:

Carrieann Lopez
Rich Fadness

North Coast Regional Water Quality Control Board

(March 31, 2011)

A: Title and Approval Sheet

Document Title Standard Operation Procedures - Enterolert ☐ Enterococci Water Analysis

Lead Organization Regional Water Quality Control Board – North Coast Region
Surface Water Ambient Monitoring Program
5550 Skylane Blvd - Suite A
Santa Rosa CA 95403

Primary Contact Rich Fadness
Regional Water Quality Control Board – North Coast Region
Regional Surface Water Ambient Monitoring Program Coordinator
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Effective Date March 31, 2011

Approvals

Originals are kept on file by the Regional Water Quality Control Board - North Coast Region (Region 1) Microbiology Laboratory Director.

Executive Officer:
Catherine Kuhlman, Regional Water Quality Control Board – North Coast Region

See Appendix C : Section A Approval Sheet Signatures _____ _____
Signature Date

Co-Laboratory Directors:

Charles Reed, Regional Water Quality Control Board – North Coast Region

See Appendix C : Section A Approval Sheet Signatures _____ _____
Signature Date

Caryn Woodhouse, Regional Water Quality Control Board – North Coast Region

See Appendix C : Section A Approval Sheet Signatures _____ _____

Signature Date

Quality Assurance Officer:
Rich Fadness, Regional Water Quality Control Board – North Coast Region

See Appendix C : Section A Approval Sheet Signatures _____ _____
Signature Date

Principal Analyst:
Melinda Pope, Regional Water Quality Control Board – North Coast Region

See Appendix C : Section A Approval Sheet Signatures _____ _____
Signature Date

B: Table of Contents

List of Appendices

C: Scope and Applicability

Enterolert® is used for the detection and quantification of enterococci in water. It may be used in drinking water, fresh and marine surface waters and wastewaters. There are no limits for enterococci in the National Primary Drinking Water Regulations for public water supplies or the Surface Water Treatment Rule promulgated under the Safe Drinking Water Act. It is approved under the CWA/NPDES regulations at 40 CFR Part 136.

D: Method Summary

The Enterolert test is based upon the ability of enterococci to metabolize a media substrate producing a fluorescent substance. Quantification is performed using the IDEXX Quantitray Most Probable Number (MPN) system. According to the manufacturer, Enterolert can detect 1 enterococcus bacteria in a 100 mL sample.

E: Definitions

Enterococci

The enterococcus group is a subgroup of fecal streptococci that includes S. faecalis, S. faecium, S. gallinaum and S. avium. Enterococci are not necessarily pathogenic but are used as indicators of the extent of fecal contamination in recreational surface waters. Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10 °C and 45 °C.

Enterolert

Enterolert is a product of IDEXX laboratories, Inc. (800-321-0207).

MPN

Enterolert can be used as either a presence/absence test, or for enumeration of Most Probable Number (MPN) per 100 ml. Enumeration is possible using IDEXX's Quanti-tray 2000.

RPD

Relative Percent Difference. The RPD between duplicates is equal to 100 times the difference divided by the arithmetic mean.

F: Health and Safety / Hazardous Waste

All laboratory operations must follow health and safety requirements outlined in the current version of the Region 1 Microbiology Laboratory Safety Manual. Potential hazards specific to this SOP as well as pollution prevention and waste management requirements are described in the following sections.

Chemical Hazards

Due to the unknown and potentially hazardous characteristics of samples, all sample handling and preparation should be performed in a well-vented area.

The toxicity and carcinogenicity of each reagent used in this method may not be fully established. Each chemical should be regarded as a potential health hazard and exposure to them should be minimized by good laboratory practices. Refer to the Material Safety Data Sheets for additional information.

- Care should be taken to avoid breathing powdered microbiology media.
- Media containers and packages should be opened pointing away from the analyst.
- Samples may contain potentially pathogenic organisms.
- Gloves, lab coats and safety glasses should be worn when handling samples and equipment.
- Samples must never be pipetted by mouth.
- Laboratory equipment and benches should be cleaned daily.

Equipment and Instruments

- A 6-watt long wave ultraviolet light is used to read Enterolert. Care should be taken not to look directly at the light, and it should be pointed away from the analyst during readings.
- Follow the manufacturer's safety instructions whenever performing maintenance or troubleshooting work on equipment or instruments.
- Unplug the power supply before working on internal instrument components.
- Use of personal protective equipment may be warranted if physical or chemical hazards are present.

Waste Management

- The Region 1 Microbiology Laboratory complies with all applicable rules and regulations in the management of laboratory waste.
- Solid and hazardous wastes are disposed of in compliance with hazardous waste identification rules and land disposal restrictions.
- All analysts must collect and manage laboratory waste in a manner consistent with Region 1 Microbiology Laboratory Safety Manual.
- Contaminated media must NEVER be discarded in the trash or dumped down the drain prior to autoclaving.

- All biologically contaminated materials in the laboratory, particularly media with growth, must be autoclaved for 30 minutes prior to disposal.
- The laboratory minimizes and controls all releases from hoods and bench operations.
- If additional guidance is needed for new waste streams or changes to existing waste streams, consult with Region 1 Microbiology Laboratory Director and Safety Officer.

This procedure generates the following waste streams:

Waste Stream Description	Waste Label	Hazard Properties
Autoclaved microbiology waste	Non-Regulated Waste	Not Applicable
Laboratory Solid Waste (Non-biologically contaminated gloves, paper towels, disposable glassware, etc.)	Non-Regulated Waste	Not Applicable

G: Sample Handling and Preservation

- Samples for microbiological analysis should be collected using aseptic sampling procedures.
- If chlorinated water is to be analyzed, sterile sample bottles must contain sodium thiosulfate to neutralize any residual chlorine.
- Hold source water, stream pollution, recreational water, and wastewater samples must be below 10°C during a maximum transport time of 6 hours.
- Samplers are required to hold water samples at 10°C during a maximum transport time of 6 hours to the laboratory.

Containers and Required Sample Volume

- Samples may be collected in sterile plastic or glass bottles. The sample bottle must have at least 1 inch of headspace for mixing.

Chain-of-Custody

- Verify sample IDs, dates and times of collection against the chain-of-custody form.

Preservation Verification

- Samples should be collected, transported and shipped with ice.
- Temperatures will be recorded upon receipt using an infrared thermometer.
- If chlorinated water is to be analyzed, sterile sample bottles must contain sodium thiosulfate to neutralize any residual chlorine.

Sample Storage and Hold Times

- Refrigerate samples upon receipt in the laboratory and process within 2 hours.

H: Interferences

The manufacturer's method requires diluting marine water samples at least 1:10 with sterile fresh water in order to reduce the possibility of interference by marine bacilli.

The potential for false positives in surface waters may be assessed by running a dilution duplicate. False positive interference should be suspected if the dilution-corrected values are significantly lower (i.e. by more than a factor of 3) at higher dilutions.

Chlorine is toxic to microorganisms and will interfere with microbiological tests. Samples bottles used for collection from chlorinated sources must contain sufficient sodium thiosulfate to neutralize residual chlorine.

I: Apparatus and Materials

This section describes recommended apparatus and materials to be used for the analysis. Minor deviations may be made in specific apparatus and materials provided that they are documented and equivalency is maintained.

Equipment and Instruments

- Quanti-Tray Sealer
- Incubator at 41 + 0.5°C
- 6 watt, 365 nm UV lamp
- Macro Pipette

Reagents

- Enterolert dry media in Snap-Packs, stored in the dark at 4-25 °C.

Standards

- No standards are run with batches of microbiological samples.

Supplies

- Quanti-Tray 2000 MPN trays.
- Quanti-tray MPN Tables and MPN generator (on PC in Lab)
- Pre-measured sterile dilution water bottles
- Sterile pipettes

J: Analytical Procedures

Instrument Operation and Calibration

- Incubators should be turned on at least 90 minutes before expected sample processing.
- The required temperature for the incubator is 41°C + 0.5°C.
- Temperatures must be recorded twice per day of use with readings at least 4 hours apart. Temperatures are recorded from the dedicated 0.1°C increment thermometer within the incubator.
- The small 0.5°C increment thermometers, used to demonstrate temperature uniformity on the top and bottom incubator shelves of the 41°C incubator if they are in use, must read 41 + 0.5°C.
- Sealer should be turned on at least 15 minutes before expected sample processing.

Sample Pretreatment/Preparation

- If the sample is chlorinated, the sample bottle must contain sufficient sodium thiosulfate to neutralize any residual prior to analysis.
- The IDEXX water sample bottles contain enough sodium thiosulfate to neutralize 10 ppm residual chlorine.

Sample Dilution

Surface water and wastewater samples may have to be diluted in order to give quantifiable results. In general, prior experience is necessary in order to select the appropriate dilution, since the hold time prevents reanalysis.

If prior information is unavailable then stream, lake and all marine samples should be diluted 1:10; 'first-flush' rain event samples should be diluted 1:100; and raw sewage should be diluted 1:1,000,000.

MPN Enumeration Test Procedure

Enterolert can be used for multiple tube Most Probable Number (MPN) analyses using serial dilutions as in the standard MPN test. However, it is easier and more accurate to use the Quanti-Tray 2000 for MPNs from 0 – 2400.

Sample Analysis

6. Carefully separate one Enterolert Snap Pack from the strip taking care not to accidentally open adjacent pack. Tap the Snap Pack to ensure all of the powder is in the bottom part of the pack. Open one pack being careful not to touch the opening of the pack.
7. Add the reagent to the water sample in a sterile, transparent, non-fluorescent 100 ml vessel. Aseptically cap and seal the vessel. Shake until dissolved.
8. Pour the sample reagent mixture into a Quanti-Tray 2000 avoiding contact with the foil tab.
9. Seal the tray according to the instructions on the Quanti-Tray sealer.
10. Be sure to use the correct rubber insert in the sealer for either the Quanti-Tray 2000.

11. Incubate for 24-28 hours at 41 °C + 0.5 °C.
12. Place the tray under the 6 watt fluorescent light to count the number of positive wells. Refer to the Quanti-Tray 2000 MPN Tables to determine the Most Probable Number of enterococci in the sample. The intensity of positive wells may vary.

Procedural Notes

If an inoculated Enterolert sample is inadvertently incubated more than 28 hours, the following guidelines apply:

- Lack of fluorescence is a valid negative test.
- Fluorescence after 28 hours is not valid and should be repeated or verified.
- Enterolert is already buffered and does not require the use of buffered water for dilutions.
- Always add Enterolert to the proper volume of diluted samples after taking dilutions.

Calculations

The number of positive wells is counted and the results are converted to the MPN Quanti-tray matrices. The MPNs are then entered on the Region 1 Microbiology Laboratory Enterolert Processing Worksheet. MPNs below 10 are reported to one significant figure. MPNs 10 and above are reported to two significant figures.

The limit of detection for the method is 1 bacterium per 100 mL. If the lowest dilution Quanti-tray has all wells positive, the result will be reported as >2400/100 mL times the dilution. If a series of dilutions are run (e.g. for wastewaters) the lowest dilution producing a countable result will be reported with the following exception: if the lowest countable dilution has all large wells and 46 or more small wells positive, then the results using the next lowest dilution are be reported.

Quality Control Review

The following actions should be taken in the event that the blank or lab duplicate QC samples do not meet the requirements. Unfortunately, the samples cannot be reanalyzed because the results will not be known until well beyond the hold time.

- If the blank is positive, report and flag all sample results and discuss the deviation, including the possible degree of bias, in a report narrative. If the relative percent difference for the lab QC duplicates is greater than 25%, check to see whether the 95% confidence limits for the duplicate results overlap. If not, report and flag all sample data.
- If the incubator temperature does not meet the requirements, report and flag all sample results and discuss the deviation, including the possible degree of bias, in the report narrative.
- If no bacteria are detected the raw quantitative result is entered as <1.
- Results are entered uncorrected for dilution.
- Calculate the final result and the MDL for each sample.

Maintenance

- The control limit for incubator temperature is 41 °C + 0.5 °C.
- When the unloaded incubator has been undisturbed for an hour or more (e.g. in the morning), the temperature reading of the calibrated internal center thermometer is typically 41 °C + 0.1 °C.
- The incubator should be recalibrated if the temperature drifts so that it is consistently 0.2 °C or more above or below 41.0.
- To recalibrate the incubator, press the 'Cal' button.
- Then press the up or down arrow until the digital display matches the internal temperature (e.g. 40.8 °C) and press enter.
- The digital display has now been recalibrated to match the internal temperature.
- After an hour, the display should read 41.0 and the internal thermometer should read 41 °C + 0.1 °C.
- The incubator should also be checked for several mornings to ensure that the calibration is adequate.

K: Quality Control

Demonstration of Capability

The Region 1 Microbiology Laboratory operates a formal quality control program. As it relates to this SOP, the QC program consists of a demonstration of capability, media QC, and the analysis of Method Blanks and duplicates as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated. A summary of QC criteria is provided in Appendix B.

A Demonstration of Capability must be in place prior to using an analytical procedure and repeated if there is a change in instrument type, personnel, or method. Follow procedures described in the Region 1 Microbiology Laboratory QAP more details.

Media Quality Control

Positive and negative controls are run on each new lot of Enterolert. Manufacturer's lot specific QC documentation showing control culture results is acceptable. The expected results for various types of bacteria are as follows:

Organism	Expected Result
Enterococcus faecium, Streptococcus faecalis	fluorescent
Serratia marcescens, E. Coli	non-fluorescent

Instrument Quality Control

- The incubator temperature is recorded twice daily during use.
- The Quanti-tray sealer is checked for leaks once per month when in use by closely examining the fluorogenic endpoints of a positive sample to ensure that no media leaks between wells.

Batch Quality Control

A batch consists of all the samples prepared on one day, up to 20 samples. The following Quality Control samples are analyzed per batch:

Blank:

- Sterile water blank is run with each batch.

Field Duplicate:

- For quantified samples, a field duplicate is also analyzed for each batch. One field duplicate is run for surface and wastewater samples with each day's samples or batch of 20 samples. The result for the field duplicate should be within the 95% confidence interval of its pair. The 95% confidence intervals are obtained from the Quanti-tray MPN chart or the IDEXX MPN generator for Quanti-tray 2000 (on the PC in lab). If the duplicate result does not meet this criterion, all results for the batch are flagged.
- The 95% confidence limit varies with the MPN, but is generally within a factor of 2, except for very low counts.

Lab Duplicate:

- One lab duplicate should be run for surface and wastewater samples with each day's samples or batch of 20 samples. The lab duplicate should be selected randomly or from a sample expected to be high. The results should agree within a confidence factor of 95%, as with field duplicates. To assess the potential for false positives in marine waters when the sample dilution used is less than 1:100, the lab duplicate may be run at a factor of 10 above the normal dilution. If the result for the lab duplicate is significantly lower than its less-dilute pair, this indicates that there may be false positive interference and all results should be flagged. No Quality Control comparison is made if the result for the sample is below the detection limit of the dilution duplicate.

Sample Quality Control

- Samples with inadequate volume or preservation are flagged.
- Samples which exceed hold time are flagged.
- Samples which show immediate color change upon addition of the media are invalidated.

Sample Bottle Quality Control

- Each lot of sample bottles is checked for sterility using sterile non-selective broth.
- The bottle is incubated for 24 hours and checked for growth.
- If there is growth, the lot is discarded.
- Each lot is also checked to ensure that the fill line is accurate to 100 mL + 2.5 mL.

- The bottle is filled to the line and then the water is poured into a Class A graduated cylinder. Alternately, the bottle is tared and then filled with DI water to the line and reweighed.
- The weight must be 100 + 2.5 grams.
- If the fill-line volume does not meet the specification, the lot is discarded.
- In addition, each lot of bottles containing sodium thiosulfate is checked to ensure that it is sufficient to neutralize 5 mg/L total residual chlorine for drinking water.
- A chlorine standard is used to prepare a 5 mg/L solution, and this is poured into the sample bottle to the fill line.
- The residual chlorine level is then measured. If the result is not below the quantitation limit of 0.1 mg/L total residual chlorine, the lot is discarded.

Method Performance

Method performance is demonstrated by acceptable analysis of blanks and control cultures.

L: Documentation

Standards

- The Media Lot Number is recorded.
- Reference organisms used to demonstrate the suitability of media lots or batches are traceable to ATCC or are obtained from other sources acceptable to the Environmental Laboratory Accreditation Program (ELAP).

Analytical Sequence

The analytical sequence is documented in the laboratory bench sheet prepared for each batch. Case Number, date of analysis, Media Lot Number, analyst initials, sample IDs, and dilution factors are recorded.

Data Package

The data package is produced from bench sheets and manual log records.

Maintenance Logbook

Maintain a maintenance logbook for each instrument covered in this SOP. Whenever corrective action is taken, record the date, the problem and resolution, and documentation of return to control. Document all preventive or routine maintenance performed, as well as repairs or corrective or remedial actions in accordance with Region 1 Microbiology Laboratory QAP.
Document any changes in the meter or incubator used.

M: References

American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998.

IDEXX, "Enterolert from IDEXX" product instructions. Number 06-02150-03, undated.

U.S. Environmental Protection Agency, Microbial Methods for Monitoring the Environment, EPA-600/8-78-017, December, 1978.

Appendix 1: Analytes and Quantitation Limits

The following table provides the target analytes list for this SOP with the CAS number and quantitation limits.

Analyte	Chemical Abstracts Registry Number (CASRN)	Water Quantitation Limit, MPN/100 mL	Soil Quantitation Limit, µg/kg
Enterococci Bacteria	NA	1	NA

Appendix 2: Quality Control Measures and Criteria

Standard Name	QC Limit
Enterococcus faecium, Streptococcus faecalis	fluorescent
Serratia marcescens, E. coli	non-fluorescent
Aerococcus viridans, Staphylococcus aueralisnon	fluorescent

Field Duplicate RPD 95%
Lab Duplicate RPD <25%
Blank <1/100mL

Incubation Temperature 41 + 0.5 °C
Incubation Period 24 - 28 hours

Appendix 3:

Section A
Approval Sheet Signatures

Appendix D:

Region 1 Microbiology Fieldsheet

SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=W0

*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_generic)

'GPS / DGPS | Lat (dd.ddddd) | Long (ddd.ddddd)

OBSERVED FLOW: NA Dry Waterbody Bed No Obs Flow Isolated Pool Trickle (<0.1cfs) | WADEABILITY: Y / N / Unk | Target: | -

0.1-1cfs 1-5cfs 5-20cfs 20-50cfs 50-200cfs >200cfs | 'Actual: | -

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

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

WATER ODOR: None Sulfides Sewage Petroleum Mixed Other

WATER CLARITY: Clear (see bottom) Cloudy (>4" vis) Murky (<4" vis)

WATER COLOR: Colorless Green Yellow Brown | Duplicate? | (RB)

OTHER PRESENCE: Vascular Nonvascular OilySheen Foam Trash Other | YES / NO | (LB)

DOM. SUBSTRATE: Bedrock Concrete Cobble Gravel Sand Mud Lnk Other | (US)

SKY CODE: Clear Partly Cloudy Overcast Fog Smoky Hazy | DupID: | (DS)

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 WI 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 = Field04; 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	VIS	YSI Datasonde: 600XL					S/N:		YSI 650MDS	
		Calibration Date							Calibration Sheet No			

COMMENTS:

*Purpose: WaterChem

Rev. 12/31/2009

Appendix E:

Region 1 Microbiology Chain of Custody Forms

Region 1 Microbiology Laboratory: Bacteria Processing Worksheet and Chain of Custody Form | **SAMPLE DATE:** | **PROCESSING DATE:**

☐ SURFACE WATER ☐ WASTE WATER ☐ OTHER _____ | **SAMPLER COLLECTOR:**

Project Site ID Number:																							
SAMPLE TIME:																							
Replicate Number:																							
Enterolert / Colilert (?):	E / C		E / C		E / C		E / C		E / C		E / C		E / C		E / C		E / C		E / C		E / C		
COLILERT Laboratory Sample ID Number:	1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		
	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	
Yellow +	# Large Wells																						
	# Small Wells																						
	Empty Wells																						
	MPN																						
	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	
Yellow + Fluorescence (+)	# Large Wells																						
	# Small Wells																						
	Empty Wells																						
	False Positives																						
	MPN																						
ENTEROLERT Laboratory Sample ID Number:	1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		1105-		
	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	
Fluorescence +	# Large Wells																						
	# Small Wells																						
	Empty Wells																						
	False Positives																						
	MPN																						

COC	Name	Signature	Date	Time	Temp	NOTES:
Sampler Relinquished						
Laboratory Accepted					Holding Time Met	
Samples Processed						
Placed in Incubator					YES - NO	
Pulled from Incubator						
Trays Read						

Revised 02/10/11

Appendix F:

Region 1 Microbiology Comparison Counting Worksheets

Colilert Comparison Counting Worksheet

Revised 10 March 2011

Total Coliform									
	Large Wells					Small Wells			
	1	2	3	4		1	2	3	4
1					1				
2					2				
3					3				
4					4				
5					5				
6					6				
7					7				
8					8				
9					9				
10					10				
11					11				
12					12				
13					13				
14					14				
15					15				
16					16				
17					17				
18					18				
19					19				
20					20				
21					21				
22					22				
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32					32				
33					33				
34					34				
35					35				
36					36				
37					37				
38					38				
39					39				
40					40				
41					41				
42					42				
43					43				
44					44				
45					45				
46					46				
47					47				
48					48				
49									

E. coli									
	Large Wells					Small Wells			
	1	2	3	4		1	2	3	4
1					1				
2					2				
3					3				
4					4				
5					5				
6					6				
7					7				
8					8				
9					9				
10					10				
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40					40				
41					41				
42					42				
43					43				
44					44				
45					45				
46					46				
47					47				
48					48				
49									

Sample ID

Date of Comparison Count

Analyst ID

Time From Start of Incubation

Results:

Total Coliform		
	Large Wells	Small Wells
1		
2		
3		
4		

E. coli		
	Large Wells	Small Wells
1		
2		
3		
4		

MPN Totals	
Total Coliform	
E. coli	

Comparison Counting Key:
1 = Dark Yellow/Dark Fluorescence
2 = Light Yellow/Light Fluorescence (Need Comparator to Determine)
3 = Colorless/No Fluorescence
4 = Colorless/No Fluorescence (Need Comparator to Determine)

Directions:

Complete the information for Sample ID, Analyst ID, and Time from start of incubation. Place the Quantitray-2000 numbered overlay over the tray to be used for Comparison Counting. Match the well to be analyzed with the well type and number identified on this worksheet. Using the Comparison Counting Key, place a "1" in the column that best fits your interpretation of the well. Once all wells have been analyzed for total coliform and *E. coli*, tally the number of each well fitting each description, and enter the result in the corresponding "Results" section. Analyst results should be within 90% of each other. For results outside this range, consult with the Program Manager for guidance.

Enterolert Comparison Counting Worksheet

Revised 10 March 2011

Enterococcus																			
	Large Wells					Small Wells						Large Wells					Small Wells				
	1	2	3	4		1	2	3	4			1	2	3	4		1	2	3	4	
1					1					1	1					1					1
2					2					2	2					2					2
3					3					3	3					3					3
4					4					4	4					4					4
5					5					5	5					5					5
6					6					6	6					6					6
7					7					7	7					7					7
8					8					8	8					8					8
9					9					9	9					9					9
10					10					10	10					10					10
11					11					11	11					11					11
12					12					12	12					12					12
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15					15					15	15					15					15
16					16					16	16					16					16
17					17					17	17					17					17
18					18					18	18					18					18
19					19					19	19					19					19
20					20					20	20					20					20
21					21					21	21					21					21
22					22					22	22					22					22
23					23					23	23					23					23
24					24					24	24					24					24
25					25					25	25					25					25
26					26					26	26					26					26
27					27					27	27					27					27
28					28					28	28					28					28
29					29					29	29					29					29
30					30					30	30					30					30
31					31					31	31					31					31
32					32					32	32					32					32
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35					35					35	35					35					35
36					36					36	36					36					36
37					37					37	37					37					37
38					38					38	38					38					38
39					39					39	39					39					39
40					40					40	40					40					40
41					41					41	41					41					41
42					42					42	42					42					42
43					43					43	43					43					43
44					44					44	44					44					44
45					45					45	45					45					45
46					46					46	46					46					46
47					47					47	47					47					47
48					48					48	48					48					48
49											49										

Sample ID

Date of Comparison Count

Analyst ID

Time From Start of Incubation

Results:

Enterococcus		
	Large Wells	Small Wells
1		
2		
3		
4		

MPN Totals

Enterococcus

Comparison Counting Key:
1 = Dark Yellow/Dark Fluorescence
2 = Light Yellow/Light Fluorescence (Need Comparator to Determine)
3 = Colorless/No Fluorescence
4 = Colorless/No Fluorescence (Need Comparator to Determine)

Directions:

Complete the information for Sample ID, Analyst ID, and Time from start of incubation. Place the Quantitray-2000 numbered overlay over the tray to be used for Comparison Counting. Match the well to be analyzed with the well type and number identified on this worksheet. Using the Comparison Counting Key, place a "1" in the column that best fits your interpretation of the well. Once all wells have been analyzed for total coliform and *E. coli*, tally the number of each well fitting each description, and enter the result in the corresponding "Results" section. Analyst results should be within 90% of each other. For results outside this range, consult with the Program Manager for guidance.

Appendix G:

Region 1 Microbiology Laboratory Certification

YET TO BE AWARDED

Appendix G – Definitions

Action levels: A measured concentration of a hazardous chemical at which certain actions such as medical surveillance or routine air sampling are required if a person is exposed, or has the potential to be exposed to the chemical in the measured concentration. This value is determined by OSHA and/or NIOSH and is typically half of the published PEL or TLV.

Acutely toxic chemicals: Chemicals that are lethal to 50% of the test population at doses equal to or less than 50 milligram per kilogram body weight (LD50 <50mg/kg).

Administrative controls: Work procedures, such as written safety policies, rules, supervision, and training, with the goal of reducing the duration, frequency, and severity of exposure to hazardous chemicals or situations.

Aerosol: Tiny particles or droplets suspended in air.

Biohazardous material: All infectious agents, vectors known to carry and transmit infectious agents, infected or potentially infected animals, infectious material, recombinant DNA, and biologically-derived toxins that present either a risk or a potential risk to the health of humans, animals, or plants either directly through infection or indirectly through damage to the environment.

Biosafety: A concept that promotes safe laboratory practices, procedures, and proper use of containment equipment and facilities by laboratory personnel in the research and instructional laboratory environment. The purpose of a biological safety program is to prevent laboratory-acquired infections.

Ceiling limit: The maximum concentration or dose of a hazardous chemical that a person should never be exposed to for any period of time.

Chemical emergency: An equipment failure, rupture of containers, or failure of control equipment that results in an uncontrolled release of a hazardous chemical into the workplace.

Chemical waste: Solid or liquid laboratory waste containing chemicals that must be disposed of through chemical waste management contract.

Chronically toxic chemical: A chemical that can produce adverse health effects through repeated exposure. Long term exposure to chronically toxic chemicals can result in localized or systemic damage.

Code of Federal Regulations (CFR): The codification of the general and permanent rules and regulations published in the Federal Register by the executive departments and agencies of the Federal Government.

Corrosive: Having a pH less than 2 or greater than 12.5 or the ability to damage or destroy body tissue upon contact.

Decontamination: Process by which contaminated surfaces, equipment, instruments, or waste are rendered non-hazardous.

Designated area: An area which may be used for work. A designated area may be the entire laboratory or an area of a laboratory.

Dose: Amount of a toxic substance that is absorbed by an individual. Dose is reported in milligrams (mg) of toxicant per kilograms (kg) of body weight (mg/kg) for acute exposures and in mg/kg per day for repeat-dose exposures.

Engineering Controls: Controls that eliminate or reduce exposure to laboratory hazards through the use or substitution of engineered machinery or equipment. Examples include, fire safety, lighting, floors, laboratory bench tops, fume hoods, and safety equipment.

Explosive Chemical: A chemical that causes a sudden, almost instantaneous release of pressure, gas, and heat when subjected to sudden shock, pressure, or high temperature.

Exposure incident: An event that results in contact with a hazardous substance via one of the following routes: inhalation, ingestion, absorption, percutaneous injury, or contact with mucous membranes (eyes, nose, mouth) or with non-intact skin.

Flammable liquids: Chemicals with a flashpoint less than 60°C (140°F).

Flashpoint: Temperature at which the vapor of a chemical is capable of being ignited momentarily.

Hazardous chemical: A chemical for which there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees. The term "health hazard" includes chemicals which are carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents that act on the hematopoietic system and agents that damage the lungs, skin, eyes, or mucous membranes.

Hazardous material: A substance or material which has been determined by the Secretary of Transportation to be capable of posing an unreasonable risk to health, safety, and property when transported.

Hazardous substance: Any material that may present a danger to human health and welfare or the environment. This includes hazardous chemicals, biohazardous materials, and sources of ionizing radiation.

Hazardous waste: A waste with properties that make it dangerous or potentially harmful to human health or the environment and exhibits at least one of four characteristics: ignitability, corrosivity, reactivity, or toxicity.

Infectious agents: All human, animal, and plant pathogens (bacteria, parasites, fungi, viruses, prions).

Infectious material: Infectious agents and all biological material that contains or has the potential to contain infectious agents. Examples of infectious material include human blood and blood components, human tissues and body fluids, cultured cells (from humans and non-human primates), infected animals and animal tissues, non-human primates and any tissues from non-human primates (can transmit Herpes B virus), sheep and any tissues derived from sheep (can transmit Coxiella burnetti, causative agent of Q-fever), and environmental samples likely to contain infectious agents.

Laboratory: A facility where the "laboratory use of hazardous chemicals" occurs. It is a workplace where relatively small quantities of hazardous chemicals are used on a non-production basis.

Laboratory personnel: staff (classified, wage, and student wage), affiliates (visiting faculty, volunteers, visiting research associates), and students (graduate students, undergraduate students, laboratory assistants, etc.) working in laboratories and laboratory support areas.

Lethal dose 50 (LD50): Quantity of material than when ingested, injected, or applied to the skin as a single dose will cause death of 50% of test animals who are exposed to it: The test conditions should be specified; the value is expressed in g/kg or mg/kg of body weight.

Local effect: Health effect restricted or limited to a specific body part or region.

Material Safety Data Sheet (MSDS): A standard formatted information sheet prepared by a material manufacturer, describing the potential hazards, physical properties, and procedures for safe use of a material. A binder located in the laboratory that contains MSDS for each chemical and biohazardous material present in the laboratory.

Organic peroxide: An organic compound that contains the bivalent -O-O- structure and which may be considered to be a structural derivative of hydrogen peroxide where one or both of the hydrogen atoms has been replaced by an organic radical.

Oxidizing material: A chemical other than a blasting agent or explosive as defined in § 1910.109(a), that initiates or promotes combustion in other materials, thereby causing fire either of itself or through the release of oxygen or other gases.

Particularly Hazardous Substances: A subset of hazardous chemicals that OSHA has identified as requiring special consideration and additional safety provisions, because of their toxic effects. Select carcinogens, reproductive toxins, and substances with a high degree of acute toxicity are Particularly Hazardous Substances.

Percutaneous injury: Injury resulting from contact with a sharp object, an animal scratch or bite, or through wounds, abrasions, or eczema.

Permissible Exposure Limits (PEL): Limits set by OSHA to protect personnel against the health effects of exposure to hazardous substances. PEL are regulatory limits on the amount of concentration of a substance in the air. They may also contain a skin designation. PEL are enforceable. OSHA PEL are based on an 8-hour time weighted average (TWA) exposure.

Personal Protective Equipment (PPE): Clothing and other work accessories designed to create a barrier against workplace hazards.

Physical hazard: Items that cause bodily harm or damage resulting from an exchange of thermal, electrical, mechanical, or other energy that exceeds the body's tolerance.

Physical properties: Characteristics of a substance such as melting point, freezing point, specific gravity, density, that cannot be changed without chemically altering the substance.

Reactive: A chemical which in the pure state, or as produced or transported, will vigorously polymerize, decompose, condense, or will become self-reactive under conditions of shocks, pressure or temperature.

Recommended Exposure Level (REL): The maximum average air concentration that a worker can be exposed to for an 8 hour work day, 40 hour work week for a working lifetime (40 years) without experiencing significant adverse health effects.

Restricted area: Area that contains unique hazards and therefore requires more stringent access restrictions.

Secondary containment: A system or container that is capable of capturing any material that is discharged or has leaked from the primary container to prevent exposure, contact with the environment, or damage property for the anticipated period of time necessary to detect and recover the discharged material.

Sensitizers: A chemical that causes a substantial proportion of exposed people or animals to develop an allergic reaction in normal tissue after repeated exposure to the chemical.

Short Term Exposure Limit (STEL): The maximum concentration personnel can be exposed to for fifteen minutes without suffering from irritation, chronic or irreversible tissue damage, or narcosis of sufficient degree to cause impairment.

Threshold Limit Value (TLV): Guidelines prepared by the ACGIH to assist industrial hygienists in making decisions regarding safe levels of exposure to various hazards found in the workplace. A TLV reflects the level of exposure that a typical worker can experience without an unreasonable risk of disease or injury. TLV are not quantitative estimates of risk at different exposure levels or by different routes of exposure.

Toxicity: The ability of a chemical to cause an undesirable effect in a biological system.

Time Weighted Average (TWA): The concentration of an airborne chemical averaged over an eight-hour workday to which personnel may be exposed to daily without sustaining injury.

Vapor pressure: The pressure exerted by a vapor in equilibrium with the solid or liquid phase of the same substance. The partial pressure of the substance in the atmosphere above the solid or liquid.

Appendix H – Abbreviations and Acronyms

ASTM	American Society for Testing and Materials
COC	Chain of Custody
CRM	Certified Reference Material
DHS	California Department of Health Services
DI	Deionized
DMT	Data Management Team
DQO	Data Quality Objective
ELAP	Environmental Laboratory Accreditation Program
EPA	U.S. Environmental Protection Agency
IMS	Information Management System
LCS	Laboratory Control Sample
LRB	Laboratory Reagent Blank
LSM	Laboratory Safety Manual
MB	Method Blank
MDL	Method Detection Limit
MPN	Most Probable Number
MQO	Measurement Quality Objective
MS	Matrix Spike
MSD	Matrix Spike Duplicate
MSDS	Material Safety Data Sheet
NCRWQCB	North Coast Regional Water Quality Control Board
n/a	Not Applicable
NIST	National Institute of Standards and Technology

PE	Proficiency Evaluation
QA	Quality Assurance
QAP	Quality Assurance Plan
QAPP	Quality Assurance Project Plan
QAPrP	Quality Assurance Program Plan
QC	Quality Control
QMP	Quality Management Plan
RL	Reporting Limit
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
RWQCB	Regional Water Quality Control Board
SOP	Standard Operating Procedure
SWAMP	Surface Water Ambient Monitoring Program
SWRCB	State Water Resources Control Board
TMDL	Total Maximum Daily Load

Appendix I: Online Resources

SWAMP Information Management System Documentation:

http://mpsl.mlml.calstate.edu/swdbase.htm

SWAMP Data Submission Documentation:

http://mpsl.mlml.calstate.edu/swdataformats.htm

Documents pertaining to SWAMP IMS data submission formats and conventions

Regional SWAMP Report Templates:

http://mpsl.mlml.calstate.edu/SWAMP_Regional_Report_QA_Section_Template_022908.doc

Narrative and tabular templates for the QA section of regional SWAMP reports

Appendix 8:

Sonoma County Laboratory Quality Assurance Plan