Regional Water Quality Control Board
North Coast Region

Russian River Human Impact Study

Quality Assurance Project Plan

Version 1.0

Originated by:

Steve Butkus

North Coast Regional Water Quality Control Board

November 7, 2012

Group A: Project Management

A1: Title and Approval Sheet

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

Lead Organization: North Coast Regional Water Quality Control Board
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Effective Date: November 7, 2012

Approvals

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

See Appendix 12: Approval Sheet Signatures

Signature Date

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

See Appendix 12: Approval Sheet Signatures

Signature Date

Renee Spears, State Board Quality Assurance Officer
California State Water Resources Control Board

See Appendix 12: Approval Sheet Signatures

Signature Date

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

See Appendix 12: Approval Sheet Signatures

Signature Date

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

See Appendix 12: Approval Sheet Signatures

Signature Date

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

See Appendix 12: Approval Sheet Signatures

Signature Date

Yeggie Dearborn, Lab Director
Cel Analytical Laboratories

See Appendix 12: Approval Sheet Signatures

Signature Date

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

See Appendix 12: 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;** **Contract Manager** Main Contact: Steve Butkus Phone: 707-576-2834 Email: SButkus@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Project Quality Assurance Officer; Main Contact: Rich Fadness Phone: 707-576-6718 Email: RFadness@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Lawrence Berkeley National Laboratory; **Laboratory Director** Main Contact: Gary Anderson Phone: 510-495-2795 Email: glandersen@lbl.gov	Lawrence Berkeley National Laboratory Ecology Department One Cyclotron Road, MS 70A-3317 Berkeley, CA 94720
Cel Analytical Laboratories; **Laboratory Director** Main Contact: Yeggie Dearborn Phone: 415-882-1690 Email: yeggie@celanalytical.com	Cel Analytical, Inc. 82 Mary Street, Suite #2 San Francisco, CA 94103
Sonoma County Public Health Laboratory - **Laboratory Director** Main Contact: Michael Ferris Phone: 707-565-4711 Email: MFERRIS@sonoma-county.org	County of Sonoma Department of Public Health Services 3313 Chanate Road Santa Rosa, CA 95404

A4: Project/Task Organization

A monitoring study has been initiated by the North Coast Regional Water Quality Control Board.

The North Coast Regional Water Board will be responsible for the collection of samples for the analysis of *E. coli*, total coliform, *Enterococcus*, *Bacteroides*, stable isotope analyses of nitrate for relative source differences in oxygen (δ18O) and nitrogen (δ15N), and fast DNA microarray (Phylochip) samples.

The North Coast Regional Water Board will be conducting laboratory analysis to include *E. coli*, total coliform, and *Enterococcus.* Sonoma County Public Health Laboratory will be responsible for the analysis of *Bacteroides* samples. Cel Analytical Laboratories will be responsible for the analysis of stable isotope analyses of nitrate samples. Lawrence Berkeley National Laboratory (LBNL) will be responsible for the analysis of Phylochip samples.

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

Table 2. Personnel responsibilities.

Name	Project Title	Organizational Affiliation	Contact Information (Telephone number, fax number, email address.)
Steve Butkus	Project Manager; Data Manager; Contract Manager	North Coast Regional Water Board	(707)-576-2834 (707)-523-0135 sbutkus@waterboards.ca.gov
Rich Fadness	Project QA Officer	North Coast Regional Water Board	(707)-576-6718 (707)-523-0135 rfadness@waterboards.ca.gov
Charles Reed	Laboratory Co-Director 1	North Coast Regional Water Board	(707)-576-2752 (707)-523-0135 creed@waterboards.ca.gov
Caryn Woodhouse	Laboratory Co-Director 2	North Coast Regional Water Board	(707)-576-2701 (707)-523-0135 cwoodhouse@waterboards.ca.gov
Gary Anderson	Contract Lab Director	Lawrence Berkeley National Laboratory	510-495-2795 510-486-7152 glandersen@lbl.gov
Yvette Piceno	Contract Lab QA Officer	Lawrence Berkeley National Laboratory	510-486-4498 510-486-7152 ympiceno@lbl.gov
Yeggie Dearborn	Contract Lab Director	Cel Analytical Laboratories	415-882-1690 415-882-1685 yeggie@celanalytical.com
Steven Tan	Contract Lab QA Officer	Cel Analytical Laboratories	415-882-1690 415-882-1685 steven@celanalytical.com
Michael Ferris	Contract Lab Director	Sonoma County Department of Public Health Services	(707)-565-4711 (707)-565-7839 mferris@sonoma-county.org
Lisa Critchett	Contract Lab QA Officer	Sonoma County Department of Public Health Services	(707)-565-4711 (707)-565-7839 mferris@sonoma-county.org

North Coast Regional Water Board

Project Manager - Data Manager – Contract Manager

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

Project Quality Assurance (QA) Officer

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

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)

Field Personnel

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

Lawrence Berkeley National Laboratory (LBNL)

Ecology Department - Laboratory Director

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

LBNL Ecology Department - Quality Assurance Officer

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

Cel Analytical Laboratories, Inc.

Commercial Laboratory Director

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

Commercial Laboratory Quality Assurance Officer

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

County of Sonoma

Sonoma Department of Health Services- Public Health Services Laboratory Director

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

Public Health Services Laboratory Quality Assurance Officer

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

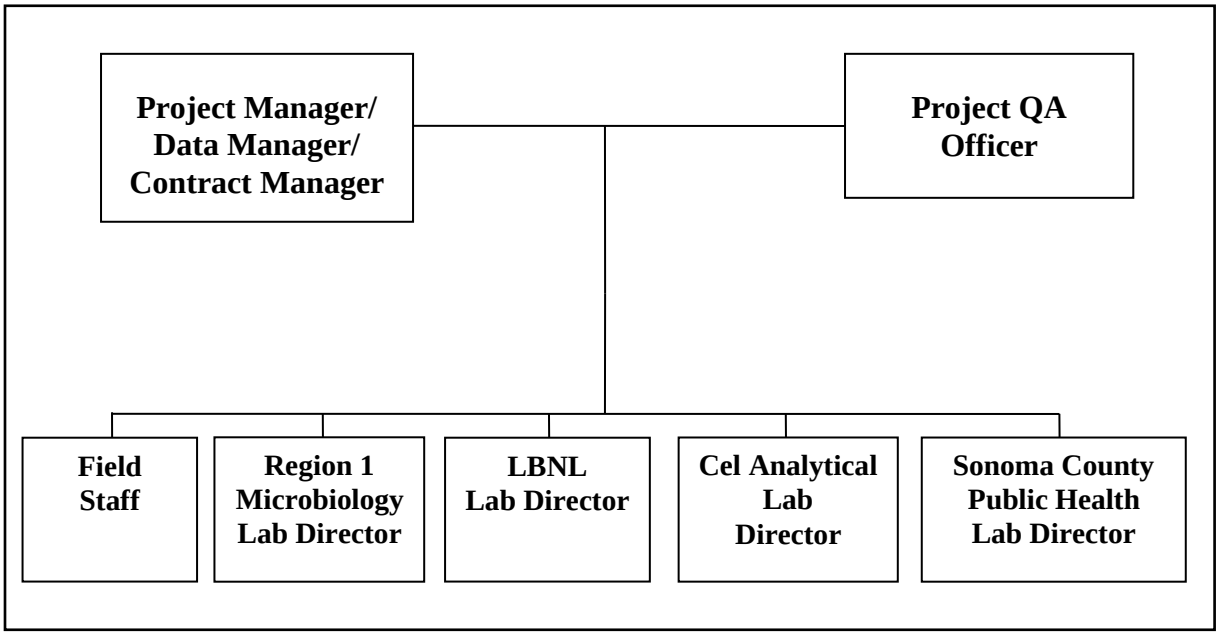


Figure 1. Project Organizational Chart

A5: Problem Definition/Background

Problem Statement.

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

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

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

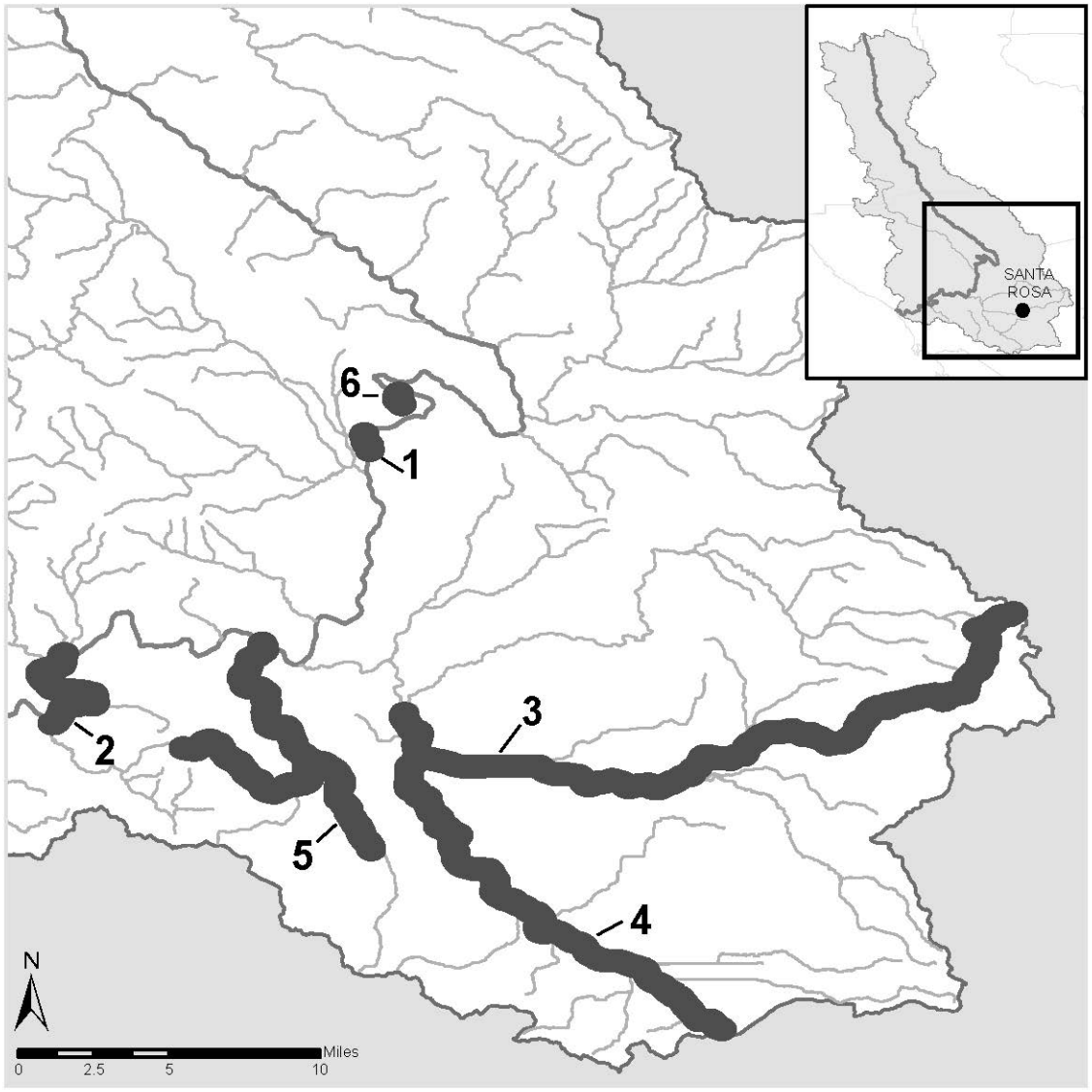


Figure 2. Russian River Watershed Surface waters impaired for REC-1

Human activity has been identified as a possible cause of the impairments:

- 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,
- Many residences and facilities along the watercourse are not supplied with sanitary sewers, and may have inadequate waste disposal capacity.

Russian River Pathogens TMDL – Preliminary Findings

North Coast Regional Water Board staff conducted a source analysis study for the development of the Russian River TMDL. The study was organized into four individual tasks and sampling plans to collect information which will address the identified management questions (NCRWQCB 2011). Task 1 evaluated the temporal and spatial variability of indicator bacteria at

high use public recreation beaches. Tasks 2 and 3 evaluated the influence of land use and beach recreational use on pathogen indicator concentrations. Results of the study were documented in report (NCRWQCB 2012). However, this report excluded the microbiome community analysis by the Lawrence Berkeley National Laboratory using the Phylochip which has not yet been completed.

Several observations were made from the study results:

1. Pathogenic indicator bacteria concentrations were higher during wet periods compared to dry periods
2. Pathogenic indicator bacteria concentrations were higher in the tributaries during wet periods than in the mainstem Russian River.
3. Human-source *Bacteroides* was detected in all sample locations and land use categories throughout the watershed.
4. Human-source *Bacteroides* was highest at Steelhead Beach and Forestville Access Beach during dry periods and at Santa Rosa Creek along the Prince Memorial Greenway during wet periods.
5. Bovine-source *Bacteroides* was uniformly low throughout the watershed except at Steelhead Beach and Forestville Access Beach during wet periods.
6. Stable Isotope Analysis results showed that the dominant sources of source water for bacteria samples are manure and septic wastes.
7. During wet periods, pathogenic indicator bacteria concentrations were modestly higher in urban sewered areas and areas with OWTS compared to less developed areas.
8. Human-source Bacteroides was significantly highest in the agricultural land designation.
9. Human-source Bacteroides was modestly higher in onsite septic areas compared to urban sewered areas.
10. No differences were observed in pathogenic indicator bacteria concentrations during high intensity recreational use.

Several logistical reasons have been identified for the tenth observation that did not match the working hypothesis for Task 3 (i.e., the data indicate no differences in pathogenic indicator bacteria concentrations due to high intensity recreational use). First, all the samples were collected at the beach location early in the morning (i.e, ~08:00) before any significant human contact for at least 10 hours. Second, the Sonoma County Water Agency removed the seasonal dam at Vacation Beach Road the Monday morning following the high intensity weekend recreational use. This dam is located between the two recreational beaches sampled and Regional Water Board staff were unaware of the planned removal. The results were confounded by the unusual temporary hydrology caused by the dam removal (i.e. flow velocity rose while the stage dropped). Therefore, Regional Board staff have proposed to conduct Task 3 again while addressing the identified logistical issues.

Decisions or Outcomes.

Goals of the Russian River Human Impact Study include:

- Collection of the information to demonstrate the degree of pathogenic indicator bacteria contributed by human sources.

- Advise the TMDL Allocation Process for developing mitigation strategies for reduction in pathogenic indicator organisms.

Monitoring tasks were identified for the following two management questions:

1. Do catchments with high density of on-site wastewater treatment (OSWT) systems contribute pathogenic indicator bacteria from human sources?
2. Do recreational beach areas contribute pathogenic indicator bacteria from human sources?

This monitoring plan is organized into two tasks to collect information to address these management questions. The data collected will be assessed with a number of statistical methods to help answer the monitoring questions. The samples will be collected concurrently with the fecal indicator bacteria samples. 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 non-parametric method 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 Policy (SWRCB, 2004) for assessing information for identifying impaired beneficial uses 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

Recent studies conducted by the Southern California Coastal Water Research Project show little to no epidemiological connection between conventional indicator bacteria and regulatory criteria. Criteria for REC-1 may be exceeded due to natural sources, especially in geographic areas with

no storm drains, or sewage systems. Analysis of the entire microbiome community will help advise the TMDL development process for developing mitigation strategies for reduction in pathogenic indicator organisms from human sources.

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 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 the development of the TMDL.

Constituents To be Monitored and Measurement Techniques.

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®.

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

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

Project Schedule

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

Table 3. Project Schedule Timeline.

Activity	Date		Deliverable	Deliverable Due Date
	Anticipated Date of Initiation	Anticipated Date of Completion		
Collect and process water samples	November 2012	October 2013	Lab Data Reports	Continuous
Draft Monitoring Plan Data Report	November 2013	January 2014	Draft Report	January 2014
Final Monitoring Plan Data Report	March 2014	June 2014	Final Report	June 2014

Geographical setting

The study area is the middle and lower Russian River watershed bounded by the Alexander Valley Bridge (upstream of Healdsburg) to the mouth at the Pacific Ocean (near Jenner).

Constraints

Water samples will be collected following a wet weather conditions. Only two of the sampling locations are perennial springs. The other thirteen sampling locations will be collected following a storm event. During storm events, runoff from the landscape surface can flood OSWT systems resulting in the direct transport of untreated human waste to surface waters. This transport occurs in ephemeral streams that exist only for a short period following a storm event. Samples will be collected from perennial, intermittent and ephemeral streams to assess these different transport mechanisms. Estimation of the hydrograph will be made by observation of nearby stream flow gage data operated by the U.S. Geological Survey available in real time on the internet (http://waterdata.usgs.gov/ca/nwis/current/?type=flow).

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

A7: Quality Objectives and Criteria for Measurement Data

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

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

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

Accuracy

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

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

Precision

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

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

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

Completeness

Completeness refers to the amount of acceptable quality data collected as compared to the amount needed to ensure that the uncertainty or error is within acceptable limits. It is expressed

as a percentage of the number of valid measurements that should have been collected. Data quality objectives require 90 percent completeness as shown in Table 4.

Sensitivity

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

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

Bias

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

Representativeness

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

Comparability

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

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

Table 4. Data quality objectives for laboratory measurements.

Parameter	Method	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Microbiome Community	Fast DNA microarray (Phylochip)	Spike-in hybridization controls, positive and negative control probes	Precision will not be assessed.	-	0.001% 16S rRNA gene PCR product	-
Bacteroides	Quantitative PCR	Proper positive and negative response	Matching duplicates on 10% of samples	-	-	90%
Nitrate-N	SM 4500 NO3 E	Standard Reference Material within 95% CI stated by provider of material.	Laboratory split sample 25% RPD	Matrix spike 80% - 120%	0.01 mg/L	90%
δ18O and δ15N	Stable isotope analysis	Check standards fall within precision limits of calibrated value (relative to IAEA)	15N: <0.4 per mL 18O: <0.8 per mL	-	1uM NO3	-
E. Coli.	Colilert®	Positive results for target organisms. Negative results for non-target organisms	Rlog within 3.27*mean Rlog	-	1 MPN/100 mL	90%
Enterococcus	Enterolert®					

A8: Special Training and Certification

Specialized Training or Certifications.

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

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

Training and Certification Documentation.

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

A9: Documents And Records

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

QAPP Updates and Distribution

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

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

Standard Operating Procedures

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

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

Documentation of Data Collection (Field) Activities

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

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

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

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

Documentation of Analytical (Laboratory) Activities

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

Laboratory Records

Each laboratory Lab Director will be responsible for documenting and tracking the aspects of samples receipt and storage, analyses, and reporting. Upon completion of laboratory analysis, laboratory data review, and data validation, the laboratory will issue a report in an electronic format describing the results of analysis for each sample submitted. Prior to issuance of the laboratory report, the laboratory's QA manager will review and approve the report. To assure

that water quality information will be available in a time frame that will allow public health advisories to be issued in a timely manner, preliminary laboratory results should be transmitted to the Project Manager within 24 hours.

Components of the laboratory report include:

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

Chain of Custody

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

Electronic Data

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

GROUP B: DATA GENERATION AND ACQUISITION

B1: Sampling Process Design

The study is organized into two individual tasks and sampling plans to collect information which will address the identified management questions. Task 1 evaluates the contribution of pathogenic indicator bacteria from areas with a high density of OSWT systems. Task 2 evaluates the influence of beach recreational use on pathogen indicator concentrations.

Task 1: On-Site Wastewater Treatment System Impact

Task 1 is designed to answer the following management question:

1. Do catchments with high density of on-site wastewater treatment (OSWT) systems contribute pathogenic indicator bacteria from human sources?

The assessment for the Russian River TMDL monitoring data collected in 2011-2012 (NCRWQCB 2012) identified the need to conduct a more robust assessment of the human contribution to exceedance of pathogenic indicator bacteria criteria. Areas that drain from catchments that have a high density of OSWT systems will be compared to catchments with a low density of OSWT systems. Three catchments of interest were also included. Fifteen (15) sample locations were selected to represent both high-density and low-density catchments throughout the study area.

Pathogenic indicator bacteria are transported to surface waters from failing OSWT systems during both wet and dry periods. During dry weather periods, perennial streams can be impacted by human-source pathogenic bacteria that migrate through shallow groundwater from failing OSWT systems. The shallow groundwater flows enter surface waters through springs or the stream hyphoreic zone. During storm events, runoff from the landscape surface can flood OSWT systems resulting in the direct transport of untreated human waste to surface waters. This transport occurs in ephemeral streams that exist only for a short period following a storm event. Samples were collected from both perennial, intermittent and ephemeral streams to assess these different transport mechanisms.

Sample Collection

Assessment of the OWTS impacts within the middle and lower Russian River will be conducted by collecting samples at each of the fifteen (15) listed stations in Table 5 and mapped in appendix 1. Field crews will locate these sampling locations with the use of the sampling location maps in Appendix 1, or by GPS if needed.

Table 5. Sampling Locations for Task 1

Category	Site ID	SWAMP ID	Latitude	Longitude	Location Description
High Parcel Density High Risk	Site 1	114DFMR68	38.6131	-122.8410	1740 Fitch Mtn Road - west of Villa Anna (Healdsburg)
	Site 2	114C01EDR	38.4776	-122.9762	River Road - culvert 100' east of Duncan Road (Monte Rio)
	Site 3	114C02SPR	38.5063	-121.0735	River Drive at Summerhome Park Road (Forestville)
High Parcel Density Low Risk	Site 4	114C03OMR	38.4781	-121.0018	19375 Old Monte Rio Road (across street from Northwood golf course)
	Site 5	114CO4TRF	38.4903	-121.1022	8612 Trenton Road (Forestville)
	Site 6	114DDRC59	38.4978	-121.0979	Along west shoulder of Del Rio Court (Forestville)
Low Parcel Density High Risk	Site 7	114C05MNS	38.4581	-122.9891	9632 Main Street (Monte Rio)
	Site 8	114C06VRG	38.5059	-121.0423	12656 River Road at Von Renner Grading (near Rio Nido)
	Site 9	114C07MRC	38.4575	-122.9531	Moscow Road box culvert - 100' west of 'Right Curve' sign (near Cassini Campgound)
Low Parcel Density Low Risk	Site 10	114CO8FRS	38.6561	-121.1264	Fredson Road south of Salvation Army driveway (Healdsburg)
	Site 11	114C09WDC	38.6467	-121.0805	3654 West Dry Creek Road (Healdsburg)
	Site 12	114C10AVR	38.6509	-121.1316	148 Alexander Valley Road (Healdsburg)
Areas of Concern	Site 13	114C11RDH	38.6238	-122.8452	West end of Redwood Drive (Healdsburg)
	Site 14	114C12FSM	38.4702	-122.9850	9632 Main Street (Monte Rio)
	Site 15	114C13LSA	38.4252	-121.0399	Lakeside Ave at Market Street (Camp Meeker)

Samples for analysis will be collected from each location five (5) times during the study period. The resulting sample size will be a total of 75 stream samples. Triplicate samples will be collected once from each sampling location during the study to assess sampling variability for an additional 30 samples.

One sample per location and sampling event will be collected and filtered for Phylochip analysis. A subset of the filtered samples will be analyzed with the Phylochip. The selection of the sample subset will be based on the results of the other bacteria analyses with a focus on samples with high measured bacteria concentrations.

Sample blanks will be collected during each sample event estimated at five (5) blanks. For the blank samples, sterile water will be poured into the sample container in the field. For the Phylochip samples, sterile water will be poured into the sample container in the field and subsequently filtered in the North Coast Regional Water Board laboratory. No sample blanks will be collected for stable isotope analysis or nitrate-N analyses.

Samples will be collected subsequently at each location at each reach for the analyses and labs listed below:

- 110 samples to Region-1 Microbiology Laboratory for Colilert®, plus 1 sample blank filtered per event (estimated 5 blanks) for a total of 115 samples.
- 110 samples to Region-1 Microbiology Laboratory for Enterolert®, plus 1 sample blank filtered per event (estimated 5 blanks) for a total of 115 samples.
- 110 samples to Sonoma County Public Health Laboratory for the analysis of *Bacteroides bacteria* (universal and human host), plus 1 sample blank filtered per event (estimated 5 blanks) for a total of 115 samples.
- 75 samples to Lawrence Berkeley National Laboratory (LBNL) for the analysis of the microbiome community with the Phylochip, plus 1 sample blank filtered per event (estimated 5 blanks) for a total of 80 sample filters.
- 75 samples to Cel Analyitcal Laboratories for the analysis of stable isotope analyses of nitrate samples. No blanks samples will be collected for a total of 75 samples.

Task 2: Beach Recreational Impacts

Task 2 is designed to answer the following management question:

- Do recreational beach areas contribute pathogenic indicator bacteria from human sources?

The assessment for the Russian River TMDL monitoring data collected in 2011-2012 (NCRWQCB 2012) identified the need to conduct a more robust assessment of the human contribution to the exceedance of pathogenic indicator bacteria criteria. The objective of this task is to assess the relative magnitude and variability of indicator bacteria levels that may be associated with increased human recreation use at public beach areas.

Sample Collection

Intensive sampling events will be conducted to assess the local impact of recreational activities on indicator bacteria levels at public beaches. Sample collection dates are planned for Tuesday, July 2, 2013 through Tuesday, July 9, 2013. Samples will be collected in the afternoon when recreational use is highest. Recreational use of these beaches during Independence Day holiday is expected to be relatively large. Samples will be collected at Veteran's Memorial Beach in Healdsburg and Monte Rio Beach in Monte Rio. Field crews will locate these sampling locations with the use of the reach maps in Appendix 4, or by GPS if needed.

Table 6. Sampling Locations for Task 2

Station ID	Station Name	Location	Latitude	Longitude
114RR2940	Veteran's Memorial Beach	Old Redwood Hwy	38.604650	-121.122922
114RR0898	Monte Rio Beach	Bohemian Hwy	38.466258	-122.990628

Triplicate samples will be collected at both beaches each day for 8 days for a total of 48 samples. Triplicate samples will be collected to assess sampling variability. Sample blanks will be collected during each sample date for a total of eight (8) blank samples. For the blank samples, sterile water will be poured into the sample container in the field. For the Phylochip samples, sterile water will be poured into the sample container in the field and subsequently filtered in the North Coast Regional Water Board laboratory. No sample blanks will be collected for stable isotope analysis or nitrate-N analyses. Due to budget limitations, no sample blanks will be collected for Bacteroides analyses and possible sample contamination will be assessed using the fecal bacteria blank samples.

One sample per location and sampling event will be collected and filtered for Phylochip analysis. A subset of the filtered samples will be analyzed with the Phylochip. The selection of the sample subset will be based on the results of the other bacteria analyses with a focus on samples with high measured bacteria concentrations.

The resulting sample size will be a total of 56 samples, including blanks. Samples will be collected subsequently at each location at each beach for the analyses and labs listed below:

- 48 samples to Region-1 Microbiology Laboratory for Colilert®, plus 8 blank samples for each day, for a total of 56 samples.
- 48 samples to Region-1 Microbiology Laboratory for Enterolert®, plus 8 blank samples for each day, for a total of 56 samples.
- 16 samples to Lawrence Berkeley National Laboratory (LBNL) for the analysis of the microbiome community with the Phylochip. Blank lab samples will be filtered each day. 16 samples collected during 8 sampling events, plus 8 blank filtered samples for each day, for a total of 24 samples.
- 16 samples to Cel Analytical Laboratories for the analysis of stable isotope analyses of nitrate samples. 16 samples collected during 8 sampling events, with no blank samples, for a total of 16 samples.
- 16 samples to Sonoma County Public Health Laboratory for the analysis of Bacteroides. 16 samples collected during 8 sampling events, with no blank samples, for a total of 16 samples.

B2: SAMPLING METHODS

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

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

Field Preparation

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

Samples for *Enterococus* and *E. coli* analysis will be collected in a 125 ml, factory sterilized and sealed polyethylene bottle. Sample volumes will be approximately 100 mL.

Samples for *Bacteroides* will be collected in sterile 100-mL irradiated nuclease-free plastic containers supplied by the Sonoma County Public Health Laboratory. The containers are enclosed in a heat-sealed plastic bag. The container will be filled to the 100mL mark on the bottles. Total sample volumes will be approximately 100 mL.

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

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

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

Sample Preservation and Holding Times

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

Sample incubation times for *Enterococcus* 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.

Responsible Person

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

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

B3: Sample Handling and Custody

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

Maximum Holding Times

Samples will be immediately placed on ice in a cooler for transport to the laboratories after collection. All samples will be delivered at the end of the field run. Analysis will begin within the holding time specified in Table 8.

Sample Handling

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

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

The following sampling technique will be used for collection of *Bacteroides* samples. Ziploc® (or other brand) plastic bags will be used to store the sample containers after collecting the water sample. Field staff will cut open with scissors the plastic bag with the sterile 100-mL plastic containers. The container will be removed and sample 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. The sample container will then be placed in Ziploc® (or other brand) plastic bags and labeled accordingly. 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.

Table 7: Sampling and Preservation

Analyte	Units	Container	Sample Volume	Preservation	Maximum Holding Time
E. coli		125 mL Sterile Plastic container	100 mL	Cool to 6 ◦C in the dark.	6 hours
Enterococcus		125 mL Sterile Plastic container	100 mL	Cool to 6 ◦C in the dark.	6 hours
Phylochip Analysis	cells/mL	Three 125mL sterile, plastic containers	300 mL	Cool to 6 ◦C in the dark Samples to be filtered Freeze filters at -80°F	6 hours
Bacteroides	cells/mL	110mL	100mL	Cool to 6 ◦C in the dark Samples to be filtered Freeze filters at -80°F	6 hours
Nitrate-N	mg/L	Polypropylene	500 mL	Cool to 6 ◦C in the dark	48 hours
Stable isotope analysis	δ18O and δ15N	Polypropylene	30 mL	Samples to be filtered (min 0.2um nylon) and frozen.	60 days

Chain of Custody Procedures

Field measurements do not require specific custody procedures since they will be conducted on site at the sample collection location. All bacteria samples will be accompanied by chain of custody forms (see Appendix 3). At the time samples are transferred, both the person receiving

and relinquishing the samples should verify that all samples collected are reflected on the chain of custody forms. The condition of the samples will also be noted and recorded by the receiver. COC records will be included in the final administrative record as prepared by the analytical laboratories. Any deviations should be explained on the field sheets and chain of custody forms, as needed.

Transport

Samples will be stored in the dark in coolers on ice, at a temperature below 6°C. Samples received by the contract laboratories will be processed immediately upon receipt and within the specified holding time.

Samples to be analyzed for *Enterococcus*, total coliform and *E. coli* will be delivered to:
Region 1 Microbiology Laboratory
5550 Skylane Blvd, Suite A
Santa Rosa, Ca 95403
Tel: 707-576-2719

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

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

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

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

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

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

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

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

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

Responsible Individuals

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

B4: Analytical Methods

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

Table 8. Laboratory Analytical Methods.

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

Corrective Actions

When failures in the laboratory occur, the individual Laboratory Managers will each be responsible for corrections in their respective laboratories. All failures will be documented on the

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

B5: Quality Control

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

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

Quality Control Samples

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

Collection of Water Samples

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

Field Blank

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

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

Laboratory Blank

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

Laboratory Duplicates

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

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

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

prior knowledge, specific microbial interactions may be identified that are key to particular changing environments.

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

B6: Instrument/Equipment Testing, Inspection, and Maintenance

Microbiological sample bottles will be provided by the contract laboratories prior to the sampling events. Laboratory equipment will be inspected, calibrated, and maintained according to the individual laboratories QAP (see Appendices 5, 6 and 7).

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

B8: Inspection/Acceptance of Supplies and Consumables

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

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

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

Staff responsible for the ordering will inspect the supplies and consumable materials for quality, and will report any that do not meet acceptance criteria to the appropriate Laboratory Director and Project QA Officer. Upon receipt of materials, a designated employee receives and signs for the materials. The items are reviewed to ensure the shipment is complete, and they are then delivered to the proper storage location. Supplies are dated upon receipt, stored appropriately, and are discarded on expiration date. Confirmation that sample bottles are laboratory certified clean will be made when received and prior to use in the field.

B9: Non-Direct Measurements

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

The National Weather Service Quantitative Precipitation Forecast will be reviewed from the internet on a daily basis for the purpose of documenting weather conditions within the project area for sampling conditions. The National Weather Service website provides a website with diel maps of precipitation forecasts over the entire study area. The National Weather Service Quantitative Precipitation Forecast website address is:
http://www.cnrfc.noaa.gov/precipForecast.php?cwa=MTR&day=1&img=5

The National Weather Service Quantitative Precipitation Forecast will be used to inform the Project Manager when storm water runoff is likely to occur. The information will be assessed by the Project Manager to determine if a sampling event will occur. The Project Manager will inform field staff and the respective Laboratory Directors of a sampling event prior to sample collection to assure that the samples can be received.

B10: Data Management

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

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

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

Data/Information Handling and Storage

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

Computerized Information System Maintenance

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

GROUP C: Assessment and Oversight

C1: Assessments & Response Actions

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

Project Assessments

Readiness reviews will be conducted prior to each sampling run by the Project Manager. All sampling personnel will be given a brief review of the sampling procedures and equipment that will be used to achieve them. Readiness reviews will consist of the following activities:

Supply Checks

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

Paperwork Checks

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

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

Field Activity Audits

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

Annual assessments of field crews will be conducted to ensure that field sampling procedures outlined in this QAPP are followed. Prior beginning any field sampling activities, the Project Manager or Quality Assurance Officer will verify that proper equipment is available for all field personnel. This includes sampling equipment, safety equipment, and field measurement equipment. It will also be verified that all personnel involved in field activities have received sufficient training and are able to properly use the equipment and follow procedures. The Project Manager or Quality Assurance may also verify the application of procedures and equipment periodically. If the Project Manager or Quality Assurance Officer finds any deficiencies,

corrective actions will be put in place and reported, and follow-up inspections will be performed to ensure the deficiencies have been addressed. Field assessments will include:

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

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

Laboratory Audits

Laboratory assessments may involve two types of activities:

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

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

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

Corrective Action

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

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

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

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

C2: Reports to Management

Interim and Final Reports

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

Table 10 – Report Due Dates

Report Type	Frequency	Responsible Party	Schedule
Data Report	Per field run	Region-1 Microbiology Lab Director	On-going
Data Report	Per field run	Cel Analytical Lab Director	On-going
Data Report	Per field run	Sonoma Public Health Lab Director	On-going
Data Report	Per Batch Delivered	LBNL Lab Director	October 2013
Draft Monitoring Plan Data Report	N/A	Project Manager	January 2014
Final Monitoring Plan Data Report	N/A	Project Manager	June 2014

Quality Assurance Reports

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

Group D: Data Validation and Usability

D1: Data Review, Verification, and Validation Requirements

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

Checking for Typical Errors

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

Checking Against DQIs

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

Checking Against QA/QC

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

Data Checking

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

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

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

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

Data Verification

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

Data Validation

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

Data Separation

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

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

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

Responsible Individuals

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

D2: Verification and Validation Methods

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

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

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

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

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

D3: Reconciliation with User Requirements

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

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

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

Appendix 1: Citations

ANSI/ASQC, 1994. American National Standard, Specifications and Guidelines for Quality Systems for Environmental Data Collection and Environmental Technology Programs. ANSI/ASQC E4-1994, American Society for Quality (ASQ), Quality Press, Milwaukee, Wisconsin.

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

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

NCRWQCB, 2011. Russian River Pathogen Indicator Bacteria TMDL – Supplemental Sampling Plan - Quality Assurance Project Plan. North Coast Regional Water Quality Control Board. Santa Rosa, CA.

NCRWQCB, 2012. Russian River Pathogen TMDL 2011-2012 Monitoring Report. North Coast Regional Water Quality Control Board. Santa Rosa, CA.

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

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

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

Appendix 2: Field Data Sheet

SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ			*Project Code: RWB1_BACTI_2011		Pg 1 of 1 Pgs
*Station ID:	Arr. Time:	*Agency: RB1	*Funding:	*Protocol:	Entered in d-base (initial/date)
Location ID:	*SampleTime:	*Group:		*PurposeFailure:	
*Date (mm/dd/yyyy):	Dep. Time:	*Personnel:			

Habitat Observations (CollectionMethod = Habitat_generic)			*GPS / DGPS	Lat (dd.ddddd)	Long (ddd.ddddd)
OBSERVED FLOW:	NA Dry Waterbody Bed No Obs Flow Isolated Pool Trickle (<0.1cfs)	WADEABILITY:	Target:		-
	0.1-1cfs 1-5cfs 5-20cfs 20-50cfs 50-200cfs >200cfs	Y / N / Unk	*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			N W E S	(RB)
OTHER PRESENCE:	Vascular Nonvascular OilySheen Foam Trash Other____		Duplicate?		(LB)
DOM. SUBSTRATE:	Bedrock Concrete Cobble Gravel Sand Mud Unk Other____		YES / NO		(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 = FieldQA; 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

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

cel analytical, inc.
water, wastewater & sludge Microbiology

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

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

Lab ID#:

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

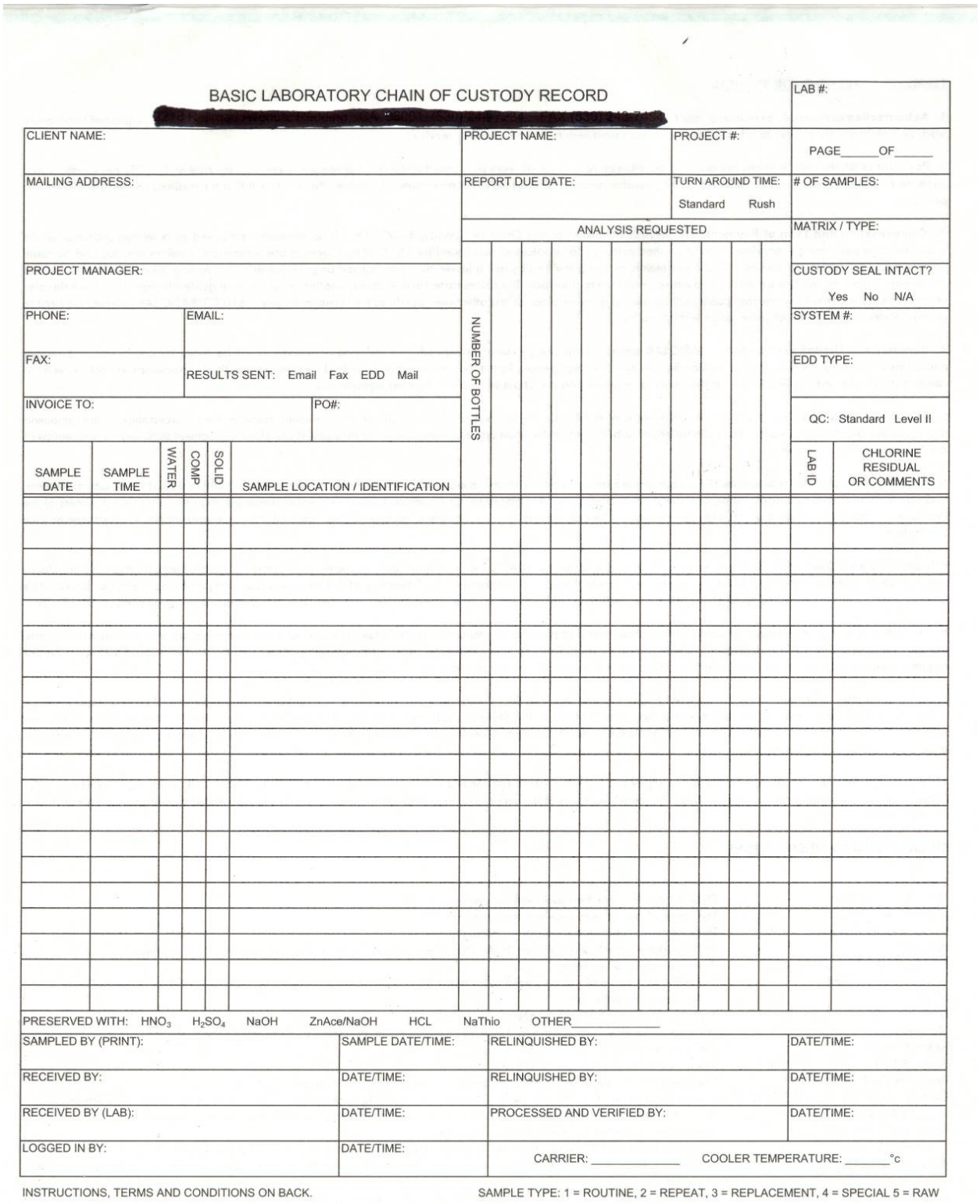
Report to:
Bill to:
Project #:
Client:
Turn around Time:

Sampled by (signature):
samples
Date:
Indicate Analysis Needed

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

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

Instructions/Comments::



BASIC LABORATORY CHAIN OF CUSTODY RECORD

LAB #:

CLIENT NAME:

PROJECT NAME:

PROJECT #:

PAGE_____OF_____

MAILING ADDRESS:

REPORT DUE DATE:

TURN AROUND TIME: Standard Rush

OF SAMPLES:

ANALYSIS REQUESTED

MATRIX / TYPE:

PROJECT MANAGER:

CUSTODY SEAL INTACT? Yes No N/A

PHONE:

EMAIL:

SYSTEM #:

FAX:

RESULTS SENT: Email Fax EDD Mail

EDD TYPE:

INVOICE TO:

PO#:

QC: Standard Level II

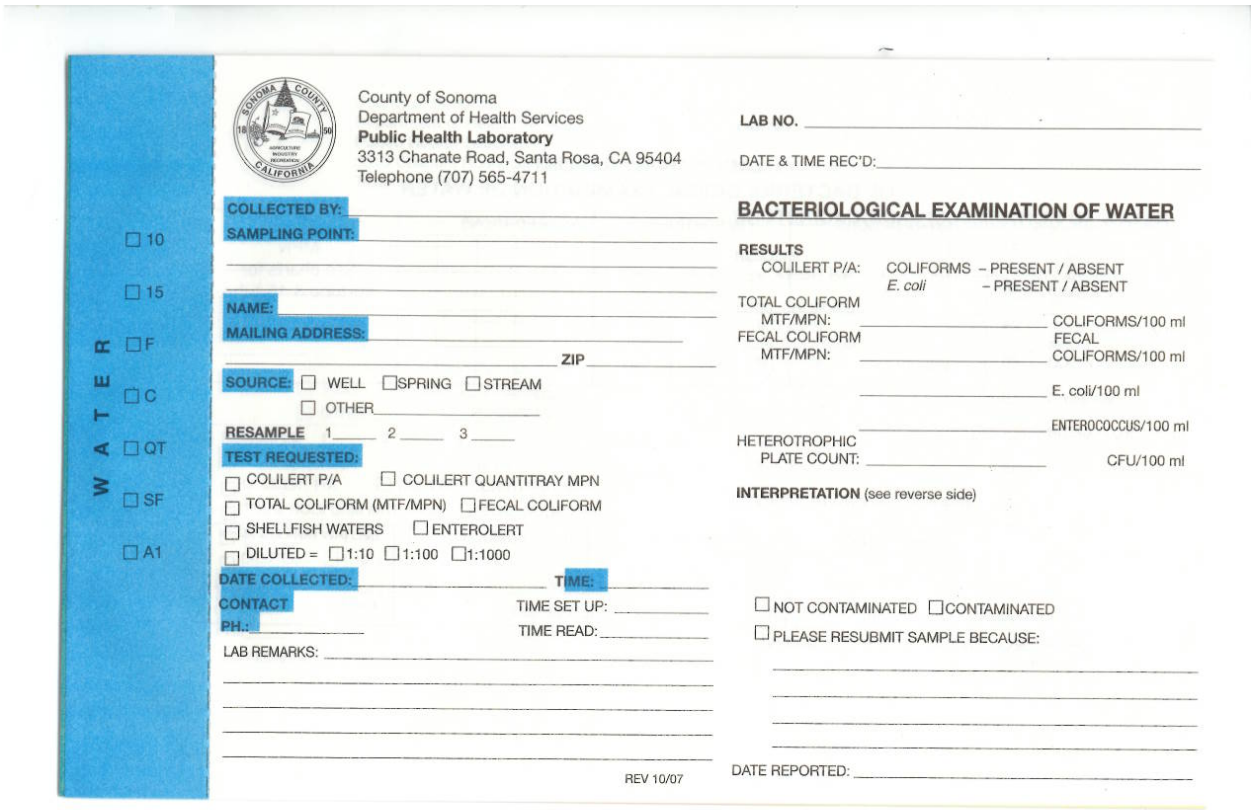
SAMPLE DATE	SAMPLE TIME	WATER	COMP	SOLID	SAMPLE LOCATION / IDENTIFICATION	NUMBER OF BOTTLES										LAB ID	CHLORINE RESIDUAL OR COMMENTS

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

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

INSTRUCTIONS, TERMS AND CONDITIONS ON BACK.

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



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

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

LAB NO. _____

DATE & TIME REC'D: _____

BACTERIOLOGICAL EXAMINATION OF WATER

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

REV 10/07

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

INTERPRETATION (see reverse side)

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

DATE REPORTED: _____

Appendix 4: Sampling Location Maps

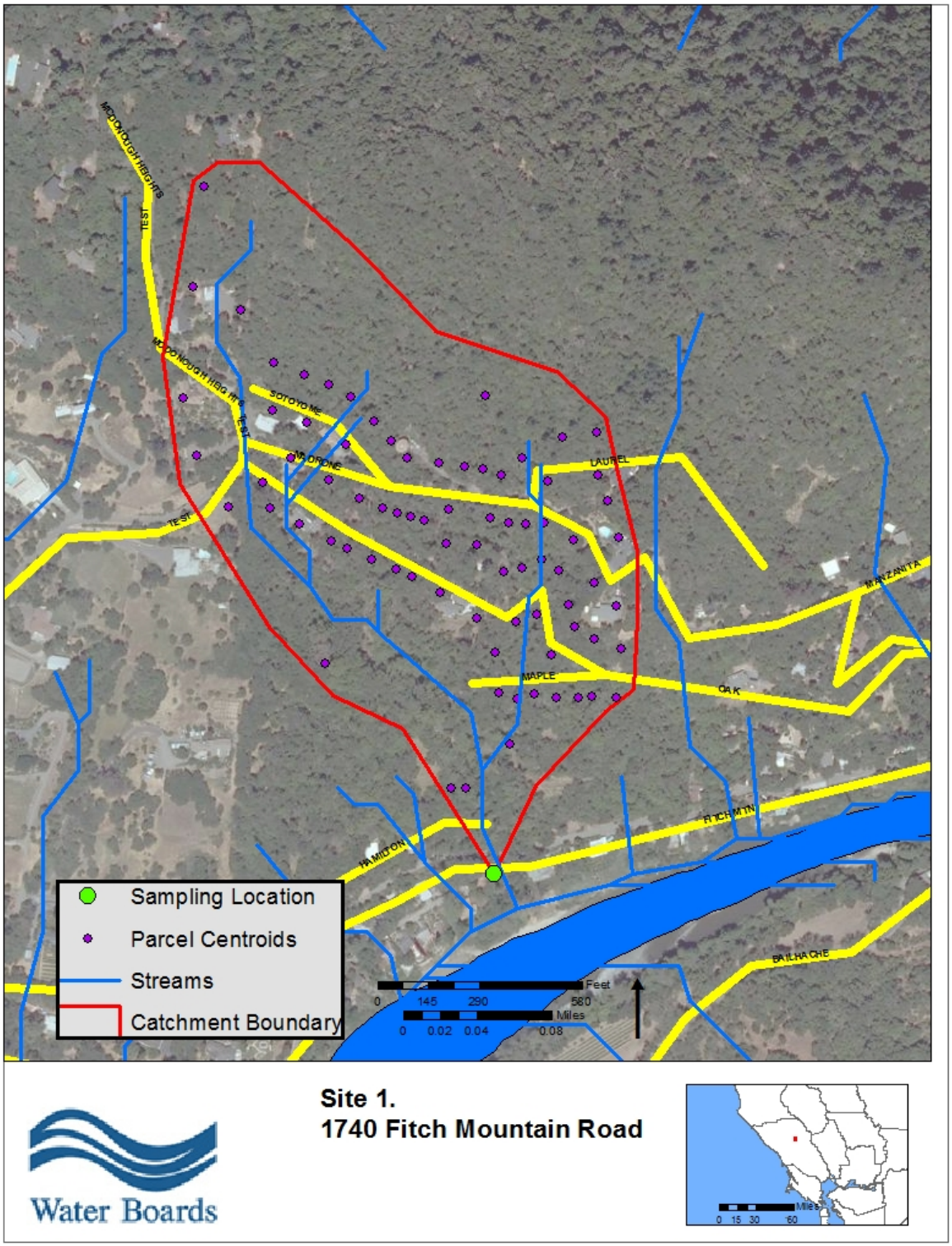


Figure A4.1. Sampling Location for Site 1 near 1740 Fitch Mountain Road, Healdsburg

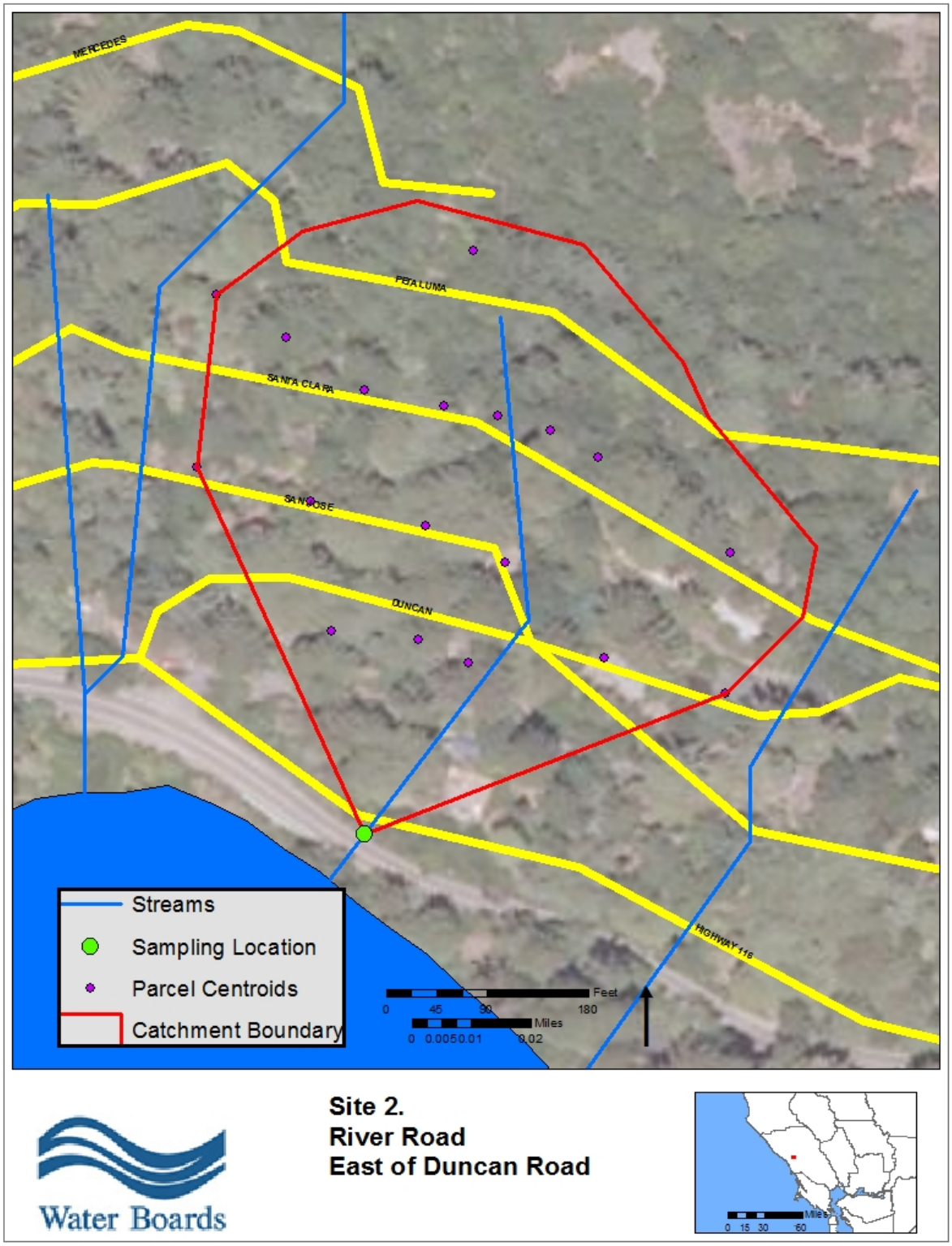


Figure A4.2. Sampling Location for Site 2 near River Road and Duncan Road, Monte Rio

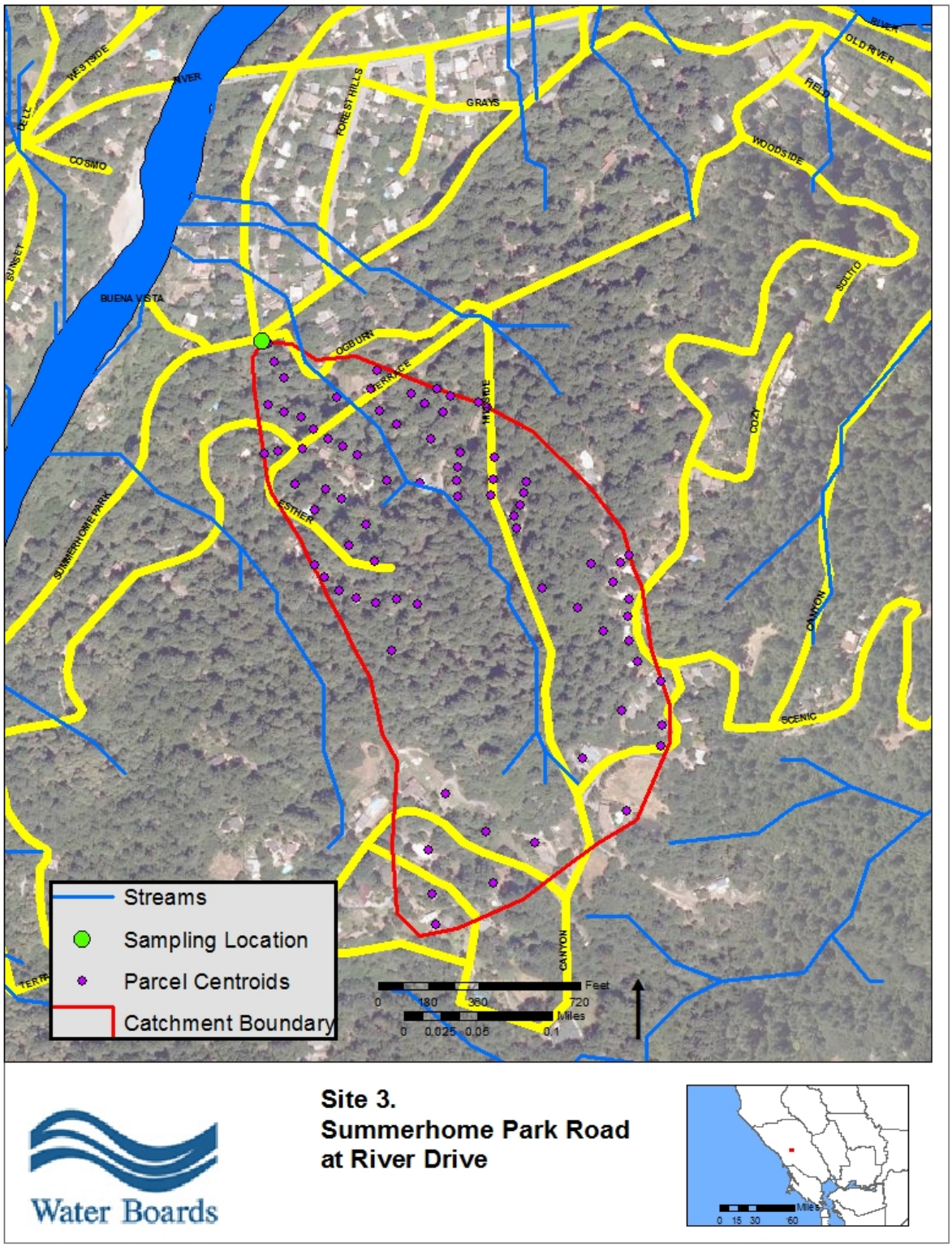


Figure A4.3. Sampling Location for Site 3 near Summerhome Park Road and River Drive, Forestville

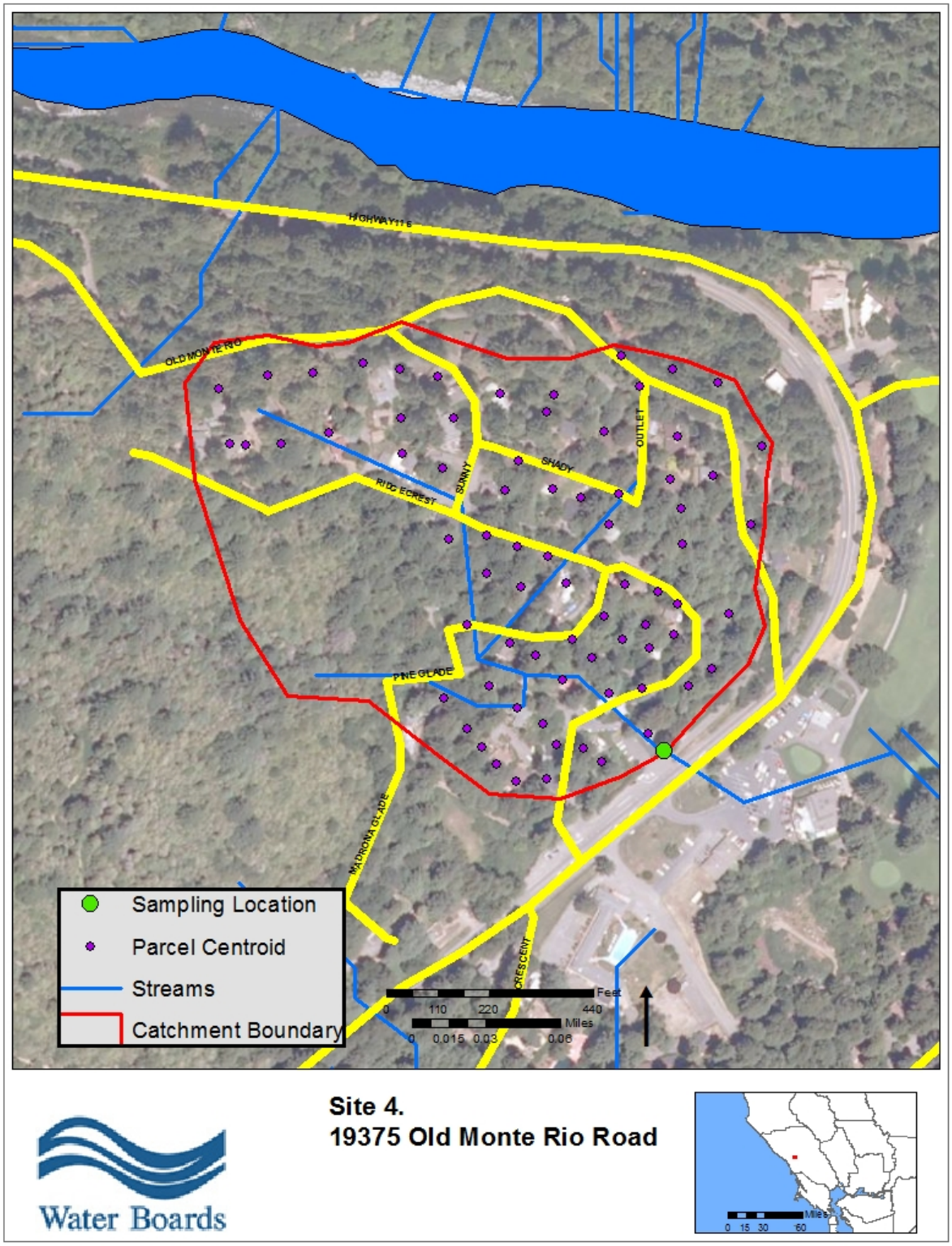


Figure A4.4. Sampling Location for Site 4 near 19375 Old Monte Rio Road, Monte Rio

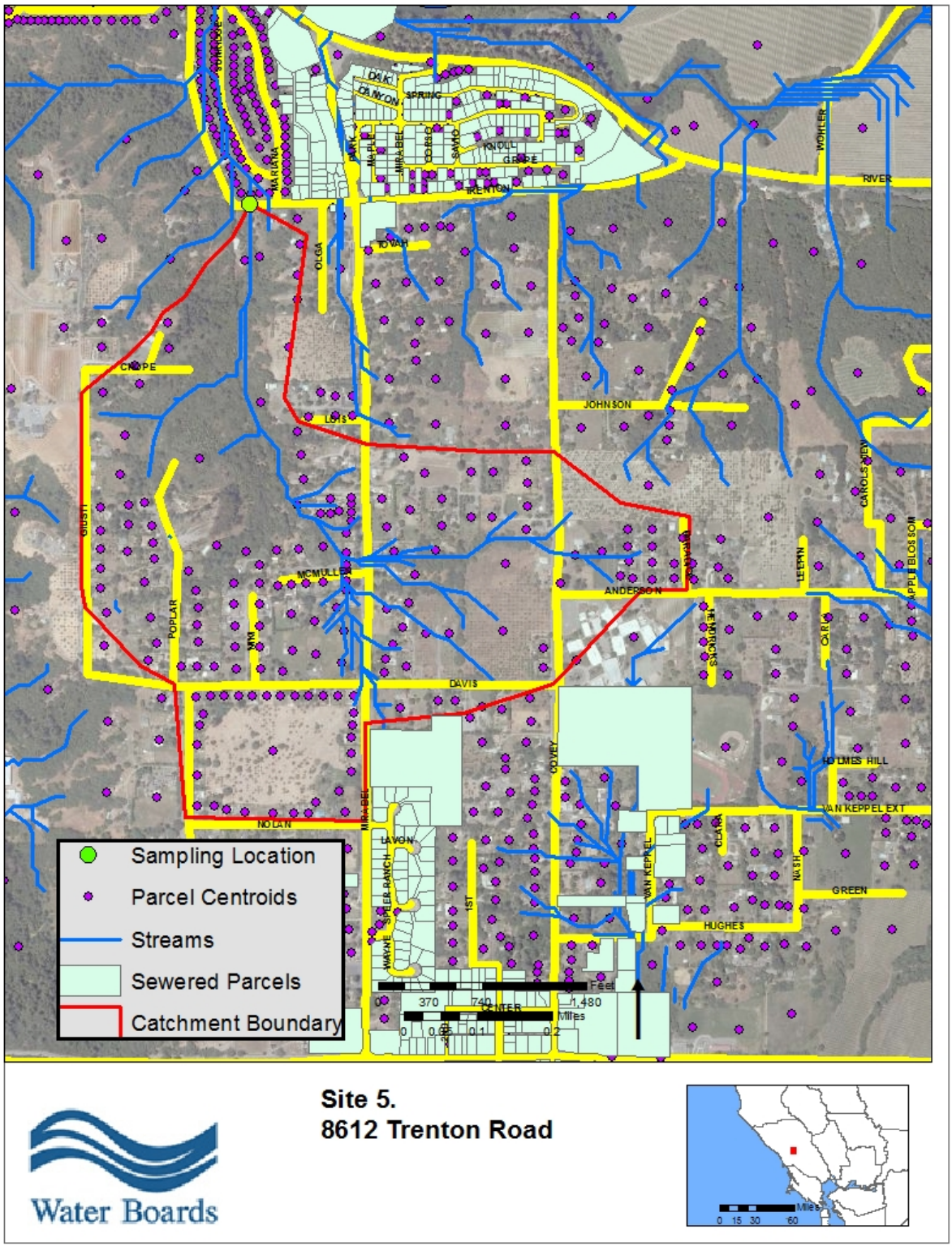


Figure A4.5. Sampling Location for Site 5 near 8612 Trenton Road, Forestville

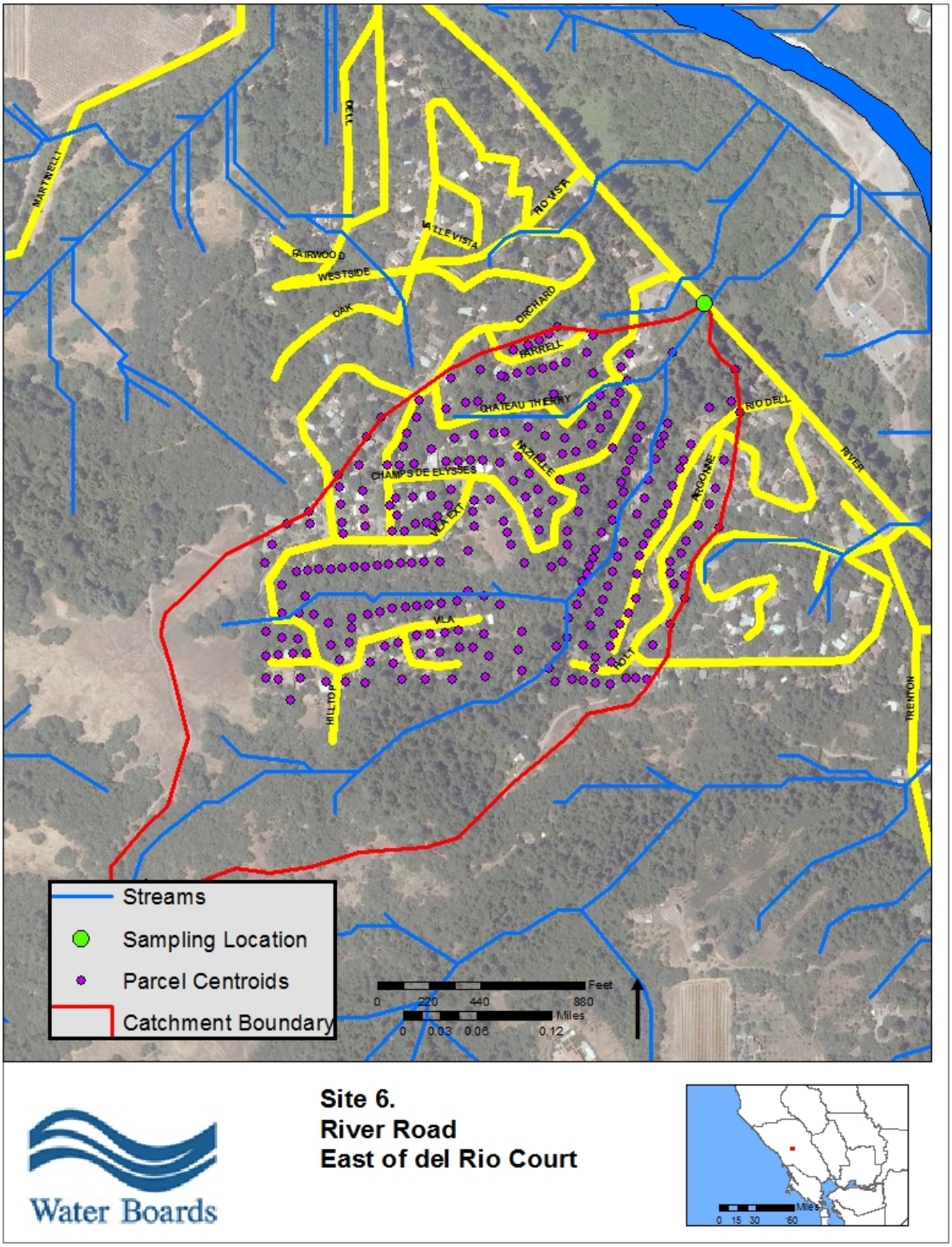


Figure A4.6. Sampling Location for Site 6 near River Road and del Rio Court, Forestville

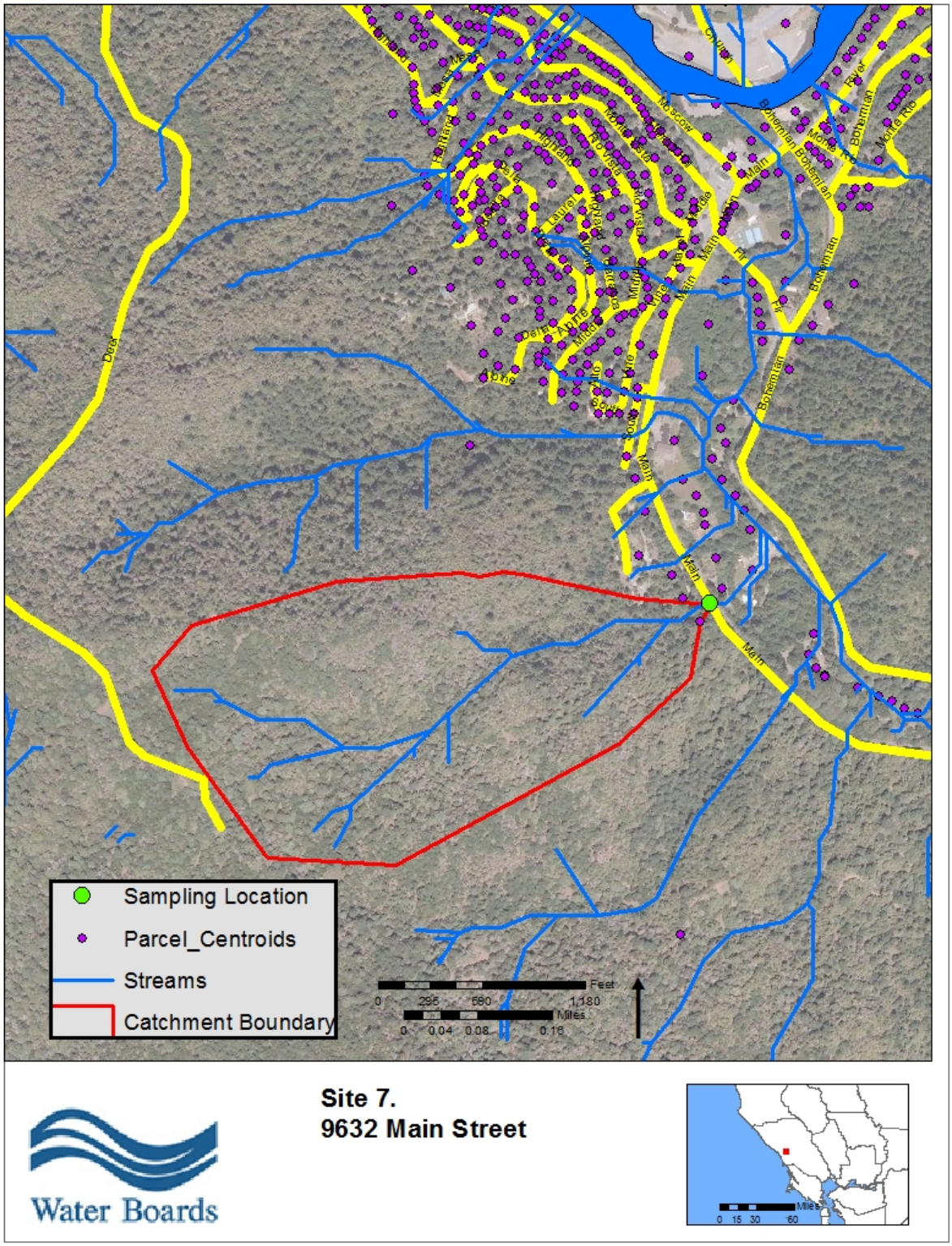


Figure A4.7. Sampling Location for Site 7 near 9632 Main Street, Monte Rio

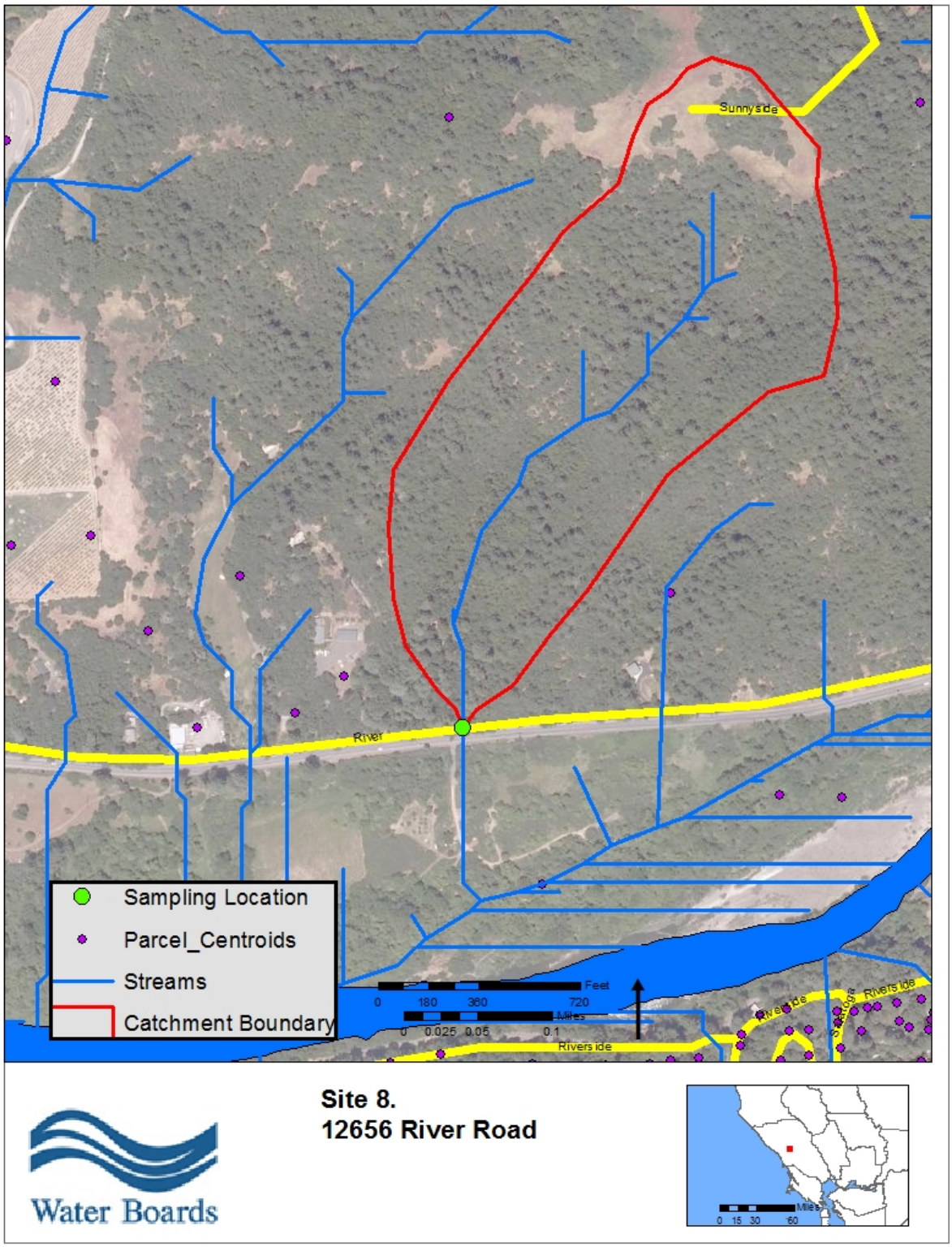


Figure A4.8. Sampling Location for Site 8 near 12656 River Road, Rio Nido

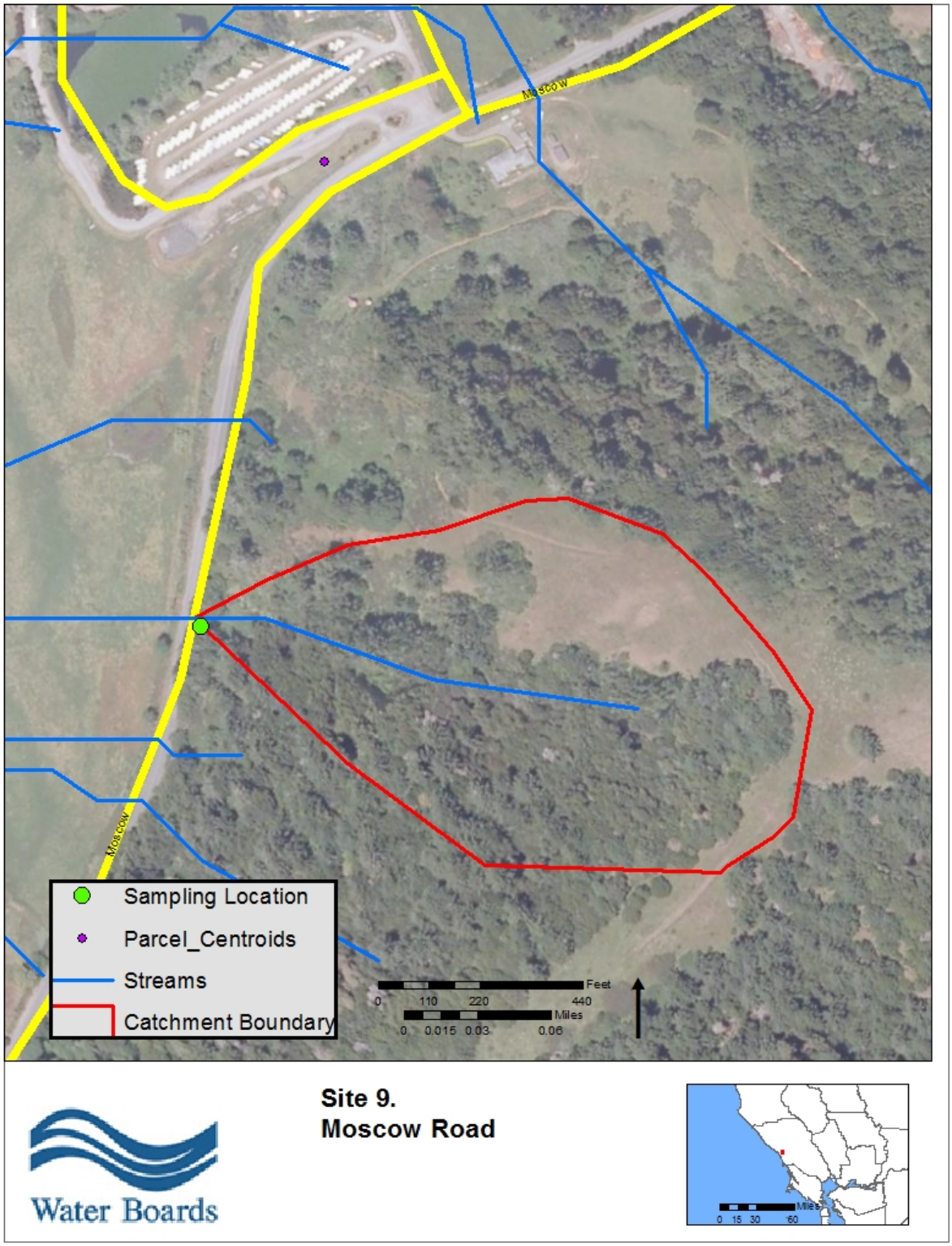


Figure A4.9. Sampling Location for Site 9 on Moscow Road, Duncan Mills

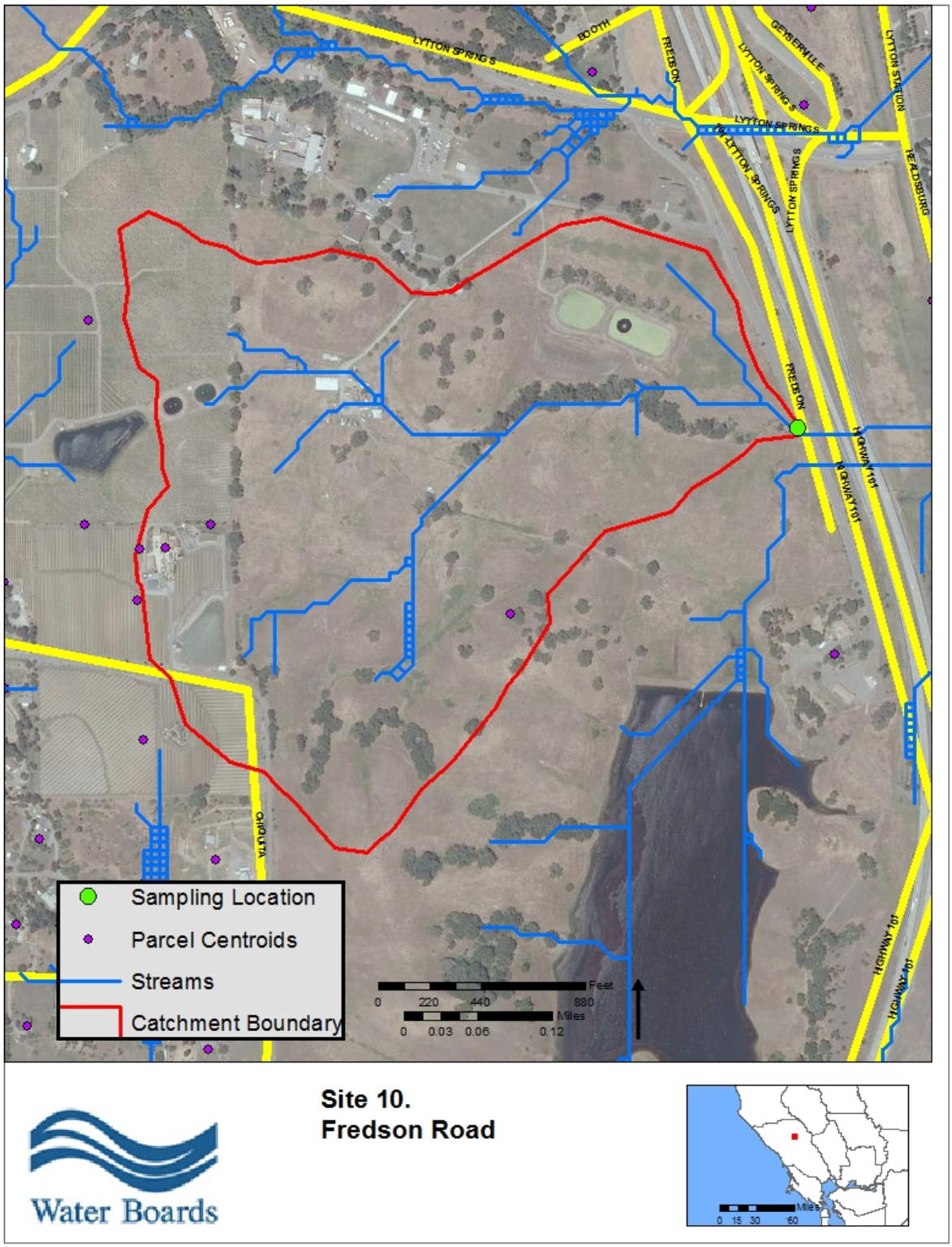


Figure A4.10. Sampling Location for Site 10 on Fredson Road, Healdsburg

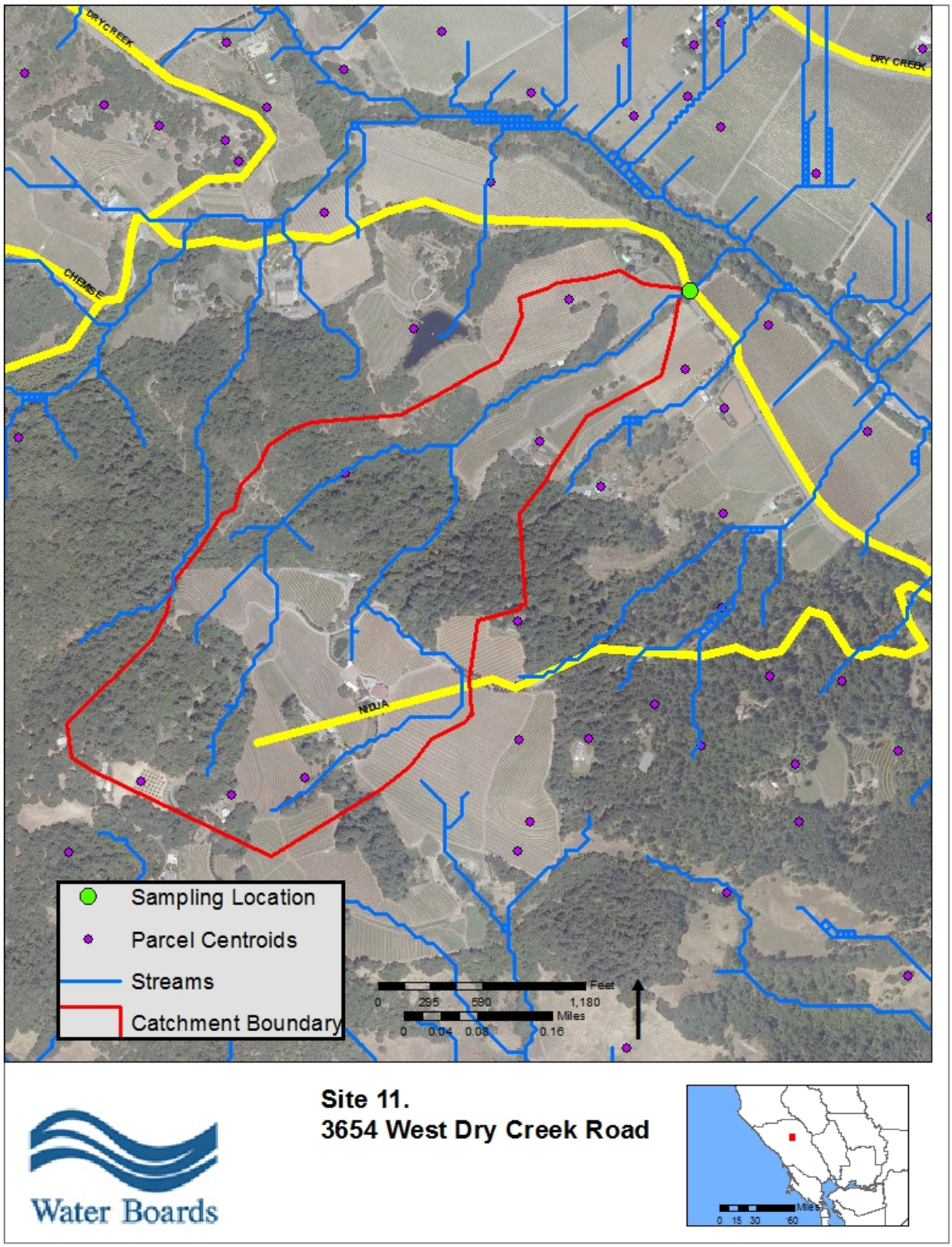


Figure A4.11. Sampling Location for Site 11 near 3554 West Dry Creek Road

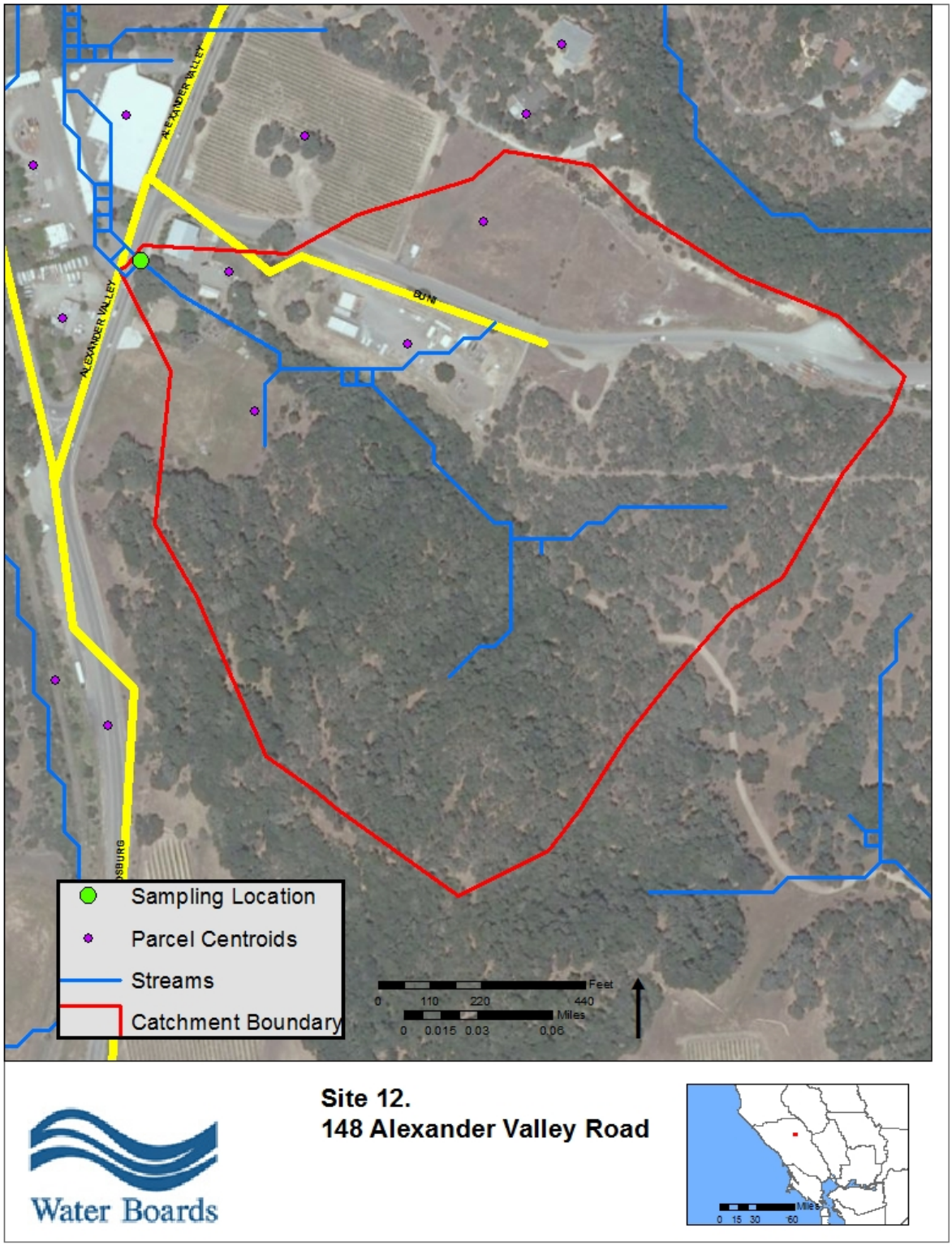


Figure A4.12. Sampling Location for Site 12 near 148 Alexander Valley Road

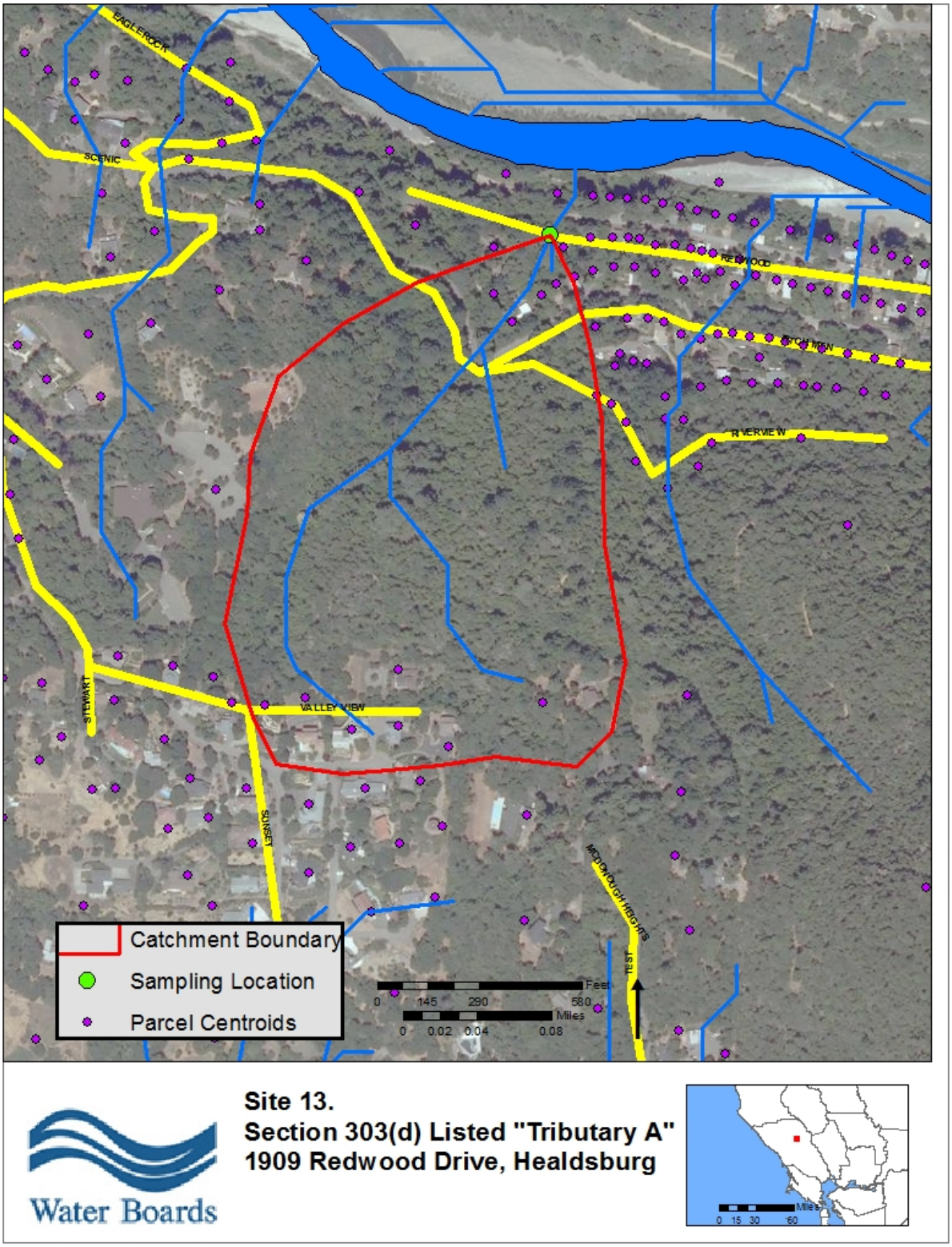


Figure A4.13. Sampling Location for Site 13 near 1909 Redwood Drive

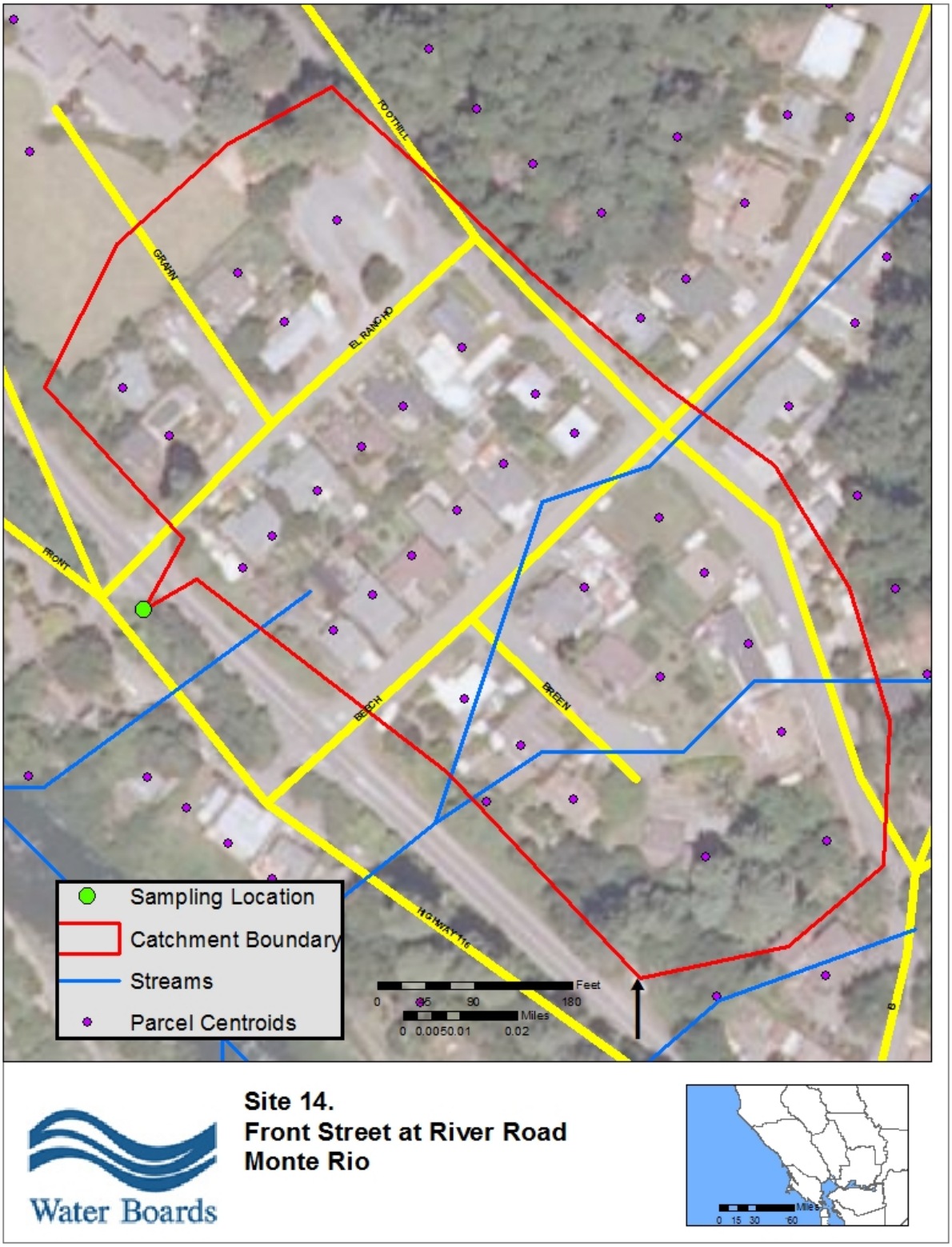


Figure A4.14. Sampling Location for Site 14 near Front Street and River Road

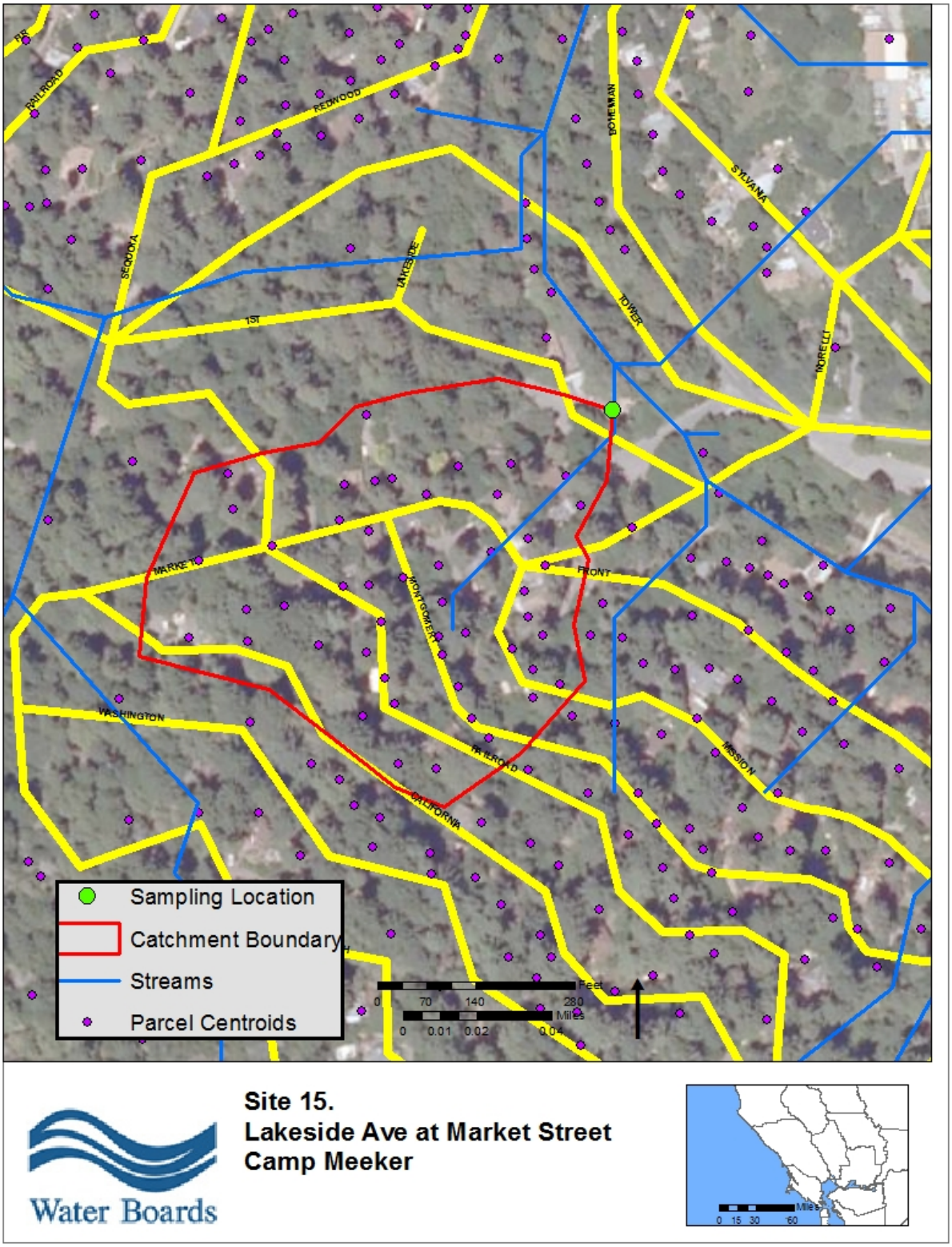


Figure A4.15. Sampling Location for Site 15 near Lakeside Ave and Market Street

Appendix 5: Standard Operating Procedure for PhyloChip Analysis

Standard Operating Procedure for Phylochip Sample Filtering:

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

Standard Operating Procedure for Phylochip Sample Filter Analysis:

G3 PhyloChip Manual: 12-07-2010 Edition:

Target Preparation.

PCR amplification of 1.5 kb product for target hybridization

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

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

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

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

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

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

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

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

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

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

TAKARA PCR for chips
PCR and/or cloning

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

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

Thermocycling program:

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

2. Quantitation of PCR amplicons

a. Invitrogen "E gels"

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

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

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

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

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

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

b. Biorad Geldoc Operating Instructions

Load the gel onto the middle of the glass surface.

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

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

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

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

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

Step 4: Save the raw image data.

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

c. DNA mass quantitation

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

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

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

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

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

3. Concentrating Samples

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

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

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

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

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

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

Step 6: Measure the retentate using a pipette.

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

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

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

Dnase I calcs

Combine Bacterial and Archaeal

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

Fragmentation Rxn (50uL) calcs

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

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

4.1 per tube

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

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

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

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

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

Recommended for each new batch of enzyme:

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

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

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

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

Fragmentation Rxn (30uL) calcs

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

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

5. Terminal labeling- same as Affymetrix protocol

Requires:

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

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

Biotynalation Rxn

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

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

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

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

6. Pre-hybe chips

Requires:

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

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

7. Target Hybridization

Requires:

- Control Oligo B2, P/N: 900301

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

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

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

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

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

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

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

Requires:

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

Before Beginning:

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

Chip Prep

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

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

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

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

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

Sample Registration

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

Washing and Staining PhyloChips

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

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

Scanning PhyloChips

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

Viewing PhyloChip Scans

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

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

Additional Info: Uracil incorporation for amplifying 16S rRNA gene

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

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

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

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

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

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

Isis
48-58C
25 cycles

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

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

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

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

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

Standard Operating Procedure UCD-SIF QA-04:

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

Author: _____

Signature: _____

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A. Protocol Summary

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

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

B. Safety

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

C. Sample Preparation

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

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

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

D. Quality Control

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

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

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

E. Instrumentation

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

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

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

F. Data Analysis and Results Reporting

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

Appendix 7: Standard Operating Procedure for Genetic Profiling of Bacteroides

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

Part I. DNA Extraction

Items needed:

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

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

Part II. A) PCR Sample Preparation

Items needed:

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

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

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

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

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

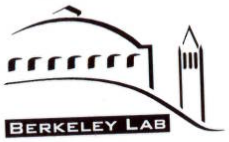
Part II. B) 7300 System Software Run Setup

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

* Default PCR Conditions:

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

Appendix 8: LBNL Laboratory QAP



OPERATING AND QUALITY MANAGEMENT PLAN

LBNL/PUB-3111, Rev. 10

EFFECTIVE DATE: October 28, 2008

Ernest Orlando Lawrence
Berkeley National Laboratory

This work was supported by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

Review and Approval

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

Signature maintained on file

James Krupnick

Director, Institutional Assurance

Lawrence Berkeley National Laboratory

Signature maintained on file

Aundra Richards

Manager, Berkeley Site Office

Department of Energy

Table of Contents

Record of Revisions

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

Statement of Laboratory Policy

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

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

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

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

Director
Ernest Orlando Lawrence
Berkeley National Laboratory

Objectives and Applicability

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

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

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

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

Offices of Institutional and Contract Assurance

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

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

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

Section 1
Organization

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

1.1 Organizational Structure

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

The description of the organization should include the following information:

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

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

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

1.2 Planning

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

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

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

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

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

1.3 Staff Proficiency

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

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

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

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

Section 2
Management Systems and Process Controls

2.1 Managing Principles

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

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

2.2 Safety Management

2.2.1 Hazards in the Work Process

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

All line managers must perform the following safety functions:

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

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

2.2.2 Formal Work Authorization

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

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

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

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

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

- Line management accountability
- Clear roles and responsibilities

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

2.2.3 Stopping Unsafe Work

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

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

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

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

2.3 Environmental Management

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

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

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

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

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

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

2.4 Safeguards and Security Management

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

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

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

2.5 Other LBNL Management Systems

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

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

2.6 Process Control

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

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

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

2.6.1 Core Functions

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

2.6.2 Descriptions, Procedures, Instructions, and Drawings

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

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

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

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

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

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

2.6.3 Consensus Standards

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

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

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

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

2.6.4 Suspect/Counterfeit Items

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

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

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

2.6.5 Safety Software Quality Assurance

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

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

The SSQA approach sets four core requirements:

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

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

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

2.6.5.1 Applicability

[A] System Software

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

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

[B] Library (Data) Files

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

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

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

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

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

[D] Acquired Software

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

[D-1] COTS Software

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

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

[D-2] Firmware

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

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

[D-3] Firmware, modified

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

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

[D-4] Vendor-Developed Software

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

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

[E] LBNL-Developed Software

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

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

2.6.5.2 Software Evaluation

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

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

2.6.5.3 Software Lifecycle

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

[A] Labeling System

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

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

[B] Change Control

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

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

[C] Software Inventory

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

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

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

[E] Software Problem Reporting

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

[F] Software Removal and Retirement

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

2.6.5.4 Software Documentation

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

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

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

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

[B] Change Control Document

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

[C] Requirements Document

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

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

[D] Design Document

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

[E] Test Plan

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

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

[F] Test Results

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

[G] User Documentation

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

2.6.5.5 Software Reviews

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

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

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

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

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

All reviews performed will be documented by the responsible organization.

2.6.5.6 Software Testing

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

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

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

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

2.6.5.7 Supplier Control

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

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

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

2.6.5.8 Records

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

2.6.5.9 Training

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

2.6.5.10 Graded Approach

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

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

Table 1: Software Quality Assurance Graded Approach

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

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

Table 2 – Software Evaluation Checklist

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

2.6.6 Function-Specific Controls

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

A. Design

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

B. Procurement

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

C. Manufactured Items Inspection and Testing

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

D. Construction Inspection and Testing

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

E. Data Collection

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

F. Asset Management

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

2.6.7 Facility-Specific Controls

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

A. Operating Practices

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

B. Facility Management

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

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

2.7 Document and Records Management

2.7.1 Document Control

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

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

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

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

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

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

Document Control Graded Approach Matrix

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

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

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

- **Document Control Implementation**

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

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

A. Documents are uniquely identified.

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

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

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

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

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

G. Approvals will be documented.

- **Document Distribution and Use**

A. Documents will be made available to affected personnel.

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

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

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

- **Document Changes**

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

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

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

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

2.7.2 Records Management

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

2.7.3 Scientific and Technical Publications

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

Section 3
Performance Assessment and Improvement

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

3.1 Management Assessment

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

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

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

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

3.2 Independent Assessment

3.2.1 Independent Assessment

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

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

Independent assessments include:

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

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

3.2.2 Peer Review

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

3.2.3 ES&H Self-Assessment

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

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

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

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

3.3 Continuous Improvement

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

3.3.1 Quality Improvement

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

A quality improvement process includes:

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

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

3.3.2 Corrective Action

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

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

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

3.3.3 Lessons Learned

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

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

Appendices

Appendix A
The Graded Approach Methodology at LBNL

Introduction

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

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

Methodology

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

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

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

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

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

Appendix B
Integrated Safety Management (ISM)

Table B-1. Seven ISM Guiding Principles

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

Table B-2. Five ISM Core Functions

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

Appendix C
Policy and Procedure for Controlling Suspect/Counterfeit Items

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

Identification

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

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

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

Procurement

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

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

Installed Items

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

Disposition and Reporting

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

Appendix D
LBNL Conformance with Quality Assurance Requirements and Standards

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

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

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

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

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

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

Appendix 9: Cel Analyitical Laboratory QAP

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

Laboratory *Quality* Assurance/Quality Control Manual

Version 5
Effective Date: 04/12/2011

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

Laboratory *Quality* Assurance/Quality Control Manual

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Acknowledgement

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

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

The manual was prepared by: ---------- *Date*----------

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

Reviewed by:

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

Reviewed by: ---------- *Date*----------

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

Reviewed by: ---------- *Date*----------

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

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1.0 INTRODUCTION

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

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

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

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

2.0 PURPOSE AND OBJECTIVES

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

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

1.0 INTRODUCTION

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

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

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

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

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

3.0 ORGANIZATION & RESPONSIBILITIES

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

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

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

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

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

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

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

Cel Analytical Laboratory currently employs:

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

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

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

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

4.0 LABORATORY FACILITY

4.1 Facility Floor Plan

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

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

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

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

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

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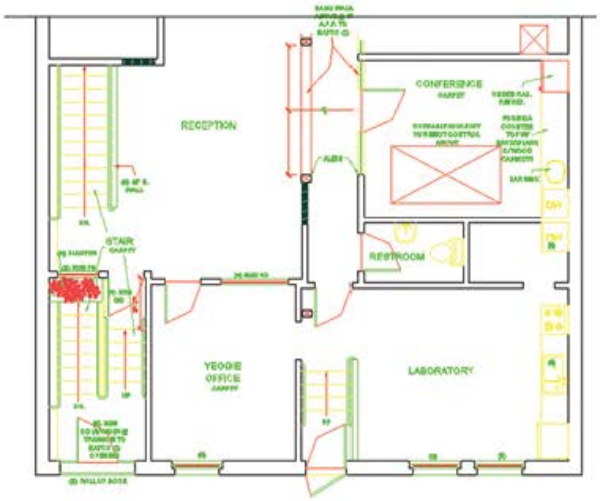


Figure 1. Facility Floor Plan

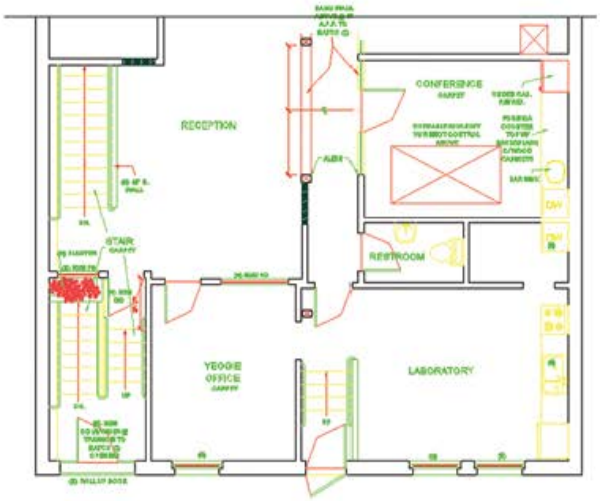
4.2 Security and Safety

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

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

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

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



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5.0 LABORATORY SAMPLE MANAGEMENT

5.1 Sampling Procedures

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

5.1.1 Microbiology

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

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

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

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

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

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

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

5.1.2 Inorganic Chemistry

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

5.1.3 Grab vs composite sampling

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

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5.1.4 Sample Plan

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

5.1.5 Source for water sampling

Distribution systems

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

Well water

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

River or stream

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

Collection

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

5.2 Sample Preservation & Sample Handling

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

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Microbiology

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

Inorganic Chemistry

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

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

5.3 Sample Acceptance, Storage & Disposal Policies

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

Table 1 Acceptance Criteria

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 2 Disposal Methods

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

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

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6.0 ANALYTICAL METHODS

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

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

6.1 Microbiology:

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

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

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

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

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

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

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

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6.2 Inorganic Chemistry

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

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

6.3 SOP List

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

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

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

Table 6.1 General Method SOPs

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

6.2 Inorganic Chemistry

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

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The establishment and verification of the calibration curve for inorganic instrumentation is follows the EPA required minimum-point calibrations for inorganic analytes measured by laboratory instrumentation.

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GAM-04	Preparation of Bacterial Smear
GAM-05	Staining Procedures (Gram Stain)
GAM-06	Staining procedures (Acid Fast)
GAM-07	Mo Bio Laboratory Water DNA Purification Kit

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

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

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

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

Table 6.2-Continued

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

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

Table 6.2-Continued

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

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

Table 6.3 SOP Lists for Inorganic Methods FOTs for Wastewater

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

Table 6.3 SOP Lists for Inorganic Methods FOTs for Wastewater

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

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

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

6.3.1 SOP Lists for Drinking water Chemistry

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

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

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

6.3.1 SOP Lists for Drinking water Chemistry

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

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

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

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

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

7.0 LABORATORY DATA REDUCTION, VALIDATION AND REPORTING

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

7.1 Data Reduction

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

7.2 Data Validation

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

Table 7.2.1

Validation Checklist	Lab Project ID#

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

7.0 LABORATORY DATA REDUCTION, VALIDATION AND REPORTING

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- ❑ Once these checks have been established, the data report is signed by the analyst and a second qualified person as described in the following section.

7.3 Data Reporting

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

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

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

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

Microbiology Reporting Requirements

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

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

7.3 Data Recording

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

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

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

Table 7.3.1

Document Recording Checklist

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

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Table 7.3.2

Data Storage Checklist

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

8.0 INTERNAL QUALITY CONTROL CHECK

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

8.1 Field Quality Controls

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

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

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

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

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

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8.2 Analytical Quality Controls

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

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

8.2.1 Microbiology

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

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8.2.2 Inorganic

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

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

   Lower control limit = x – 3s (lower warning limit -2s)

   Use the control limits specified in the method until data is acquired for 20 to 30 samples.

6) **Quality Control Samples** for Instrument analysis (Spectrophotomers/Inductively Couple Plasma-ICP-AES) Include:

   6.1) Calibration Standard (CS) A standard containing known quantities of target analyses, prepared from traceable stock materials of known, certified quality obtained from a reliable source or sources. Used to calibrate analytical instrument response.

   6.2) Initial (ICV) An initial calibration verification (ICV) is performed to check the accuracy of the calibration curve immediately after it

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is prepared. The ICV comes from a different source than the initial calibration standard. Normally the ICV concentration obtained from the calibration curve should agree within ±5% of the true value of the ICV solution. Failure to obtain acceptable ICV results may be due to:

- Concentrations of either the calibration standard(s) or ICV solution are not accurate.
- There is a problem with the calculation of the calibration curve.
- The instrument developed a problem between the completion of the calibration and the analysis of the ICV.
- An instrument problem which existed during the calibration was corrected prior to the ICV analysis.
- The analysis of the ICV was botched.

6.3) The continuing calibration verification (CCV) is used to determine that the initial calibration is holding. The source of the CCV should be a standard containing known quantities of target analyses, prepared from traceable stock materials of known, certified quality obtained from a reliable source or sources independent from those associated with the corresponding calibration standards. (See also *Laboratory Control Standard.*)
The minimum frequency for the CVV will be specified by the method but at least once per use.

7. **Initial Demonstration of Capability**: Must be performed for each method prior to analyzing compliance samples. IDC should be performed for each analyst and each piece of equipment. Precision, accuracy should be determined for each analyst. IDC includes demonstration of ability to achieve low background, the precision and accuracy required by the method, and satisfactory performance on an unknown sample. The IDC should be performed again if there is a change of analyst, instrument or significant change to the method.

   IDC for Trace Metal analysis in drinking water and wastewater is provided in Appendix E

8. **Periodic MDL calculation**: The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte accordance with the procedure given in 40 CFR 136 Appendix B. The procedure requires a complete, specific, and well defined analytical method and may vary as a function of sample type.

9. **Low Level Quantitation (LOQ)**: is the level above which quantitative results may be obtained with a specified degree of confidence. The LOQ is mathematically defined as equal to 10 times the standard deviation of the results

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for a series of replicates used to determine a justifiable **limit of detection**. Limits of quantitation are matrix, method, and analyte specific.

10. **Instrument Detection Limit (IDL)** is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise by a particular instrument. The IDL should always be below the **method detection limit**, and is not used for compliance data reporting, but may be used for statistical data analysis and comparing the attributes of different instruments. The IDL is similar to the "critical level" and "criterion of detection" as defined in the literature. (Standard Methods, 20th edition)

11. **Limit of Detection (LOD)** or detection limit, is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence). The LOD is typically determined to be in the region where the **signal to noise ratio** is greater than 5. Limits of detection are **matrix**, method, and analyte specific. (ss. NR 140.05(12) & 149.03(15)

Note: For the purposes of laboratory certification, the LOD is approximately equal to the **MDL** for those tests, which the **MDL** can be calculated.

9.0 PERFORMANCE AND SYSTEM AUDITS

As part of Cel-A's QA/QC program the following audits are conducted:

- Internal and external system audits to evaluate and validate qualitatively the laboratory's methods and overall performance, to identify any problems, and to provide technical support to the laboratory staff.
- Performance audits consisting of intra-laboratory performance evaluation and participation in the environmental laboratory proficiency testing sponsored by recognized authorities at the California Department of Health Services, Bureau of Environmental Laboratory Management (ELAP program).
- In addition, for all tests conducted at the laboratory the analyst(s) must show initial demonstration of capability (IDC) for the method(s) in question prior to analysis of environmental samples. At Cel Analytical we highly commend and encourage our staff to remain current with advances in technology and methodology by attending workshops, seminars, and educational courses and to participate in-house in new method development or training for new methods.

9.1 Internal Audit Program

Cel-A's internal audit program is designed to evaluate the staff's observance of the quality assurance plan and determine possible weaknesses in the plan itself. These audits are conducted annually by the Quality Assurance Manager and consist of a general review of all aspects of sample management, sample analysis, calculation and reporting. Notebooks and SOPs are reviewed to ensure protocols as outlined in SOPs are being followed. If any errors are discovered through the internal audit, corrective action goes

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- In addition, for all tests conducted at the laboratory the analyst(s) must show initial demonstration of capability (IDC) for the method(s) in question prior to analysis of environmental samples. At Cel Analytical we highly commend and encourage our staff to remain current with advances in technology and methodology by attending workshops, seminars, and educational courses and to participate in-house in new method development or training for new methods.

9.1 Internal Audit Program

Cel-A's internal audit program is designed to evaluate the staff's observance of the quality assurance plan and determine possible weaknesses in the plan itself. These audits are conducted annually by the Quality Assurance Manager and consist of a general review of all aspects of sample management, sample analysis, calculation and reporting. Notebooks and SOPs are reviewed to ensure protocols as outlined in SOPs are being followed. If any errors are discovered through the internal audit, corrective action goes

into effect without delay and is documented. The laboratory director must inform clients in writing if errors affect the integrity of the laboratory's results.

9.2 External Audit Program

Cel-A is audited every other year by the California Department of Health Services Environmental Laboratory Accreditation Program (ELAP). The external audit program assesses the overall effectiveness of the laboratory quality assurance program and provides suggestions to correct any deficiencies that may be observed during site visits and document reviews. Cel-A's QA/QC manager is responsible for implementing the recommended corrective actions.

Furthermore, if desired, clients can conduct their own audit to ensure fulfillment of their specific quality control requirements or contract specifications.

9.3 Performance Audit and Initial Demonstration of Capability

Cel-A Lab stands committed to establishing compatibility levels of routine analytical results by participating in Proficiency Testing (PT) and accreditation programs. Laboratory staff will analyze reference material(s) obtained through authorized vendors where their performance is evaluated through inter-laboratory comparisons. The performance audit results are then reviewed by ELAP authorities and accreditation status is granted upon successful analysis of referenced material. Accreditation status, once granted must be renewed through the same process every 2 years.

As part of the general QC requirements of Cel-A, the laboratory director and individual analysts participate in an in-house initial demonstration of capabilities (IDC) test for each method SOP, prior to engaging in field sample analysis. Samples used for IDC testing will be either water of known microbial or analyte content, or blind samples. Blind samples are water samples with a known quantity of a particular organism or analyte yet unknown to the analyst. Results of each IDC test will be recorded in such a way that trends such as variability, reproducibility and accuracy of methods are detectable and reviewed by statistical techniques described in section 11 of this manual.

10.0 PREVENTIVE MAINTENANCE

10.1 Equipment Maintenance, Calibration Procedures and Frequency

The Cel Analytical laboratory QA/QC manager sets policies for routine checks and repairs of laboratory equipment depending on the manufacturer's recommendations and prior to sample analysis when applicable. Equipment performance is monitored and logged to ensure they meet the appropriate levels of accuracy and precision. A maintenance log is kept for equipment to record all checks and repairs. QA/QC logbooks are kept in the laboratory for autoclaves, microscopes, pipettors, biological hoods, vacuum pumps, the laboratory water purification system, incubators, refrigerators,

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freezers and the PCR machine. Balances, thermometers and the pH meter are routinely calibrated to meet the NIST national standards and EPA standards.

A log is kept near each piece of equipment in accordance with the USDA/FSIS Microbiology laboratory, Guidebook 3rd Edition 1998. The logbook contains:

- Type of equipment
- Manufacturer's name and product's serial number or other identification
- Receipt and service date
- location
- Copy of manufacturer's instructions
- Copy of dates and results of calibrations and/or verification
- Copy of date of next calibration and/or verification
- Description of maintenance performed in past and scheduled for future
- Product history with respect to any damage, malfunction, modification or repair

Laboratory personnel are required to record daily temperatures of incubators and refrigerators and to ensure that all equipment is functioning properly prior to the start of any analysis. Expired maintenance schedules or equipment malfunctions must be brought to the attention of the laboratory director.

Standard operating procedures for the recording log that contains the date of use, event, corrective action taken, and the initials of the person making the entry is described below for each piece of equipment. Recordings are kept for five years past last entry.

A brief description of the operation and maintenance of the equipment currently in use at Cel-A is described below with details provided in SOPs referenced in this section. The SOPs are found in appendix C of this manual and are also are maintained electronically under the name of equipment in the equipment file (File location: C drive/Cel-A-lab file/Equipment Log forms file).

10.2 Equipment:

Calibration of all measurement devices is traceable to national standards whenever possible.

Autoclave: Operation of the autoclave must be done according to manufacturer's instructions. Each autoclave cycle is recorded in a log book (placed next to the autoclave) that indicates the date, content, sterilization time, temperature and operator's initials. With each cycle of use heat sensitive tape is placed on the items being sterilized to assure the proper temperature is reached.

Sterilization procedures should be followed as described in the SOP # EA-01. All items such as media and reagents requiring sterilization are sterilized in the autoclave at 121ºC for the designated time. The minimum sterilization time is 12-15 min for most carbohydrate containing media with the exception of A-1 medium which is 10 minutes.

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Biohazard waste is sterilized for a minimum of 30 minutes. Efficiency of sterilization is monitored monthly using spore stripes or ampoules.

Maintenance records and corrective actions required along with the initial of the person making the entry should be recorded on the maintenance log form (SOP # EA-02).

Balance (top loader): Calibrated monthly using ASTM (NIST traceable) type weight (type 1, 2 and 3) and recorded in a log book. In addition, professional calibration will take place annually. The balance should be cleaned prior to and following each use. Calibrate non-reference weights with reference weights every six months. Mass measurement should be recorded daily using a single weight. A logbook displaying daily checks on cleaning, maintenance, performance deviations, and corrective actions should be recorded and kept next to the balance (Ref SOP # EB-01).

Phase/Light Microscope: The microscope is equipped with lenses to provide approximate 20X and 40X to 100X magnification. Manufacturer's protocol and maintenance is strictly followed and logged in the binder provided next to the microscope. SOPs # EM-01 & EM-02 provide detail of routine in house QC and the corrective action log respectively. The accuracy and life time of the microscope depends on proper maintenance after each use and prompt reporting of any mal-function observed during use.

Conductivity meter, pH meter: Before each use standardize the pH meter with pH 7.0, 4.0 and 10.0 standard buffers. All pH buffers (NIST Traceable) are aliquotted for single use. The original containers when opened are dated and the lot number recorded. Expired buffer must be discarded. Maintain electrodes according to the manufacturer's recommendations. Each pH meter has a corresponding log (SOP#EpH-01) containing dated entries with each use, change of buffers or electrodes, service and corrective action taken in case of performance deviation.

The conductance meter is capable of measuring conductivity within 1μ Ohm per centimeter and is used routinely for testing reagent grade water. Similar to the pH meter, it is maintained according to the manufacturer's protocol. The conductivity meter is calibrated with every use using certified standard KCl solutions as described in Standard Method of Examination of Water and Wastewater 20th edition (APHA page 2-45) and recorded in the log book (SOP# Econ-01).

If a calibration curve is being used, standard solutions should be analyzed from time to time within the required range of concentration. The ideal calibration curve is linear within the range most often used.

Class II biological hood: Serviced at installation and annually thereafter. The pressure gauge reading should correspond approximately to the same level as the annually recorded level on the calibration sticker. If pressure is significantly higher, it indicates that the filters are dirty and need to be changed. If pressure is significantly lower, it may indicate an electric problem. Monthly check of airflow; test the filters for obstruction and dirt accumulation; clean and replace as needed. A light weight paper strip attached

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to the window can also indicate the inward air flow daily. Record maintenance and corrective action taken as outlined in SOP#EH-01.

Expose open agar plates to flow and air for an hour each month. Check contamination by incubating plates at 35 degrees Celsius for 24 hours. Clean and sanitize hoods and cabinets following each use, and turned UV light on. Lift up the work surface and clean under this area periodically.

Ultraviolet lamp in the hood: Disconnect and clean monthly, with soft cloth moistened with ethanol. Replace lamp if agar spread plate containing 200 to 250 microorganisms is exposed to UV light for 2 minutes and does not show a count reduction of 99%. For sanitization (SOP# EH-02), conduct spread plate irradiation test quarterly.

Thermometers: working thermometers include bulb thermometers immersed in glycerol, full immersion thermometer for water bath and maximum registered thermometer for autoclave and hot air oven. Thermometers are NIST calibrated and are re-certified whenever they have been exposed to temperature extremes. A reference certified thermometer is used to check the working thermometer. Referenced thermometer is recalibrated every 5 years.

Incubator Units (35ºC & 41.5ºC, 44.5ºC): All incubators are maintained at their desired temperature (+ or – 0.5 degrees Celsius) or (+ or – 0.2 degrees Celsius) depending on the application. Temperatures are recorded on daily log forms twice per day 3-4 hrs difference(SOP#EI-01). Any nonconforming temperature, possible cause, and corrective action are entered in a log book (SOP#EI-02).

Refrigerator/Freezer, -20 degrees Celsius: Calibrated at installation. Freezer maintains temperature of –20 + or – 5 degrees Celsius. Refrigerators are maintained between 2-8 degrees Celsius. Place NIST calibrated thermometer bulb immersed in glycerol on the shelf near the work area. Record temperature once per day at minimum for each day it is in use (SOP#ERF-01). Normal housekeeping once a week and clean and rearrange once a month. Annually check the overall condition of freezer and refrigerator, with a corresponding log of maintenance dates, problems, and corrective action (SOP#ERF-02).

Inoculating equipment: Use sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipette tips. Metal inoculating loops and needles are made of nickel alloy or platinum. Note: for oxidase test DO NOT use nickel alloy loops as they may interfere with the oxidase tests.

Pipettes (Mechanical): Use sterile, calibrated micropipette tips to ensure delivery of the correct volume per manufacturer's recommendation. Delivery volumes (depending on the size) should be within a 2.5% tolerance. Verify delivery volumes monthly by applying mass/volume measurement is near mass/volume expected in accordance with USDA/FSIS Microbiology Laboratory, Guidebook 3rd Edition 1998 (SOP#EP-01). Recalibrate in accordance with manufacturer's recommendations if performance verification failed. Return to manufacturer for recalibration. Keep records of maintenance, service, calibration, and verification measurements (SOP#EP-02).

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J-6 Centrifuge: The maximum speed of the J-6 centrifuge is 2700 rpm with a maximum rotor capacity of 2 liters. Maximum centrifugal speed is 1520 x g. Maintain the rpm and the corresponding g-force according to the chart attached to the instrument. For each centrifuge run, log speed, time of centrifugation, run temperature, and operator's initials (SOP#ECEN-01).

Record of calibrations, services, and corrective maintenance are kept as described in SOP# ECEN-02.

Vacuum Pump: Maintain and operate per manufacturer's instructions. Following each use clean and sanitize. Check annually and record maintenance, performance deviations, and corrective actions in log (SOP#EPU-01).

Membrane Filtration Equipment: Use membrane filters approved by manufacturer for total coliform water analysis; (approval based on data from tests for toxicity, recovery, retention, and absence of growth-promoting substances). Filters: cellulose ester, white, grid marked, 47 mm diameter, 0.45 µm pore size. Use stainless steel, glass, or autoclavable plastic. Before use, make sure they are not scratched, corroded or leaking. Use a standard graduated cylinder to check the accuracy of graduation marks on clear glass or plastic funnels used to measure sample volume (tolerance: less than or equal to 2.5%; retain calibration records.) The lot number for membrane filters and date received must be logged (SPO#EMF-01).

Water Purification System for Reagent Grade Water: The Millipore water purification system utilized at the laboratory consists of three components: Elix 5 with a reverse osmosis (RO) membrane that receives tap water and feeds purified deionized water into a 5 gallon water container that feeds the high quality water into the MilliQ Cell membrane system with a molecular weight cut off of 5000 Dalton, producing pyrogen free water. MilliQ water is further purified through a 0.2 µm filter at the dispenser tip for bacteria free water. The system is maintained according to manufacturer's instructions. With each use, conductivity (>0.5 megohms-cm at 25 degrees Celsius) of the reagent grade water is tested. The DI/RO system-cartridge is replaced as recommended by the manufacturer. A daily log of the conductivity reading (SOP# EW-01) and record of any scheduled maintenance or corrective action taken (SOP#EW-02) are maintained.

Spectrophotometer: The wavelength range of the SP 830 plus spectrophotometer is 330 to 999 nm. Allow the instrument to warm up for 30 minutes prior to use. Follow manufacturer's instructions for instrument validation and lamp adjustment. Record lamp use routine maintenance, instrument validation, lamp adjustment or any corrective action taken in the logbook located near the spectrophotometer (SOP SP-01). Standards are also employed to verify the accuracy of the optical densities at each given wavelength in use.

Inductive Couple Plasma Atomic Emission Spectroscopy ICP AES: An inductively coupled plasma is a plasma that contains a sufficient concentration of ions and electrons to make the gas electrically conductive. The plasmas used in spectrochemical analysis are essentially electrically neutral, with each positive charge on an ion balanced by a free

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One of the most frequent forms of routine maintenance is changing out sample and waste tubing on the peristaltic pump, as these tubes can get worn fairly quickly resulting in holes and clogs in the sample line, resulting in skewed results. Other parts that will need regular cleaning and/or replacing are sample tips, nebulizer tips, sample cones, skimmer cones, injector tubes, torches and lenses. It may also be necessary to change the oil in the interface roughing pump as well as the vacuum backing pump, depending on the workload put on the instrument. The ICP is due for an annual maintenance check by the instrument provider (Varian-Agilent Technology).

10.3 Laboratory Reagents and Supplies

Reagent and supplies are routinely checked for expiration dates when applicable, contaminant levels when applicable, and inventory checks to ensure all reagents and supplies are in stock and in good condition at all times. SOPs referenced in this section are found in appendix D of this manual.

Reagent Water (dIH_20): Cel Analytical uses only pyrogen free MilliQ ultrapure water (dIH_20) to prepare media, reagents, and dilution rinse water. Reagent grade water is microbiologically suitable (MS) water, free from traces of dissolved metal, bactericidal, and inhibitory compounds. Water quality shall conform to the EPA standards as shown in

Table 10.2 EPA Standards for Quality of Reagent Water

EPA Standards for Quality of Reagent Water

Parameter*	Limits	Frequency
Conductivity	< 2 microhms/cm at 25°C	Daily
Pb, Cd, Cr,	No greater than 0.05 mg/L/ contaminant	Annually
Cu, Ni, Zn	collectively, no greater than 0.1 mg/L	Annually
Total Chlorine Residual	<0.1 mg/L	Monthly
Heterotrophic Plate Count	<1000 CFU/mL	Monthly

If a test requires bacterial free water, the MilliQ ultrapure deionized water suitable for the application since a 0.22µ filter is already installed at the dispenser for immediate use. This water is also suitable for trace metal analysis since it has been filtered. Store deionized water in sterilized in glass bottles only for bacterial use..

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Dilution/Rinse Water: Use prepared stock buffer solution/peptone water prepared in accordance with the EPA's Standard Methods under Section 9050C in Standard Methods for the Examination of Water and Wastewater. Details of the preparation are described in the appropriate SOPs. In general use stock buffers that have been autoclaved or filter-sterilized. The prepared water must be labeled and dated on the container. Stock buffers are refrigerated and are checked to ensure they are free of turbidity. Sterility of each batch of dilution/rinse water is checked by adding 50 mL of water to a double strength non-selective medium such as trypticase soy or tryptose broth. Check growth by incubating at 35 degrees Celsius + or – 5 degrees Celsius for 24 hours. Discard water if growth is discovered.

2% HNO3 water: For metal analysis, standards, quality control samples, spikes and dilutions of unknown samples will be prepared by using the 0.22 micron filtered water acidified to 2% with Nitric acid (HNO3).

Media: Dehydrated media or commercially prepared media are purchased from Fisher Scientific. Powder media in use should not be more than a year old. Therefore, media are ordered in smaller containers from the suppliers to reduce shelf-life. Document the expiration dates, when received and opened, lot number, type, quantity, and appearance of each media in a log book (SOPRSM-01). In general prepared media will be purchased from manufacturer/suppliers with the certificate of quality assurance and quality control for the batch received. Once received at the lab, the information is recorded in the media log book. Ensure that lot number, date opened and the expiration dates are clearly marked on the media. New lots of dehydrated or commercially prepared media are checked before use with positive and negative control cultures. In addition, laboratory prepared media should include positive and negative culture controls with each batch. These organisms are purchased commercially as disks impregnated with organisms or are available in ampoule form.

Quality control pertaining to each media is further described in detail in analytical method SOPs.

Disposable Culture Dishes: Plastic dishes are certified by the manufacturer to be clean, sterilized, and accurate. Incubate loose-lid petri dishes in tight-fitting containers, such as a plastic crisper containing a moistened paper towel to prevent dehydration of membrane filter and media. Dispose of opened packs of disposable culture dishes between uses.

Glassware and plastic ware: Prior to use examine, (and if necessary discard) glassware and flasks to ensure they are not chipped, broken or etched. Check glassware and flasks following washing to ensure water beads are not excessive on the surface. Always use pre-sterilized plastic ware and ensure plastic items do not contain foreign residues and that they contain visible gradations. **For metal analysis, a dedicated set of sample containers or disposable conical tubes shall be used. Plastic or glass containers shall be rinsed in acid-water to ensure all residues have been removed prior to a final rinse.**

Sample containers: All sample containers, whether glass or plastic are tested to ensure their sterility through random selection of at least one sample container of a set of sterile

Dilution/Rinse Water: Use prepared stock buffer solution/peptone water prepared in accordance with the EPA's Standard Methods under Section 9050C in Standard Methods for the Examination of Water and Wastewater. Details of the preparation are described in the appropriate SOPs. In general use stock buffers that have been autoclaved or filter-sterilized. The prepared water must be labeled and dated on the container. Stock buffers are refrigerated and are checked to ensure they are free of turbidity. Sterility of each batch of dilution/rinse water is checked by adding 50 mL of water to a double strength non-selective medium such as trypticase soy or tryptose broth. Check growth by incubating at 35 degrees Celsius + or – 5 degrees Celsius for 24 hours. Discard water if growth is discovered.

2% HNO3 water: For metal analysis, standards, quality control samples, spikes and dilutions of unknown samples will be prepared by using the 0.22 micron filtered water acidified to 2% with Nitric acid (HNO3).

Media: Dehydrated media or commercially prepared media are purchased from Fisher Scientific. Powder media in use should not be more than a year old. Therefore, media are ordered in smaller containers from the suppliers to reduce shelf-life. Document the expiration dates, when received and opened, lot number, type, quantity, and appearance of each media in a log book (SOPRSM-01). In general prepared media will be purchased from manufacturer/suppliers with the certificate of quality assurance and quality control for the batch received. Once received at the lab, the information is recorded in the media log book. Ensure that lot number, date opened and the expiration dates are clearly marked on the media. New lots of dehydrated or commercially prepared media are checked before use with positive and negative control cultures. In addition, laboratory prepared media should include positive and negative culture controls with each batch. These organisms are purchased commercially as disks impregnated with organisms or are available in ampoule form.

Quality control pertaining to each media is further described in detail in analytical method SOPs.

Disposable Culture Dishes: Plastic dishes are certified by the manufacturer to be clean, sterilized, and accurate. Incubate loose-lid petri dishes in tight-fitting containers, such as a plastic crisper containing a moistened paper towel to prevent dehydration of membrane filter and media. Dispose of opened packs of disposable culture dishes between uses.

Glassware and plastic ware: Prior to use examine, (and if necessary discard) glassware and flasks to ensure they are not chipped, broken or etched. Check glassware and flasks following washing to ensure water beads are not excessive on the surface. Always use pre-sterilized plastic ware and ensure plastic items do not contain foreign residues and that they contain visible gradations. **For metal analysis, a dedicated set of sample containers or disposable conical tubes shall be used. Plastic or glass containers shall be rinsed in acid-water to ensure all residues have been removed prior to a final rinse.**

Sample containers: All sample containers, whether glass or plastic are tested to ensure their sterility through random selection of at least one sample container of a set of sterile

bottles. 25mL of sterile, non-selective broth is added and then incubated for 24 hours at 35 degrees Celsius, + or – 5 degrees Celsius. The growth is subsequently checked and where growth is detected re-sterilized. Sufficient amounts of the appropriate preservatives must be added to sample bottles prior to sterilization if water samples are suspected to contain chlorine or heavy metals. Refer to sample collection SOP.

Dyes and Stains: Use organic chemicals as indicators (e.g., phenol red) and microbiological stains (e.g., gram stains) certified by the Biological Stain Commission.

Chemical and Reagents: Chemicals employed are ACS or an equivalent grade to prevent impurities and undesirable reactions. All reagents and mixtures are labeled as shown in Table 10.2.4 and 10.2.3. Also see SOP#Chem-01 & Chem-02.

Table 10.2.2
Labels for Reagents:

Identity:
Concentration:
Date Opened: Initials: Expiration Date:
Storage Conditions: RT/4°C/-20°C

Table 10.2.3
Labels for Solutions:

Identity:
Concentration:
Date Prep.: Initials: Expiration Date:
Storage Conditions: RT/4°C/-20°C

An inventory list of all chemicals with the associated **material safety data sheets** (MSDS) are maintained in a hard copy binder on a shelf in the lab. In addition, an inventory list shall be maintained electronically that includes the chemical name, lot number, date purchased and reordering dates. The laboratory holds the most current versions as hard copies and in electronic format.

Glassware Washing: Wash glassware with special laboratory detergent. Thoroughly rinse glassware with tap water. Use pyogen free MilliQ ultrapure distilled/deionized water purified through a reverse osmosis filter for the final rinse.

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Glassware Washing: Wash glassware with special laboratory detergent. Thoroughly rinse glassware with tap water. Use pyogen free MilliQ ultrapure distilled/deionized water purified through a reverse osmosis filter for the final rinse.

Microbiology:

Complete the glassware inhibitory residue test in accordance with EPA's Standard Methods for the Examination of Water and Wastewater under section 9020B (internal SOP#GW-01).

Chemistry:

Consult individual parameter testing method for special glassware handling procedures. Maintain separate glassware for nitrate analysis. Some parameters require acid rinse to remove dissolved metals that may interfere with analysis.

10.4 Laboratory Cleanliness

Cel-A lab commits itself to maintaining high standards of cleanliness. The laboratory is scheduled for weekly cleaning, and removal of non-hazardous garbage. Floors are wet-moped and washed with disinfectant, and at no time swept or dry-moped. The laboratory air is tested on a weekly base to screen bacterial counts in the work area, as well as a background area. Biological wastes are autoclaved in the laboratory prior to disposal. Disinfection of laboratory benches and biological cabinets are performed before and after each use by the laboratory personnel. A monitoring log sheet is used to record and document the routine autoclaving of the biological waste in the laboratory.

11.0 LABORATORY PROCEDURES USED TO ASSESS PRECISION, ACCURACY AND COMPLETENESS OF DATA

Statistical methods outlined below are used for determining precision and accuracy of data. These methods are based on established EPA guidelines for statistics in the inorganic[3] or microbiology[11] testing laboratory. The methods outlined below help to establish trends and quality control limits that will improve the quality of the data generated by the laboratory.

11.1 Precision

Precision of quantitative methods is a measure of reproducibility of data through analysis of replicate samples, method blanks, and control samples. Precision is checked by running duplicate analyses and expressing results as a range (difference between duplicate analyses). For each different sample such as drinking water, waste water or surface water the precision of its duplicate analysis is determined, using the most recent values.

Microbiology:

Complete the glassware inhibitory residue test in accordance with EPA's Standard Methods for the Examination of Water and Wastewater under section 9020B (internal SOP#GW-01).

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11.1.1 Range

Range is commonly used to evaluate an analyst's precision for methods and sample types commonly used in the laboratory. Range is determined using the following protocol:

1. Duplicate samples are analyzed on the first 15-20 sample types with positive responses, preferably over time, depending how many samples are processed per week in the laboratory. Duplicate samples are designated D1 and D2.

2. The results are log- transformed for subsequent statistical calculations because logarithmic transformation of data tends to normalize the skewed distribution of results from duplicate/replicate analysis. This will even-out the deviation normally observed among samples with different microbial densities. If either of a set of duplicate results is <1, add 1 to each value prior to transforming to the logarithm value.

   The logarithm (Log_{10}) of results:

   $L1 = Log_{10}$ of D1
   $L2 = Log_{10}$ of D2

3. Calculate the range (R) for each pair of transformed duplicates as the mean (R) range as follows:

   a) Calculate the range difference between duplicates:
   $$R_{log} = (L2-L1)$$
   b) Calculate the sum of all log transformed duplicates:
   $$\bar{} \quad \sum \text{ of } R_{log}$$
   c) Calculate R (mean range):
   $$\bar{R} = \sum R \; Log_{10}/n$$, where n is the number of samples.

5. Control limits for sample variability are established using the first 15 duplicate samples by

   Multiplying the mean range ($\bar{R}$) by the student "t" factor value of 3.27 for a 95% confidence interval:

   Control Limit ≡ R x 3.27

   Any duplicate range that exceeds the set control limit would indicate an error has occurred.

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   Any duplicate range that exceeds the set control limit would indicate an error has occurred.

6. Control limits and precision calculations are conducted and compared with the initial established limits for 10% of sample types analyzed routinely. If the range is greater than 3.27(R) then this variability has to be investigated. The initial control limits are periodically updated by repeating the procedures using the most recent set of 15 duplicate samples.

The QC manager will track the precision of each method by evaluating historical data for any indication of variability caused by the analyst's performance, change in reagents/supplies or the need for equipment maintenance.

11.2 Accuracy

While precision measures the closeness of duplicate sample analysis, it does not ensure the accuracy of the data generated at the laboratory. Accuracy of data reflects the degree to which the analytical measurement represents the actual concentration of the tested sample or its "true value".

Microbiology

Microbial samples pose a special problem of reproducibility due to natural variations in the number of organisms found from sample to sample. Apart from blank samples (laboratory blank or field blank), additional control samples (described below), are utilized to determine accuracy when deemed necessary to establish method performance:

a) Control samples: Water samples inoculated with a known number of organisms from a referenced culture (obtained from commercial sources, American Type Culture Collection-ATCC grade) are analyzed simultaneously. Recovery from the Control sample is compared with Buffered water samples on the non-selective medium and expressed as Percent Recovery (P).

Inorganic Chemistry

Laboratory Fortified Blanks: A reagent water sample that has been fortified with a known concentration of analytes of interest is a LFB. Calculate the percent recovery (P) of the analyte in the Laboratory Fortified Blanks (LFB).

$$P = \frac{(\text{LFB} - \text{Sample Result})}{\text{Known LFB conc. added}} \times 100$$

When duplicate spiked samples are run along with unspiked samples (particularly for drinking water samples) the relative percent difference (RPD) between the control spiked samples can be calculated:

$$\text{RPD} = \frac{\text{(Spiked Sample A) - (Spiked Sample B)}}{\text{(Spiked A + Spiked B)/2}} \times 100$$

b) For Instrument analytical methods where known reference with known values or "true values" are available commercially, accuracy is established by comparing the relative percent difference (RPD) between the laboratory measured value and the known "true value".

$$\%\ \text{RPD} = \frac{\text{True Value - Measured Value}}{\text{(True Value + Measured Value)/ 2}} \times 100$$

11.3 Mean & Standard Deviation

To establish control limits and determine variations expected in the laboratory data the mean and standard deviation are often calculated. Approximately 20 control replicate samples are analyzed over time, for each test method to determine an estimate of the central tendency or "the mean value" of the data set. Any shift or drift from the mean value may compromise the accuracy of the method and lead to errors. Control limits are updated by periodically calculating the mean and standard deviation using the most recent set of 20 replicate samples.

Mean

In microbiology, the data often does not show a normal statistical distribution. The data is skewed because the frequency of low bacterial counts is favored. In order to obtain a more symmetrical distribution of the data, the values are converted to their logarithms. The preferred method to calculate the mean of the dataset is the **geometric mean** $\bar{x}_{(g)}$ instead of **arithmetic mean** $\bar{x}_{(a)}$ (used for normal distribution) since it is less influenced by outliers (extreme values) occasionally observed in datasets. The geometric mean may be calculated using either procedure described below:

1) Calculate the anti log of the arithmetic mean of log transformed data:

a) $\log \bar{x}_{(g)} = (\sum \log x_i)/n$ $\log \bar{x}_{(a)}$ = logarithm of mean calculated value

$\sum \log x_i$ = sum of individual log transformed values = $(\text{Log } x_1 + \log x_2 + \log x_3 \ldots + \log x_n)$

n = total number of values

b) $\bar{x}_{(g)} = \text{antilog}\ (\log \bar{x}_{(g)})$

2) Calculate the nth root of the product of the results:

$$\bar{x}_{(g)} = \sqrt[n]{(x_1)\ (x_2)\ (x_3) \ldots .\ (x_n)}$$

The same results can be obtained using either (1) or (2).

Note: arithmetic mean may be used if required by regulatory bodies

$\bar{x}_{(a)} = \sum xi\ /n$ where $xi = x1 + x2 + x3 \ldots . + .xn$

Standard Deviation

Once the mean is calculated, the standard deviation is determined by taking the difference of each control result from the mean, squaring that difference, dividing by n-1, then taking the square root. All these operations are shown in the following equation:

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{(n - 1)}}$$

where **s**= is the standard deviation,
$\sum$ means summation of all the $(xi - \bar{x})^2$ values,
x_i = an individual control result,
$\bar{x}$= the mean of the control results,
n = the total number of control results included in the group.

A larger standard deviation (s) reflects a greater dispersion of data and an increased frequency of random error. A smaller standard deviation (s) reflects narrower and sharper distribution of data and a lower frequency of random error.

For a measurement procedure, it is generally expected that the distribution of control results will follow log normal distribution such that control limits and warning limits can be established:

Warning Limit= Observed result ± 2(s)

This limit indicates that with 95% confidence limit, the observed result is within +/- 2 standard deviations of the mean.

Control limit = Observed result ± 3(s)

Establishes the acceptance limit with 99% confidence such that the observed result falls within plus/minus 3 standard deviations of the mean.

These limits provide monitoring of the accuracy of the results for the methods used at Cel-A. If variation is observed beyond these limits, the data is rejected and the cause of dispersion is investigated.

The mean and standard deviation are calculated using the EPA software ProUCL (software to calculate upper confidence limits).

11.4 Completeness of Data

Completeness of data is established by the percentage of laboratory results backed up by accepted quality assurance data. Completeness of data however relies also on the sampling process, transportation and treatment of samples prior to receipt by laboratory in addition to the analytical procedures.

11.5 Method Detection Limit (MDL)

MDL Calculation: Three important things to remember about calculating MDLs for inorganic parameters are:
1) use the sample standard deviation
2) use the correct Student's t-value and
3) use all significant figures.

The sample standard deviation, s, must be used when calculating MDLs. One of the most common mistakes is using the population standard deviation, s.

The EPA's MDL procedure derives the 95% confidence interval
estimates for the method detection limit using the percentiles of the chi square over the degrees of freedomdistribution.

For seven replicates, the 95% confidence intervals listed in 40 CFR 136 are:
Lower Critical Limit (LCL) = 0.64 x MDL
Upper Critical Limit (UCL) = 2.20 x MDL

These limits vary depending on the number of replicates. For the purposes of determining whether multiple MDL determinations are equivalent, the laboratory calculates the UCL and LCL for the lowest MDL value. If the other MDLs fall below the UCL of the lowest MDL, it is acceptable to report the highest MDL value as the detection limit, as long as the highest MDL meets all of the necessary regulatory requirements.

MDL is calculated as shown in the following equation:

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MDL is calculated as shown in the following equation:

$$MDL = (s)(t\text{-value})$$

Where: MDL = Method Detection Limit
s = Sample stadnadard deviation
t-value = Student t values provide in the EPA MDL method for the number of tests performed

THE FIVE POINT CHECK

In addition to analyst experience, the calculated MDL should be evaluated using several checks to determine if it will meet all of the necessary criteria. The following five items, which will be referred to as the "FivePoint Check" are simple ways to check a calculated MDL.

1. Does the spike level exceed 10 times the MDL? If so, the spike level is high.
2. Is the MDL higher than the spike level? If so, the spike level is too low.
3. Does the calculated MDL meet regulatory requirement for the necessary program(s)?
4. Is the signal/noise (S/N) in the appropriate range?
5. Are the replicate recoveries reasonable?

If a particular analyte does not have a maximum required MDL the third item can be disregarded. For environmental programs setting maximum MDLs, it is important to check the appropriate Administrative Code or analytical methods for the current requirements. Items 4 and 5 are not required, but are useful for evaluating the data used to generate the MDL.

Other ways to evaluate whether or not a calculated MDL is a good estimate of the detection limit exist. The MDL procedure in 40 CFR 136 gives an iterative procedure, utilizing pooled standard deviations, forevaluating the MDL. This procedure is found in Appendix D. Another validation method is the analysis of serial dilutions.

The S/N Test - The MDL procedure in 40 CFR Part 136 recommends that the detection limit be estimated somewhere in the range where the signal to noise ratio is 2.5 to 5. The S/N is not only useful for estimating the initial detection limit, but it is also useful for evaluating the final MDL determination. The signal to noise ratio describes the effect of random error on a particular measurement, and estimates the expected precision of a series of measurements. Samples spiked in the appropriate range for an MDL determination typically have a S/N in the range of 2.5 to 10. A signal to noise ratio less than 2.5 indicates that the random error in a series of measurements is too high, and the determined MDL is probably high. In this instance, the samples should be spiked at a higher level to increase the signal. If the signal to noise ratio is greater than 10, the spike concentration is *usually* too high, and the calculated MDL is not necessarily representative of the LOD. In this case, the samples should be spiked at lower level. In some instances, especially with highly precise analytical techniques, the S/N may always be higher than ten. Again, the analyst's experience is crucial for determining when the S/N ratio is too high. The S/N ratio for a series of measurements can be **estimated** by:

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$$(S/N)_{est} = X_{ave}/s$$

Where
Xave = the average of either the calculated concentrations or analytical signals for the replicates
S= the sample standard deviation for the replicates.

The S/N ratio is a useful test for MDL validity, but a high signal to noise ratio does not necessarily indicate that the MDL is invalid.

Percent Recoveries Notes - One of the drawbacks of the MDL procedure is that it doesn't take into account the effects of high or low bias in a series of measurements. The effects of a bias are usually most notable in samples other than reagent water, due to matrix interferences. Bias can be measured by the average percent recovery of a series of samples. In order for an MDL to be realistic, the average percent recovery for the samples should be reasonable. A reasonable
recovery is subjective, and will be defined differently for different situations. Since MDL calculations involve low level analysis, the recoveries may not be comparable to samples spiked somewhere well within the "quantification" region of the calibration curve. An analyst familiar with the analytical system should be able to judge whether or not the average percent recovery falls within the expected range for low level samples. The average percent recovery can be calculated using the following equation:

$$Ave.\%R = (X_{ave}/spikelevel) x100\%$$

Where X ave = the average concentration of the samples
spike level=the initial spike concentration.

Some analytical methods specify appropriate control limits for low-level precision and accuracy samples. If a method does not specify appropriate low level control limits, it is often useful to evaluate the average percent recovery using previously established control limits. The appropriate control limits for reagent water spikes could be the limits for fortified blank recovery. If the determination was performed in a matrix other than reagent water, use the control limits specific for the matrix spikes. The results for low-level analysis may not always fall within the acceptable range, but if a calculated MDL is questionable, evaluating the recoveries in this manner may reveal a bias which could affect the calculation.

11.6 Laboratory Reporting Limits (RL)

The RL is the in-house determination of control limits described in the previous sections which take into account the precision and the accuracy of the data. The RL is reported to clients for each method, when applicable. The Method Detection Limit (MDL) is the lowest level of detection determined by inter-laboratory collaborative studies for certain methods. Where the MDL is available it is reported along with the laboratory reporting limit (RL).

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In general, results below the reporting limit are interpreted as either

a) the level of contaminants is very low or
b) not present in the sample.

Results above the RL mean the contaminant is not only there, but the lab is very confident of the reported result.

If the results are too high, such as too-numerous to count as in membrane filtration method, results are reported with data qualifiers defined at the bottom of the report with an explanation of the results and pertinent information about the sample or analytical procedures that may have affected the reported results. The laboratory often requests additional samples to repeat the analysis.

For inorganic parameters, Reporting limits are not acceptable substitutes for detection limits unless specifically approved by the Department for a particular test.

12.0 CORRECTIVE ACTION

12.1 Laboratory Procedures

If a problem is identified at the laboratory, corrective action is initiated by the QA/QC officer. In general most problems are associated with equipment, sample management, analytical results and data reporting where the associated internal SOPs outline what to do when anomalies are observed. Corrective actions affecting the integrity of the analytical results must be brought to the attention of the client, in writing by the laboratory director. The effectiveness of the corrective action must be established by follow up procedures and an internal audit and verified by the QA/QC manager. SOP CA-1 outlines the laboratory procedure for corrective actions.

12.2 Customer Complaint

At all times our company's policy promises to provide customers with the best possible service in a courteous and thoughtful manner. Possible complaints are reviewed and taken seriously, as we are open to feedback from clients who may suggest areas of improvement. Potential customer complaints are forwarded immediately to the laboratory director, who ensures the execution of corrective action to prevent recurring failures, and who communicates findings to the customer in a good faith effort to resolve the complaint. With the customer's satisfaction and approval, the dispute will be marked as resolved. A complaint investigation form (SOP CA-2) will be maintained in the customer file.

In general, results below the reporting limit are interpreted as either

a) the level of contaminants is very low or
b) not present in the sample.

Results above the RL mean the contaminant is not only there, but the lab is very confident of the reported result.

If the results are too high, such as too-numerous to count as in membrane filtration method, results are reported with data qualifiers defined at the bottom of the report with an explanation of the results and pertinent information about the sample or analytical procedures that may have affected the reported results. The laboratory often requests additional samples to repeat the analysis.

For inorganic parameters, Reporting limits are not acceptable substitutes for detection limits unless specifically approved by the Department for a particular test.

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13.0 QUALITY ASSURANCE REPORT TO MANAGEMENT

The QA/QC officer is responsible for gathering all quality control information pertinent to the day to day laboratory operations monthly in addition to a yearly report and to present them to the laboratory director for her review. The QA/QC reports in general include:

- Any updates and revisions to the QA/QC manual with dates and revision number
- Performance audit results and rating, including annual performance evaluation
- Annual internal audit report for each analysis conducted at the laboratory
- Precision and accuracy report of each method of testing
- Revisions or any updates on SOPs
- Corrective action reports

Annual Report Contains:

- Evaluation and revision of the laboratory's Quality Assurance Manual.
- Assessment and modification of the laboratory's Standard Operating Procedures.
- Review of the internal quality assurance audits.
- Synopsis of deviations and remedial action, including analyst non-compliance with laboratory policies
- An abstract of instrument calibration and maintenance.
- Review of temperature monitoring data for incubators and refrigerator up keep.
- Assessment of reagent water quality and maintenance schedules
- Assessment of performance results.
- Evaluation of external audit results

The laboratory director shall review all the reports, comment on any irregularities and ensure implementation of corrective action(s) when needed in a timely manner.

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- Any updates and revisions to the QA/QC manual with dates and revision number
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- Synopsis of deviations and remedial action, including analyst non-compliance with laboratory policies
- An abstract of instrument calibration and maintenance.
- Review of temperature monitoring data for incubators and refrigerator up keep.
- Assessment of reagent water quality and maintenance schedules
- Assessment of performance results.
- Evaluation of external audit results

The laboratory director shall review all the reports, comment on any irregularities and ensure implementation of corrective action(s) when needed in a timely manner.

General References:

The following sources were used in preparation of this quality assurance manual and the internal standard operating procedures:

[1] US EPA Microbiology Home Page: http://www.epa.gov/nerlcwww/

[2] US EPA Publication G-065: Manual for the Certification of Laboratories Analyzing Drinking Water -5th edition, January 2005, On line copy: http://www.epa.gov/safewater/methods/pdfs/manual_labcertification.pdf

[3] Standard Methods for the Examination of Water and Waste Water 20th edition 1998. Published jointly by American public health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF)

[4] Guidelines Establishing Test Procedures for the Analysis of Pollutants: Analytical Methods for Biological Pollutants in Ambient Water; Final Rule Fact Sheet (EPA-821-F-03-009) July 2003; http://www.epa.gov/ost/methods/biological/fsbio.htm

[5] USEPA Control of Pathogen and Vector Attraction in Sewage Sludge http://www.epa.gov/ORD/NRMRL/Pubs/1992/625R92013appF.pdf

[6] Association of Official Analytical Chemists. (AOAC) https://ecam.commer.net/aboutecam.asp

[7] Total Coliform Rule: http://www.epa.gov/OGWDW/tcr/pdf/qrg_tcr_v10.pdf

[8] Surface Water Treatment Rule: http://www.epa.gov/OGWDW/therule.html#Surface

[9] California Department of Health Division of Drinking Water and Environmental Management http://www.dhs.ca.gov/ps/ddwem/beaches/Beaches_appendices/

[10] Efiok, B., Eduok, E. 2000: Basic Calculations for Chemical and Biological Analysis, Second Edition. AOAC international publication

[11]. US EPA publication 600/878017 "Microbiological Methods for Monitoring the Environment, Water and wastes" Part IV Section B: Statistics for Microbiology, 1978

[12] US EPA Publication SW846 3rd edition- Solid waste Hazardous Waste - Test Methods

General References:

The following sources were used in preparation of this quality assurance manual and the internal standard operating procedures:

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[4] Guidelines Establishing Test Procedures for the Analysis of Pollutants: Analytical Methods for Biological Pollutants in Ambient Water; Final Rule Fact Sheet (EPA-821-F-03-009) July 2003; http://www.epa.gov/ost/methods/biological/fsbio.htm

[5] USEPA Control of Pathogen and Vector Attraction in Sewage Sludge http://www.epa.gov/ORD/NRMRL/Pubs/1992/625R92013appF.pdf

[6] Association of Official Analytical Chemists. (AOAC) https://ecam.commer.net/aboutecam.asp

[7] Total Coliform Rule: http://www.epa.gov/OGWDW/tcr/pdf/qrg_tcr_v10.pdf

[8] Surface Water Treatment Rule: http://www.epa.gov/OGWDW/therule.html#Surface

[9] California Department of Health Division of Drinking Water and Environmental Management http://www.dhs.ca.gov/ps/ddwem/beaches/Beaches_appendices/

[10] Efiok, B., Eduok, E. 2000: Basic Calculations for Chemical and Biological Analysis, Second Edition. AOAC international publication

[11]. US EPA publication 600/878017 "Microbiological Methods for Monitoring the Environment, Water and wastes" Part IV Section B: Statistics for Microbiology, 1978

[12] US EPA Publication SW846 3rd edition- Solid waste Hazardous Waste - Test Methods

[12] The U.S. Environmental Protection Agency Method Detection Limit (MDL) procedure found in Title 40 Code of Federal Regulations Part 136 (40 CFR 136, Appendix B, revision 1.11

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Appendix 10: Sonoma County Laboratory Bacteroides Analysis QAP

Bacteroides Quality Assurance Program (QAP)

5/12/11

The Sonoma County Public Health Laboratory performs molecular assays on recreational water samples for the quantitative determination of Human (HuBac), Bovine (Bobac) and Total (Allbac) bacteroides species.

Organization of the laboratory

The Sonoma County Public Health Laboratory staff is currently comprised of four public health microbiologists, three laboratory technicians a secretary and a laboratory director. The microbiologists are certified by the State of California and perform numerous assays in accordance with CLIA, ELAP and the CDC. Job descriptions and responsibilities are on file.

Molecular Testing Responsibilities

All of the molecular assays are performed by certified public health microbiologists. As members or the laboratory response network (LRN) each microbiologist and the lab director's molecular technique and skills are reviewed at a minimum of every other month. This review includes extraction, PCR, interpretation and final reporting of results. These competency reviews are observed and critiqued as part of our routine bio-terrorism readiness.

Safety

The entire staff receives annual safety training. The trainings include both biological agent and chemical hygiene. The microbiologists also annually review the proper use of all personnel protective equipment (PPE). This includes the proper use and care of PAPR's used inside the BSL 3, molecular laboratory.

All trainings consist of, but are not limited to:

1. Blood borne pathogens
2. Personnel Protective Equipment
3. Incident response
4. MSDS information
5. Select agent reviews
6. Chemical Hygiene

Data Quality Objectives:

The objective of the Sonoma County Public Health Laboratory is to produce good quality, reproducible data. Measures in place to ensure that the reported data is of the highest quality include; quality control procedures, system controls, assay controls, technical competency reviews, corrective action program and participation in numerous laboratory proficiency testing programs.

Sample receiving and handling

Water samples are collected by the submitting agency, business, organization or private clients. Once the samples arrive at the laboratory, the paper work and or chain of custody is dated and time stamped in. The temperature of the sample(s) is taken with a NIST traceable infra-red thermometer and recorded onto the sample log sheet. The samples are immediately placed into a refrigerator at 4 degrees centigrade. All samples are given a laboratory number and hand written onto a log sheet. The log sheet will capture at a minimum the date, the time, the temperature, the submitter and the name of the employee logging in the sample. Additional information may be required (chain-of-custody or location of sample) and would be captured at this time. Sample processing will begin as soon as possible once the samples arrive and are logged in. Any sample received that does not meet acceptable standards will be flagged on the log sheet and the final report may be qualified.

Performance and System Audits:

Audits are performed annually or whenever there is a change in SOP. The performance audit consists of checking SOP protocols against actual practice. The performance audit will ensure that the analysts are following the written SOP procedures.

The systems audit entails following archived records of a sample from receipt of the sample into the laboratory to the final report. The system audit ensures that protocols are followed, thermometers used were certified, molecular reagents employed were not out of date, the assay controls fell within their normal range, the system control gave an expected result and that the final report was accurate and issued within an acceptable time frame.

Corrective Action:

Corrective action is taken whenever there is a known deviation from the SOP or when the assay controls or system controls fail or fall outside the normal range of acceptable performance. Corrective action involves a critical analysis of "what went wrong". Was the problem procedural or technical in nature? Was there human error? Once the problem is diagnosed a corrective action is employed to rectify the problem. Additional measures may be taken to prevent the problem from happening again. All corrective actions are recorded and archived in the laboratory.

Proficiency Testing:

The laboratory subscribes to a proficiency testing program through the College of American Pathologists (CAP) and we receive unscheduled proficiencies from the Centers for Disease Control (CDC) that involve all aspects of our molecular testing protocols. The results are graded and reviewed.

Preventative Maintenance of Instruments and Equipment.

Our ABI 7500 fast dx instrument is used for the bacteroides qPCR assay. This instrument is maintained and serviced by ABI technical service representatives. As partners in the federal Laboratory Response Network for bio-terrorism and bio-watch we are required to have current service contracts in place for all of our technical equipment.

All of our molecular testing is performed in a BSL 3 level lab. The room maintains a continuous negative air pressure. The BSL 3 also houses two biological safety cabinets (BSC) for safety and preservation of samples. The room and the BSC are annually re-certified by a private contractor. Records are available for review.

Temperatures of freezers, refrigerators and incubators housed within the BSL 3 are taken daily. Thermometers are calibrated to meet NIST traceable standards.

There are two ***Clean-Spots*** TM used for molecular testing. One *clean-spot* is dedicated for the preparation of master mix only. The second is dedicated for the addition of template only. Both of these clean spots are wiped before and after use with a DNase product to minimize carry-over. By dedicating clean-spots in this way we reduce the potential of molecular carry-over.

All disposable components in our molecular lab are certified by our vendors to be DNase free.

System Controls and Assay Controls:

Each molecular run will contain both a system control as well as positive and negative assay controls. The system control is designed to detect carry over (false positives) from any component used in the assay from extraction (manual or auto) through the making of master mix. This system control goes through the entire protocol and should yield a negative result. This ensures that the "system" is working and that no singular component is contaminated with template. System controls can also include a known DNA target inserted into the sample to detect any possible molecular inhibition (false negatives) in the sample itself.

The assay controls are simply known positive and negative templates. This ensures the instrument and the microbiologist followed proper protocols and reagent sequences to yield the correct expected results.

If any combination of these controls does not yield the correct expected result the run is in invalid. Once the problem has been identified a corrective action would be employed and the run repeated.

With quantitative assays, the standards are run in duplicate to ensure reasonable reproducibility. Each sample is run in duplicate to also demonstrate reasonable reproducibility.

If applicable outdates and lot numbers of molecular reagents are checked each day of use and recorded.

Assessment of Data Precision, Accuracy, Validation and Reporting:

The laboratory director or designee reviews worksheets and test data for any discrepancies. Mathematical calculations are reviewed for accuracy. The final report is reviewed to ensure the correct interpretation has been made. All raw data and worksheets are archived and available for future review. Upon request copies of all raw data, worksheets and control performances will be submitted along with the final report.

Richard Gruen 5/12/11

Appendix 11: Region 1 Microbiology Laboratory QAP

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

(March 25, 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 March 25, 2011

Approvals

The approvals below were submitted separately, preventing their inclusion in this signature block. Instead, they appear in Appendix J: *Approval Signatures* of this document.

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

_____ _____
Signature Date

Co-Laboratory Directors:

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

_____ _____
Signature Date

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

_____ _____
Signature Date

Region 1 Microbiology Laboratory
Laboratory Safety Manual – Version 1.0

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

_____ _____
Signature Date

Principal Analyst:
Bella Neufeld, Regional Water Quality Control Board – North Coast Region

_____ _____
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

List of Figures

List of Tables

List of Appendices

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).

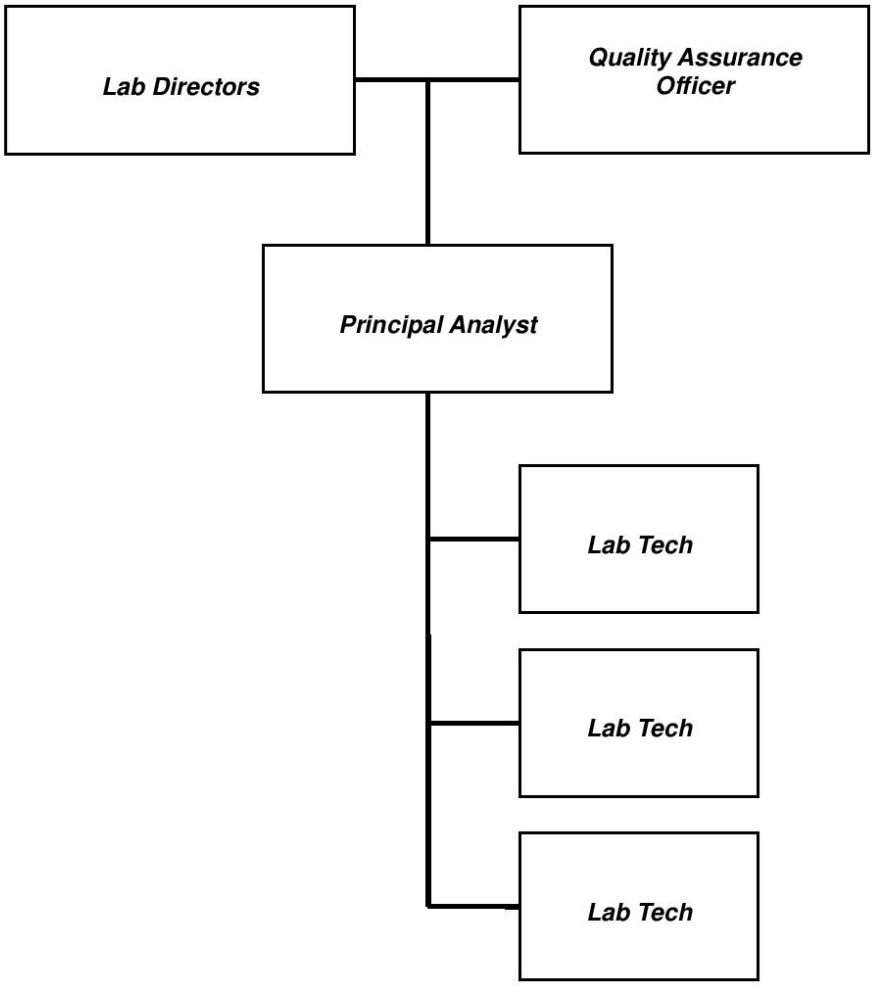
Table 1: QAP Distribution List Primary Contact Information

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

Region 1 Microbiology Laboratory
Laboratory Safety Manual – Version 1.0

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 MSDS sheets.
- 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

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 (MB)

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 (LRB)

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.

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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	NIST	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

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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 on going 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 asses 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 duplicates (MS/MSDs)
- Laboratory duplicates
- Surrogates
- Certified reference material (CRMs)
- Laboratory control samples (LCSs)
- Method blanks
- Field QC samples
- Reporting limits (RLs)

Appendix A:

Region 1 Microbiology 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

(March 25, 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
Phone Number: 707-576-6718
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Effective Date March 25, 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

_____ _____
Signature Date

Co-Laboratory Directors:

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

_____ _____
Signature Date

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

_____ _____
Signature Date

Region 1 Microbiology Laboratory
Laboratory Safety Manual – Version 1.0

Principal Analyst:
Bella Neufeld, Regional Water Quality Control Board – North Coast Region

_____ _____
Signature Date

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

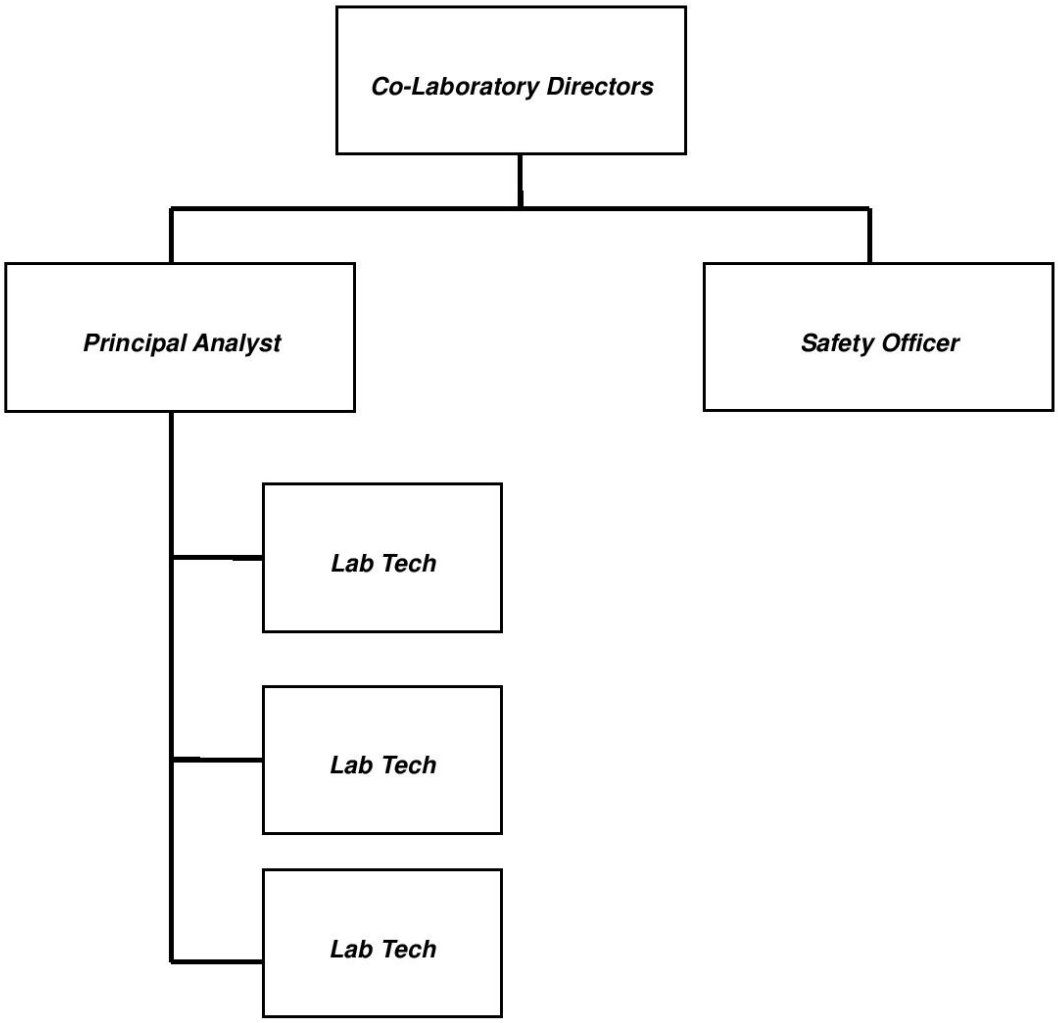
_____ _____
Signature Date

A2. Table of Contents

List of Figures

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.

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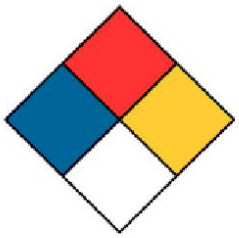
Figure 2: NFPA Safety Diamond



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.

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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, halogentated 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 chords 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 can not 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 clean up 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

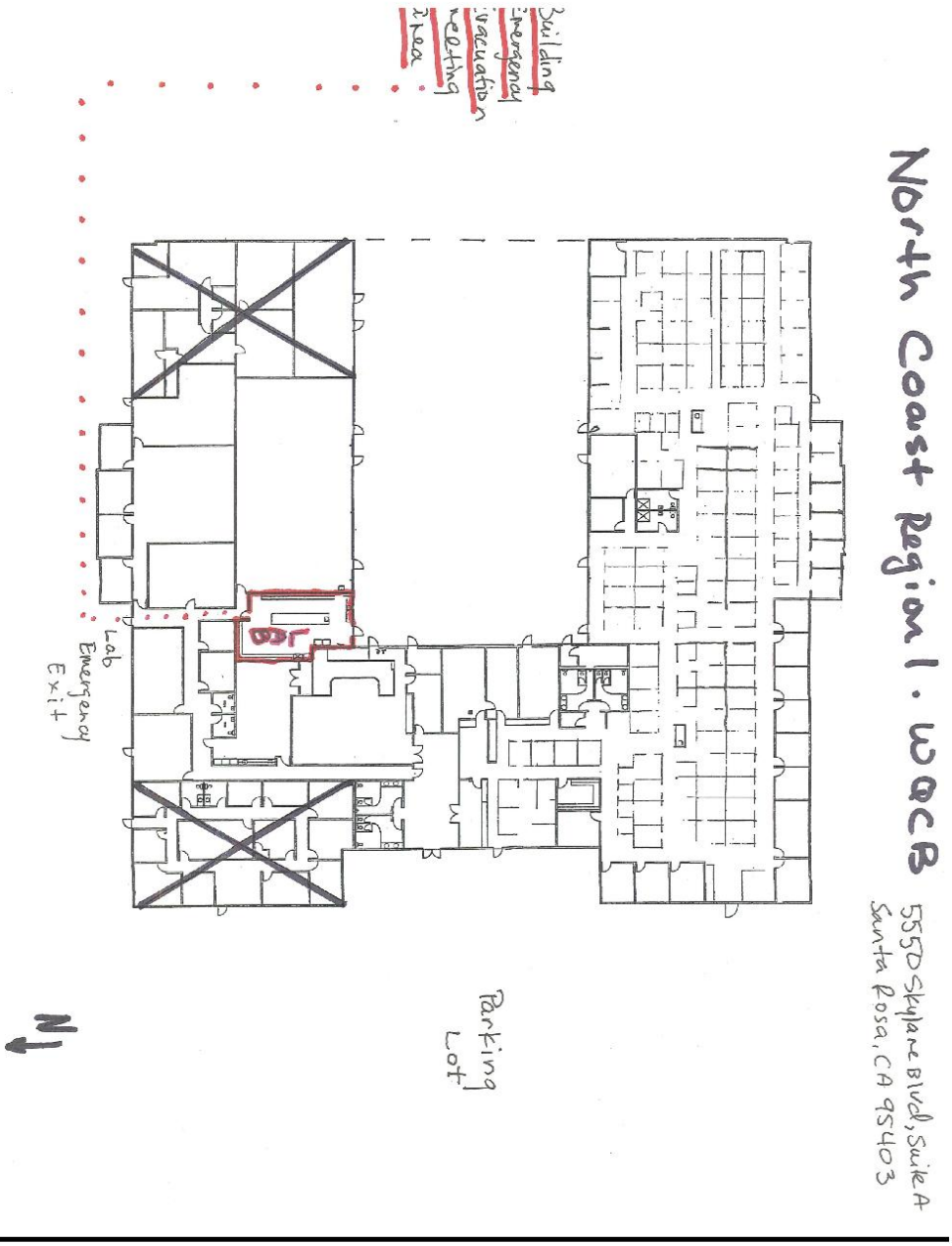
If laboratory building ventilation fails, all operations concerning chemicals within that laboratory or building must be discontinued. Laboratory operations may resume in the laboratory or building once ventilation has been restored and if it is confirmed that all ventilation systems are operating correctly.

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 B:

Region 1 Microbiology Evacuation Plan


North Coast Region 1 · WQCB
5550 Skylane Blvd, Suite A
Santa Rosa, CA 95403
Building Emergency Evacuation Meeting Area
Lab Emergency Exit
B31
Parking Lot
N

Appendix C:

Region 1 Microbiology Laboratory

Standard Operating Procedures - Colilert ®

Coliform and E. coli Water Analysis

and

Standard Operating Procedures - Enterolert ®

Enterococci Water Analysis

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 25, 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
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Effective Date March 25, 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

_____ _____
Signature Date

Co-Laboratory Directors:

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

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Signature Date

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

_____ _____
Signature Date

Region 1 Microbiology Laboratory
Laboratory Safety Manual – Version 1.0

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

_____ _____
Signature Date

Principal Analyst:
Bella Neufeld, Regional Water Quality Control Board – North Coast Region

_____ _____
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

Colilrt 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 (Nonbiologically 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.

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.

J-6 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.

J-7 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 (eg. 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 more details.

K-2 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.

K-7 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 A: 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 B: 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

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 25, 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
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Santa Rosa CA 95403

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Effective Date March 25, 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

_____ _____
Signature Date

Co-Laboratory Directors:

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

_____ _____
Signature Date

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

_____ _____
Signature Date

Region 1 Microbiology Laboratory
Laboratory Safety Manual – Version 1.0

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

_____ _____
Signature Date

Principal Analyst:
Bella Neufeld, Regional Water Quality Control Board – North Coast Region

_____ _____
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 (Nonbiologically 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 (eg. 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 A: 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)	WaterQuantitation Limit, MPN/100 mL	Soil Quantitation Limit,µg/kg
Enterococci Bacteria	NA	1	NA

Appendix B: 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

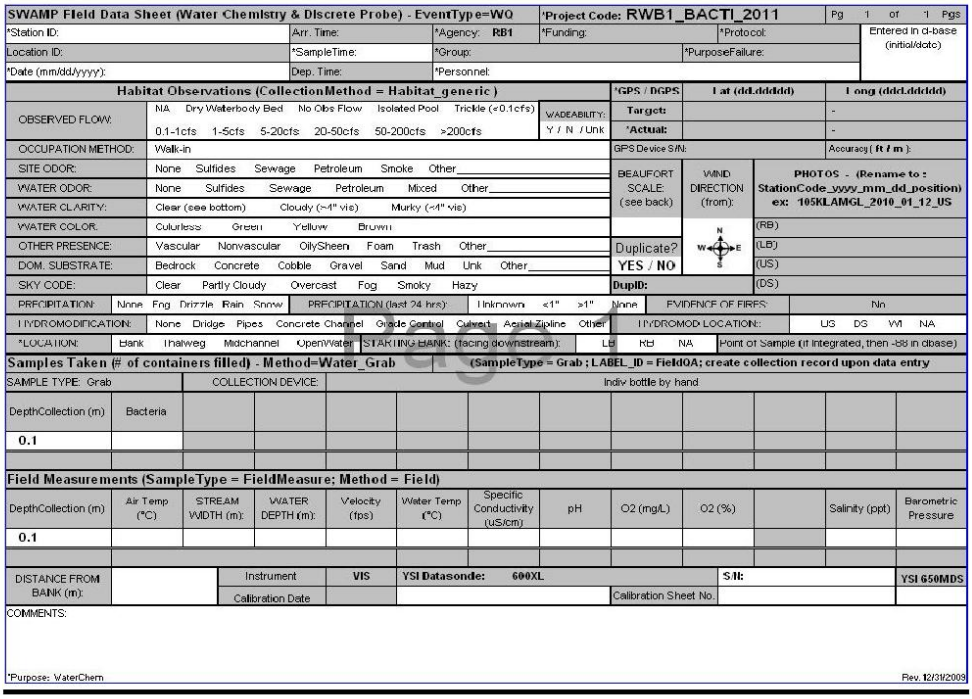
Field Duplicate RPD 95%
Lab Duplicate RPD <25%
Blank <1/100mL

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

Appendix D:

Region 1 Microbiology COCs and Fieldsheets

Region 1 Microbiology Laboratory
Laboratory Safety Manual – Version 1.0



SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ				*Project Code: RWB1_BACTI_2011		Pg 1 of 1 Pgs
*Station ID:	Arr. Time:	*Agency: RB1	*Funding:		*Protocol:	Entered in cl-base (initial/date)
Location ID:	*SampleTime:	*Group:			*PurposeFailure:	
*Date (mm/dd/yyyy):	Dep. Time:	*Personnel:				

Habitat Observations (CollectionMethod = Habitat_generic)			*GPS / DGPS:	Lat (dd.dddddd)	Long (ddd.dddddd)
OBSERVED FLOW:	NA Dry Waterbody Bed No Obs Flow Isolated Pool Trickle (<0.1cfs) 0.1-1cfs 1-5cfs 5-20cfs 20-50cfs 50-200cfs >200cfs	WADEABILITY: Y / N / Unk	Target:		-
			*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)				(RB)
WATER COLOR:	Colorless Green Yellow Brown				
OTHER PRESENCE:	Vascular Nonvascular OilySheen Foam Trash Other		Duplicate?		(LB)
DOM. SUBSTRATE:	Bedrock Concrete Cobble Gravel Sand Mud Unk Other		YES / NO		(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 FIRE?:	No
HYDROMODIFICATION:	None Dridge 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 cbase)	

Samples Taken (# of containers filled) - Method=Water_Grab											(SampleType = Grab ; LABEL_ID = FieldQA; 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

Region 1 Microbiology Laboratory
Laboratory Safety Manual – Version 1.0

Region 1 Microbiology Laboratory: Colilert Processing Worksheet and Chain of Custody Form							SAMPLE DATE:				PROCESSING DATE:			
Project Site ID Number:														
SAMPLE TIME:														
Replicate Number:														
Laboratory Sample ID Number:		1105-		1105-		1105-		1105-		1105-		1105-		1105-	
		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
Yellow + Fluorescence (+)	# Large Wells														
	# Small Wells														
	False Positives														
	MPN														

MEDIA LOT #		FIELD REPLICATES				LAB REPLICATES			Incubator Temperature Checks (see back for instructions)		
		MPN	95% CI Lower	95% CI Upper		MPN	RD% – {[X1 - X2] / Xave} x 100		CHECKS	1	2
Total Coliform	Replicate 1				Replicate 1(X1)		Xave =		INITIALS		
	Replicate 2				Replicate 2 (X2)		RD% =		TIME		
	Replicate 3				Difference (X1 - X2)		Pass[1]	Needs Review	TEMP		
	Mean		Pass	Needs Review							
E. COLI	Replicate 1				Replicate 1(X1)		Xave =		CHECKS	3	4
	Replicate 2				Replicate 2 (X2)		RD% =		INITIALS		
	Replicate 3				Difference (X1 - X2)		Pass[1]	Needs Review	TIME		
	Mean		Pass	Needs Review					TEMP		
BLANK	Field Blank #		Pass	Needs Review	Lab Blank #		Pass	Needs Review			

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

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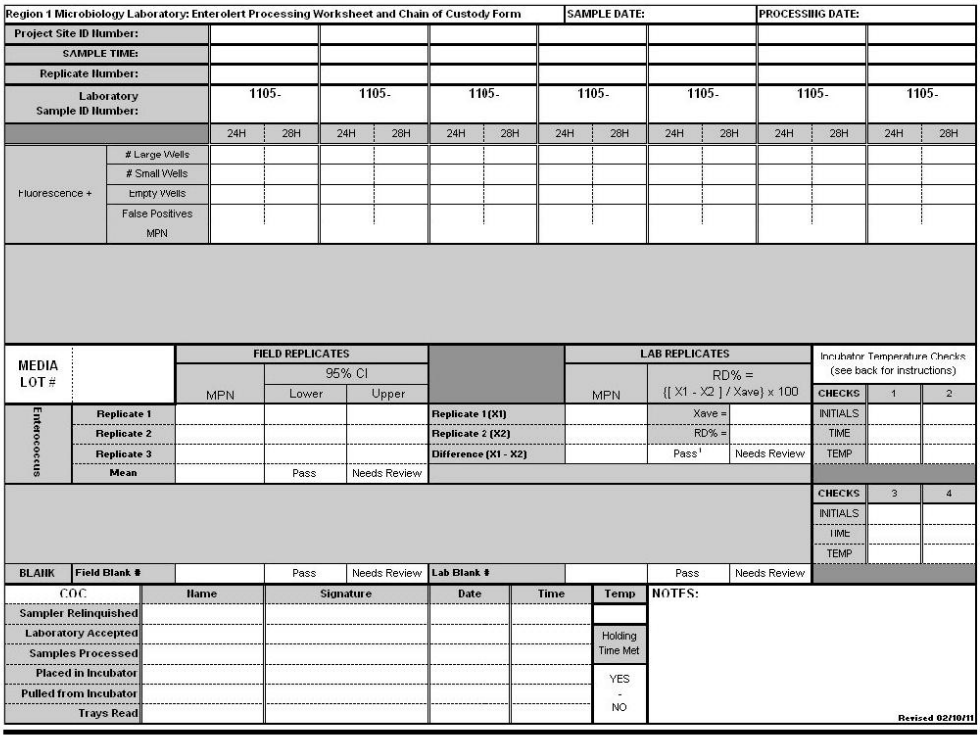

Region 1 Microbiology Laboratory: Enterolert Processing Worksheet and Chain of Custody Form							SAMPLE DATE:				PROCESSING DATE:			
Project Site ID Number:														
SAMPLE TIME:														
Replicate Number:														
Laboratory Sample ID Number:		1105-		1105-		1105-		1105-		1105-		1105-		1105-	
		24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H	24H	28H
Fluorescence +	# Large Wells														
	# Small Wells														
	Empty Wells														
	False Positives														
	MPN														

MEDIA LOT #		FIELD REPLICATES				LAB REPLICATES			Incubator Temperature Checks (see back for instructions)		
		MPN	95% CI Lower	95% CI Upper		MPN	RD% = ([X1 - X2] / Xave) x 100		CHECKS	1	2
Enterococcus	Replicate 1				Replicate 1 (X1)		Xave =		INITIALS		
	Replicate 2				Replicate 2 (X2)		RD% =		TIME		
	Replicate 3				Difference (X1 - X2)		Pass[1]	Needs Review	TEMP		
	Mean		Pass	Needs Review							
									CHECKS	3	4
									INITIALS		
									TIME		
									TEMP		
BLANK	Field Blank #		Pass	Needs Review	Lab Blank #		Pass	Needs Review			

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 E:

Region 1 Microbiology Comparison Counting Worksheets

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Colilert Comparison Counting Worksheet

Revised 10 March 2011

	Total Coliform									*E. coli*								
	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				
2					2					2					2				
3					3					3					3				
4					4					4					4				
5					5					5					5				
6					6					6					6				
7					7					7					7				
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46					46					46					46				
47					47					47					47				
48					48					48					48				
49										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.

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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				
2					2					2					2				
3					3					3					3				
4					4					4					4				
5					5					5					5				
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46					46					46					46				
47					47					47					47				
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 F:

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 can not 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 Objectives
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 12: Approval Sheet Signatures

Appendix 12: Title and Approval Sheet

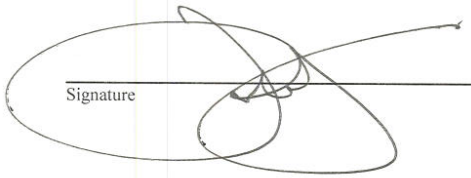
Steve Butkus, Project Manager – Data Manager – Contract Manager
North Coast Regional Water Quality Control Board

Steven Butkus 7 Nov 2012

Signature Date

Appendix 12: Title and Approval Sheet

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



Signature

11-7-12
Date

Appendix 12: Title and Approval Sheet

Renee Spears, Board Quality Assurance Officer
California State Water Resources Control Board

Renée Spears — Signature

11.16.2012 — Date

Appendix 12: Title and Approval Sheet

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

11/19/12

Signature Date

Appendix 12: Title and Approval Sheet

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

Signature

11/20/2012
Date

Appendix 12: Title and Approval Sheet

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

Signature Date 11/12/12

Appendix 12: Title and Approval Sheet

Contract Laboratory Director

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

Michael Ferris 11/8/2012
Signature Date

Appendix 12: Title and Approval Sheet

Veggie Dearborn, Lab Director
Cel Analytical Laboratories

Signature 11/08/12 Date