Regional Water Quality Control Board
North Coast Region

Coastal Watershed Pathogen Indicator Study

Quality Assurance Project Plan

Version 1.0

Originated by:

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North Coast Regional Water Quality Control Board

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Group A: Project Management

A1: Title and Approval Sheet

Project Title: Coastal Watershed Pathogen Indicator Study

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Effective Date: November 1, 2015

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See Appendix 9: Approval Sheet Signatures

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Signature Date

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Signature Date

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See Appendix 9: Approval Sheet Signatures

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Jeremy Corrigan, Humboldt County Public Health Laboratory, Lab Manager
County of Humboldt

See Appendix 9: Approval Sheet Signatures

Signature Date

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A3: Distribution List

Table 1. QAPP Distribution List Primary Contact Information

Contact Information	Organization's Mailing Address
Project Manager; **Data Manager;** Main Contact: Steve Butkus Phone: 707-576-2834 Email: SButkus@waterboards.ca.gov	Regional Water Quality Control Board North Coast Region 5550 Skylane Blvd. Suite A Santa Rosa, CA 95403
Contract Manager Main Contact: Bryan McFadin Phone: 707-576-2751 Email: BMcfaden@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
State Board Quality Assurance Officer; Main Contact: Renee Spears 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
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
Humboldt County Public Health Laboratory - Laboratory Manager Main Contact: Jeremy Corrigan Phone: 707-268-2179 Email: JCorrigan@co.humboldt.ca.us	County of Humboldt Department of Health & Human Services 529 I Street Eureka, CA 95501

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 water samples for the analysis of *E. coli*, total coliform, *Enterococcus*, and *Bacteroides* bacteria.

The North Coast Regional Water Board will be conducting laboratory analysis to include *E. coli*, total coliform, and *Enterococcus.* Sonoma County and Humboldt County Public Health Laboratories will be responsible for the analysis of the water samples for concentrations of *E. coli*, total coliform, *Enterococcus. Bacteroides* bacteria.

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

Table 2. Personnel Responsibilities

Name	**Project Title**	**Organizational Affiliation**	**Contact Information:** **Telephone number** **Fax number** **Email address**
Steve Butkus	Project Manager; Data Manager;	North Coast Regional Water Board	(707)-576-2834 (707)-523-0135 SButkus@waterboards.ca.gov
Bryan McFaden	Contract Manager	North Coast Regional Water Board	(707)-576-2751 (707)-523-0135 BMcFaden@waterboards.ca.gov
Rich Fadness	Project QA Officer	North Coast Regional Water Board	(707)-576-6718 (707)-523-0135 rfadness@waterboards.ca.gov
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
Jeremy Corrigan	Contract Lab Director, Contract Lab QA Officer	Humboldt County Department of Health & Human Services	(707)-268-2179 (707)-445-7640 jcorrigan@co.humboldt.ca.us

North Coast Regional Water Quality Control Board

Project Manager - Data Manager

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

Contract Manager

Bryan McFaden – He is responsible for managing the financial contracts between the North Coast Regional Water Quality Control Board and the Sonoma County and Humboldt County Public Health Laboratories for the analysis of water samples.

Project Quality Assurance (QA) Officer

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

Field Personnel

North Coast Regional Water Board staff will conduct all water sampling and data collection activities for Tasks 1 & 3. Water sample collection for Task 2 will be collected by trained staff of the Sonoma, Mendocino, and Humboldt Public Health Labs concurrently with sampling conducted for Clean Beach Initiative Program.

County of Sonoma

Sonoma Department of 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 and the Sonoma County Public Health Laboratory QAP (Appendices 4, 6 & 7).

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 Service, by communicating QA/QC issues contained in this QAPP.

County of Humboldt

Humboldt Department of Health & Human Services- Laboratory Manager & Laboratory Quality Assurance Officer

Jeremy Corrigan – 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 Humboldt County Public Health Laboratory Standard Operating Procedure and the Humboldt County Public Health Laboratory QAP (Appendices 5, 6 & 7). He will be responsible for the QA/QC procedures found in this QAPP as part of the sampling analysis.

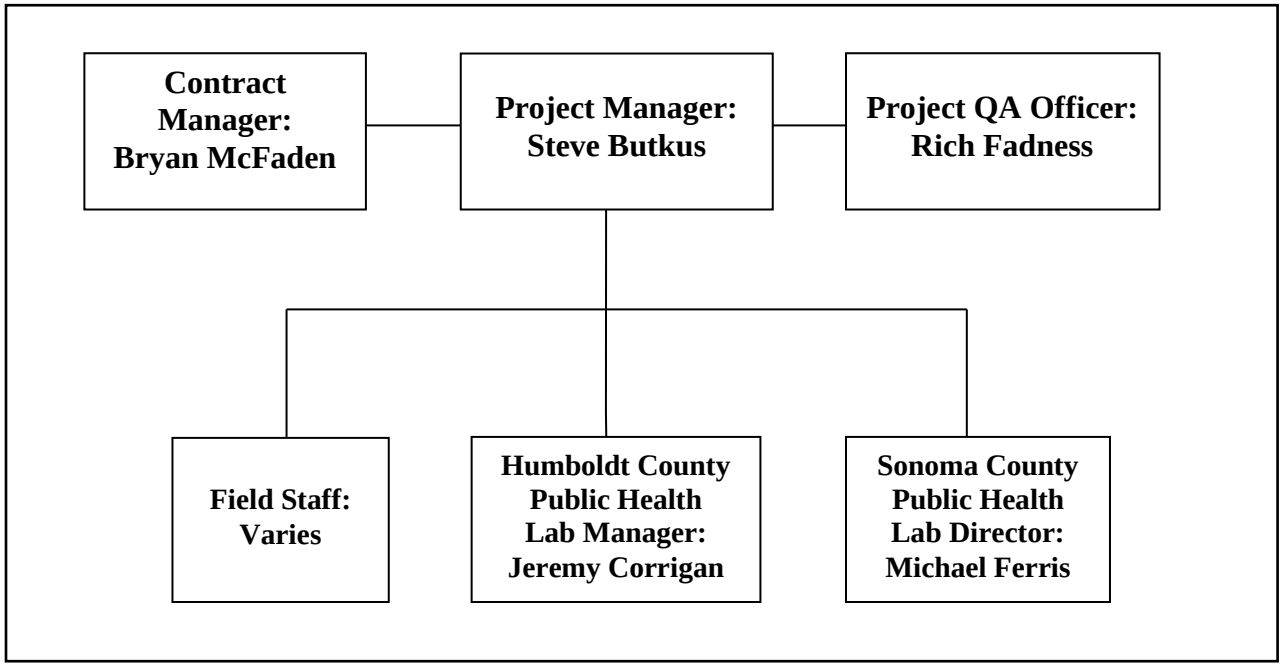

Figure 1. Project Organizational Chart

A5: Problem Definition/Background

Problem Statement.

The North Coast Regional Water Board staff are developing the Coastal Watershed Total Maximum Daily Loads (TMDLs) for pathogen indicators to identify and control contamination. Potential pathogen contamination has been identified several beaches and fresh water streams draining to marine waters. 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), and shellfish consumption (SHELL) designated beneficial uses.

Water Contact Recreation (REC-1) includes uses of surface water for recreational activities involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, swimming, wading, water-skiing, skin and scuba diving, surfing, white-water activities, fishing, or use of natural hot springs. Non-Contact Water Recreation (REC-2) include uses of surface water for recreational activities involving proximity to water, but not normally involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, picnicking, sunbathing, hiking, beachcombing, camping, boating, tidepool and marine life study, hunting, sightseeing, or aesthetic enjoyment in conjunction with the above activities. The SHELL beneficial use is defined as water suitable for the collection of filter-feeding shellfish (e.g., clams oysters, and mussels, but not crabs) for human consumption.

The 2012 Section 303(d) list identifies twenty-eight (28) surface waters as impaired for REC-1 or SHELL beneficial use from pathogen indicator bacteria. A separate TMDL Project will address the nine (9) listed waters located in the Russian River watershed. The remaining eighteen (19) surface waters will be addressed in this Coastal Pathogen Indicator TMDL Project (Table 1; Figures 1 - 3).

Table 3. Coastal Waters Impaired from Pathogen Indicator Bacteria

Hydrologic Unit	Listed Water Body Name	Impaired Beneficial Use	Sampling Location
Trinidad	Trinidad State Beach	SHELL	Trinidad St. Beach at Mill Creek
	Old Home Beach	SHELL	Old Home Beach at Scenic Drive
	Luffenholtz Beach	SHELL	Luffenholtz Beach at Luff. Creek
	Moonstone County Park	SHELL	Moonstone Beach at Little River
	Little River	REC-1	Little River at Hwy 101
			Little River at Crannell Road
	Clam Beach (near Strawberry Creek)	SHELL	Clam Beach at Strawberry Creek
Mad River	Clam Beach (near Mad River mouth)	SHELL	Clam Beach at Mad River
	Norton Creek	REC-1	Widow White Creek at Central Ave
			Widow White Creek at Murray Road
Eureka Plain	Jolly Giant Creek	REC-1	Jolly Giant Creek at Foster Road
			Jolly Giant Creek at Samoa Blvd
			Jolly Giant Creek at Granite Ave
	Gannon Slough	REC-1	Campbell Creek at 14th & Union Street
			Campbell Creek at 7th Ave
	Lower Elk River and Martin Slough	REC-1	Martin Slough at Campton & Fern Streets
			Martin Slough at Fairway Street
			Elk River at Berta Road
			Elk River at Zanes Road
Mendocino Coast	MacKerricher State Park (near Virgin Creek)	SHELL	MacKerricher State Park at Virgin Creek
	Pudding Beach	SHELL	Pudding Beach at Pudding Creek
	Pudding Creek	REC-1	Pudding Creek at Hwy 1
	Hare Beach	SHELL	Hare Beach at Hare Creek
	Caspar Headlands State Beach	SHELL	Caspar Beach at Caspar Creek
	Big River Beach at Mendocino Bay	SHELL	Mendocino Bay at Big River
Bodega	Campbell Cove	REC-1	Campbell Cove State Beach

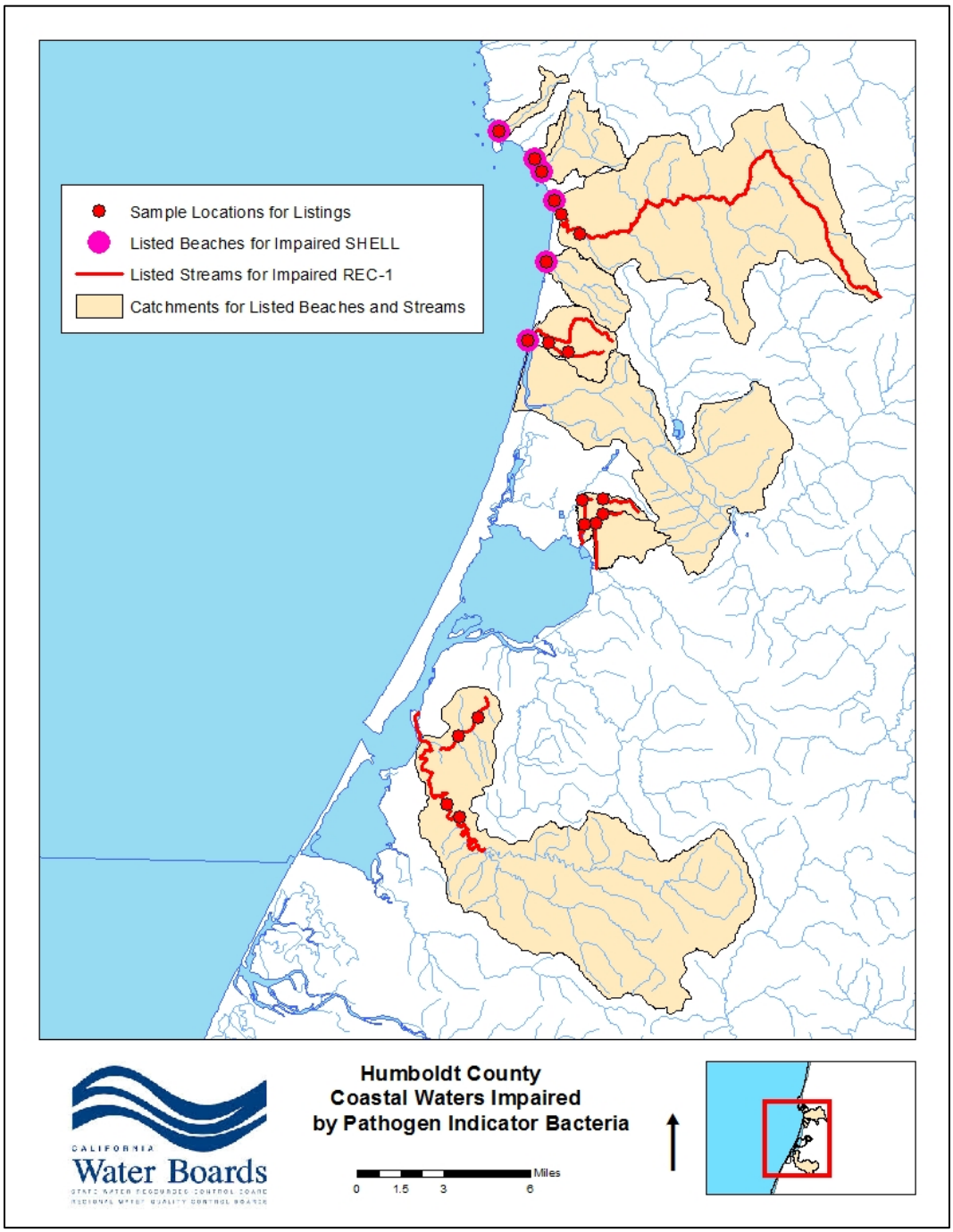

Figure 2. Humboldt County Coastal Waters Impaired by Pathogenic Indicator Bacteria

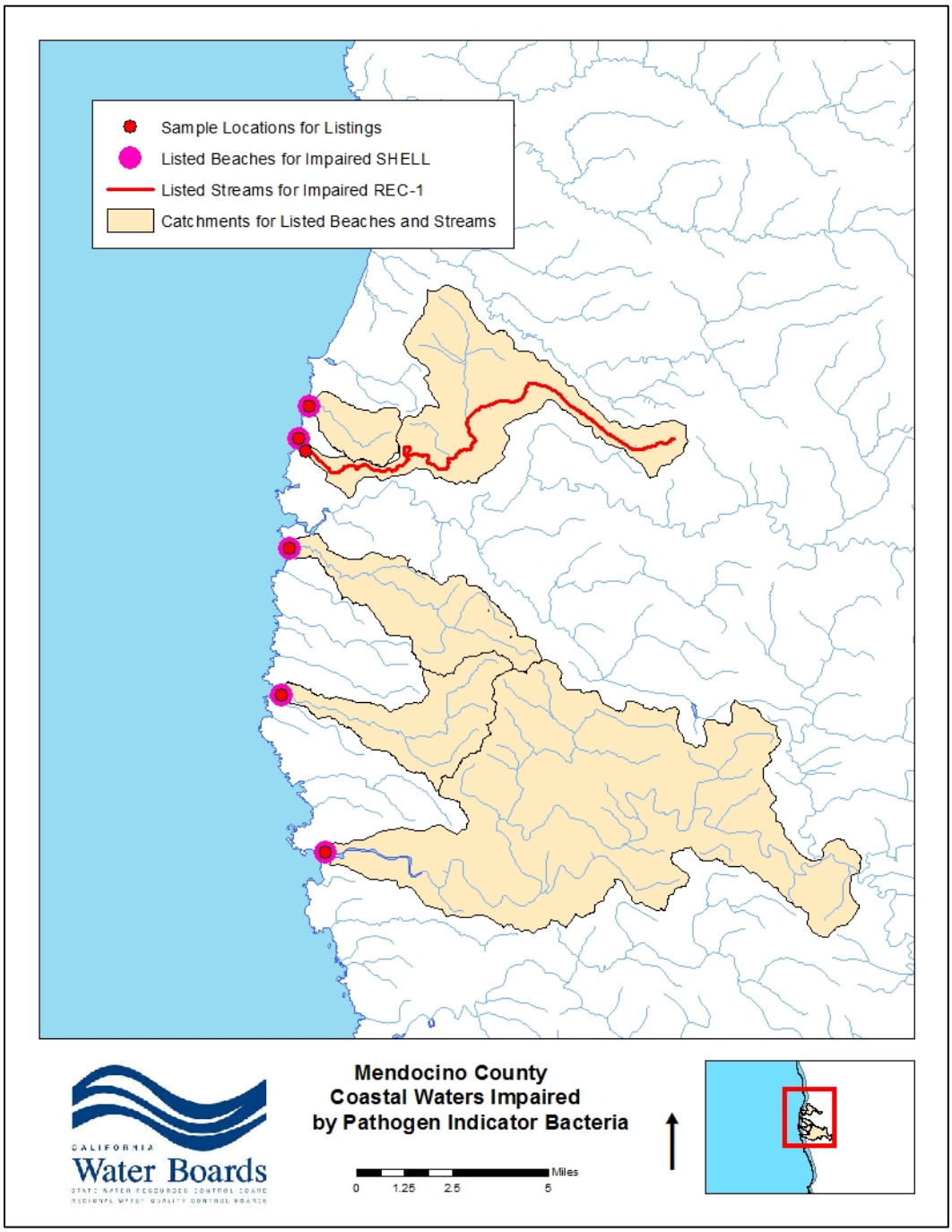

Figure 3. Mendocino County Coastal Waters Impaired by Pathogenic Indicator Bacteria

Figure 4. Mendocino County Coastal Waters Impaired by Pathogenic Indicator Bacteria

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

- Residences lacking proper or fully functioning septic disposal;
- Improperly connected sewer lines;
- Direct discharges of waste from residential/commercial/long-term camping facilities;
- Leaks and spills of wastewater from permitted facilities;
- Runoff from landscape applications of manure;
- Irrigation creating direct runoff to surface waters;
- Homeless and itinerant worker encampments along streams which lack sanitary disposal facilities;
- Livestock, pets, and wildlife.

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

Decisions or Outcomes.

Goals of the Coastal Watershed Pathogen Indicator Study include:

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

Monitoring tasks were identified for the following four management questions:

1. What is the natural background of pathogenic indicator bacteria concentrations?
2. What is the spatial variability of pathogenic indicator bacteria concentrations?
3. What is the temporal variability of pathogenic indicator bacteria concentrations?
4. Which anthropogenic sources have the greatest influence pathogenic indicator bacteria concentrations?

This monitoring plan is organized into tasks to collect information to address these management questions. This monitoring plan is organized into tasks to collect information to address these management questions. The data collected will be assessed with a number of statistical methods to help answer the monitoring questions.

Non-parametric statistical methods will be used for all assessments. The Mann-Whitney U Test is a non-parametric test for assessing whether two samples of observations come from the same distribution. The Kruskal-Wallis Test is a one-way analysis of variance conducted using ranked data. The non-parametric methods will be used for testing equality of population medians among groups.

Water Quality or Regulatory Criteria

Section 303(d) of the federal Clean Water Act requires states to develop a list of surface waters where required pollution control mechanisms are not sufficient enough to meet water quality standards applicable to such waters. According to the Clean Water Act, each state must develop a Total Maximum Daily Loads (TMDL) for all the waters on the Section 303(d) list.

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

The Basin Plan (NCRWQCB, 2011) promulgates specific Water Quality Objectives (WQOs) for pathogenic indicator bacteria. These WQOs are established to protect REC-1 and SHELL beneficial uses. WQOs specified in the Basin Plan apply to inland waters, enclosed bays, and estuaries. The Basin Plan for inland surface waters includes both narrative and numeric WQOs for inland surface waters, enclosed bays, and estuaries, as described below. Standards from the California Ocean Plan (SWRCB 2012) apply to ocean beaches, as described below.

Narrative Bacteria Criteria

The Basin Plan narrative WQO for inland surface waters, enclosed bays, and estuaries states:

> "The bacteriological quality of waters of the North Coast Region shall not be degraded beyond natural background levels.

Natural background is interpreted to mean the quality of water that in the absence of significant human disturbance or alteration is in a minimally disturbed condition. This matches the definition of a "reference condition for biological integrity" or "minimally disturbed condition" as used by the Water Board's Surface Water Ambient Monitoring Program (Ode & Schiff 2009) and expressed by Stoddard et al. (2006). Natural background does not equal a pristine, unpolluted, or anthropogenically undisturbed state with zero human waste or domestic animal waste discharges to waterbodies. Humans are part of the natural landscape, both historically and today.

Bacteroides bacteria are another group of pathogen indicator organisms that are used to measure fecal contamination in water. *Bacteroides* is the genus name of the bacteria from the phylum Bacteroidetes and order Bacteroidales. *Bacteroides* bacteria are anaerobic (i.e., they do not live or grow in the presence of oxygen) and make up a substantial portion of the gastrointestinal flora of animals (Wexler 2007). *Bacteroides* bacteria are not found in ambient surface waters without sources of animal waste.

Due to their anaerobic-nature, *Bacteroides* bacteria have a low potential for survival and regrowth in the environment. *Bacteroides* bacteria are especially useful to as a tool to identify specific animal waste sources. The percentage of the *Bacteroides* bacteria population that originates from specific animal hosts can be determined using real-time quantitative polymerase chain reaction (qPCR) methods, which amplify specific DNA sequences of the 16S rRNA gene marker (Molina 2007). *Bacteroides* bacteria assay primers have been developed for most domestic animal hosts including cattle, swine, chicken, dog, and horse (Griffith et al. 2013). Commercial laboratories are available that conduct these animal host analyses.

Because of the short life span, *Bacteroides* bacteria concentrations are often used to indicate recent fecal contamination of surface waters. *Bacteroides* bacteria are a suitable indicator of a waterbody's bacteriological quality since the bacteria come from the gastrointestinal systems of mammals, they degrade rapidly outside of the body, and technology is available to trace the bacteria back to specific types of mammals, including humans and domestic animals. Host-specific *Bacteroides* bacteria can used to help assess the natural background of pathogenic indicator bacteria in minimally disturbed waterbodies.

Recent studies conducted by the Southern California Coastal Water Research Project show little to no epidemiological connection between conventional pathogen indicator bacteria (e.g., total coliform and fecal coliform bacteria) and regulatory criteria. There are also likely to be many cases of exceedances of the State standards that are caused by natural sources, especially in geographic areas with no storm drains, or sewage systems. Analysis of host-specific bacteria (e.g., *Bacteroides* bacteria) will help advise the TMDL process for developing mitigation strategies for reduction in pathogenic indicator organisms.

Water Contact Recreation (REC-1) Criteria

The Basin Plan numeric WQO for the protection of REC-1 for inland surface waters, enclosed bays, and estuaries states:

> "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."

Fecal coliform bacteria concentration measurements were used to identify six (6) streams as impaired for REC-1 (Table 1). Measurements were made from samples collected by the Humboldt Baykeeper's Citizen Monitoring Program and the Mendocino County Beach Watch Program. These measurements were compared to the Basin Plan WQO for the protection of REC-1 for inland surface waters, enclosed bays, and estuaries. The assessment was used to place these streams on the 2012 Section 303(d) list of impaired waters.

The fecal coliform value described in the Bacteria Water Quality Objective for the protection of water contact recreation is based on outdated science thresholds from the 1970s. Since 1976, several key epidemiological studies evaluated the criteria for effectiveness at protecting public health from water contact recreation (Cabelli et al. 1982; Cabelli et al. 1983; Dufour 1983;

Favero 1985; Seyfried et al. 1985a, Seyfreid et al. 1985b) The studies concluded that the 1976 U.S. EPA recommended fecal coliform bacteria criteria had no scientific basis. As a result, the U.S. EPA changed the criteria recommendation in 1986 to use the pathogen bacteria indicators of *E. coli* and *Enterococcus* bacteria, instead of fecal coliform bacteria. Additionally, detection of fecal coliform bacteria in recreational waters may overestimate the level of fecal contamination because this bacteria group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* bacteria are commonly associated with soils and the surfaces of plants, so that areas with allochthonous organic debris may show high levels of fecal coliform bacteria that do not have a fecal-specific bacteria source.

E. coli and enterococci are types of bacteria that are found in the fecal material from humans and other animals. Epidemiological studies have demonstrate a link between *E. coli* and enterococci bacteria concentrations and gastrointestinal illness. The U.S. EPA (2012) recommends criteria as an indicator of health risk to water contact recreation.

The U.S. EPA (2012) criteria for water contact in recreational waters were established for both the geometric mean and the statistical threshold value (STV)(Table 2). The geometric mean criterion is compared to the logarithmic average of the bacteria concentration distribution. The STV criterion is compared to the 90^{th} percentile of the bacteria concentration distribution. Criteria were also published for two different levels of illness risk. The first level of risk (36 estimated illnesses per 1,000 recreators) is the same risk level applied with the previous recreational criteria (i.e., U.S. EPA 1986). The 1986 U.S. EPA criteria correspond to the level of risk associated with an estimated illness rate of the number of highly credible gastrointestinal illnesses (HCGI) per 1,000 primary contact recreators.

The information developed for the 2012 U.S. EPA criteria use a more comprehensive definition of GI illness, referred to as NEEAR-GI (NGI), which includes diarrhea without the requirement of a fever. Because NGI is broader than HCGI, more illness cases were reported and associated with recreation using the NGI definition of illness, at the same level of water quality observed using the previous illness definition (i.e., HCGI). The U.S. EPA (2012) also recommends criteria that correspond to an illness rate of 32 NGI per 1,000 primary contact recreators to "encourage an incremental improvement in water quality." The current draft of the statewide amendment to the Inland Surface Waters, Enclosed Bays, and Estuaries Plan recommends using the more protective criteria (i.e., 32 estimated illnesses per 1,000 recreators).

Table 4. Recreational Water Quality Criteria (U.S. EPA 2012)

Pathogen Indicator Bacteria	Recommendation 1 Estimated Illness Rate 36 per 1,000 recreators		Recommendation 2 Estimated Illness Rate 32 per 1,000 recreators	
	Geometric Mean (cfu/100mL)	Statistical Threshold Value (cfu/100mL)	Geometric Mean (cfu/100mL)	Statistical Threshold Value (cfu/100mL)
E. coli	126	410	100	320
Enterococci	35	130	30	110

The California Ocean Plan (SWRCB 2012) also establishes indicator bacteria standards for the protection of REC-1 for ocean beaches.

> "Within a zone bounded by the shoreline and a distance of 1,000 feet from the shoreline or the 30-foot depth contour, whichever is further from the shoreline, and in areas outside this zone used for water contact sports, as determined by the Regional Board (i.e., waters designated as REC-1), but including all kelp beds, the following bacterial objectives shall be maintained throughout the water column:
>
> 30-day Geometric Mean – The following standards are based on the geometric mean of the five most recent samples from each site:
>
> i. Total coliform density shall not exceed 1,000 per 100 mL;
> ii. Fecal coliform density shall not exceed 200 per 100 mL; and
> iii. Enterococcus density shall not exceed 35 per 100 mL.
>
> Single Sample Maximum:
>
> i. Total coliform density shall not exceed 10,000 per 100 mL;
> ii. Fecal coliform density shall not exceed 400 per 100 mL;
> iii. Enterococcus density shall not exceed 104 per 100 mL; and
> iv. Total coliform density shall not exceed 1,000 per 100 mL when the fecal coliform/total coliform ratio exceeds 0.1."

Shellfish Harvesting (SHELL) Criteria

The California Ocean Plan (SWRCB 2012) also establishes indicator bacteria standards for the protection of shellfish that may be harvested for human consumption (SHELL) at ocean beaches. The Basin Plan identifies all ocean waters (i.e., marine beaches) to have existing SHELL beneficial use.

> "At all areas where shellfish may be harvested for human consumption, as determined by the Regional Board, the following bacterial objectives shall be maintained throughout the water column: The median total coliform density shall not exceed 70 per 100 mL, and not more than 10 percent of the samples shall exceed 230 per 100 mL."

Total coliform bacteria concentration measurements were used to identify eleven (11) ocean beaches as impaired for SHELL (Table 1). Measurements were made from samples collected by the Humboldt County and the Mendocino County Beach Watch Programs. These measurements were compared to the Ocean Plan standard for the protection of SHELL for ocean waters. The assessment was used to place these streams on the 2012 Section 303(d) list of impaired waters.

A6. Project/Task Description

Work Statement and Produced Products.

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

Constituents to be Monitored and Measurement Techniques.

Analysis of water samples for *E. coli* and *Bacteroides* bacteria concentrations will be conducted by Sonoma County Public Health Laboratory and the Humboldt County Public Health Laboratory.

E. coli bacteria concentrations will be measured utilizing the IDEXX, Colilert® test. *Bacteroides* bacteria concentrations will be measured using quantitative polymerase chain reaction (qPCR) techniques. Animal host-specific bacterial source markers for human, cow, dog, and gulls will be used to quantify the sources of the *Bacteroides* bacteria in the water sample (Griffith et al 2013).

For the beach locations listed for impaired SHELL use in Table 1, samples will be collected for analysis of *Bacteroides* bacteria concentrations concurrently with the Beach Watch Program sampling conducted by County staff. The Beach Watch Program collects waters samples for the analysis of total colform, *E. coli* and enterococci bacteria concentrations.

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

Table 5. Project Schedule Timeline

Activity	Date		Deliverable	Deliverable Due Date
	Anticipated Date of Initiation	Anticipated Date of Completion		
Collect and process water samples	Oct 2015	May 2017	Lab Data Reports	Continuous
Draft Monitoring Plan Data Report	May2017	September 2017	Draft Report	September 2017
Final Monitoring Plan Data Report	September 2017	January 2018	Final Report	January 2018

Geographical setting

The study area is the coastal watersheds draining to the ocean and estuaries in Sonoma, Mendocino, Humboldt, and Del Norte counties.

Constraints

Water samples will be collected during wet and dry weather conditions. Wet period sampling will take place during or following storm events that are predicted to generate 0.2 inch or greater of rainfall. Dry period sampling must be preceded by 72 hours of dry weather.

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

A7: Quality Objectives and Criteria for Measurement Data

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 6. Data Quality Objectives for Laboratory Measurements

Parameter	Method	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Bacteroides	Quantitative PCR	Proper positive and negative response	Triplicates within 10%	Not available	Not yet available	90%
E. Coli.	Colilert®	Positive results for target organisms. Negative results for non-target organisms	Rlog within 3.27*mean Rlog		1 MPN/100 mL depending on sample dilution	
Enterococcus	Enterolert®					
Total coliform	Colilert®					

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.

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).

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 field sheets at each sampling site and COC forms (see Appendix 3) for the samples collected. COC forms will accompany water samples to the appropriate laboratories for analysis. 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).

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 Monitoring Plan is organized into four individual tasks and sampling plans to collect information which will address the identified management questions.

- **Task 1** evaluates the temporal and spatial variability of pathogen indicator bacteria concentrations draining from "reference" catchments that are in a minimally disturbed condition.

- **Task 2** evaluates the temporal variability of indicator bacteria from the listed beaches to determine percent reductions needed to achieve WQOs.

- **Task 3** evaluates the temporal variability of indicator bacteria from the listed streams near the mouth of the catchments to determine load reductions needed to achieve WQOs.

Task 1: Reference Catchment Assessment

Task 1 is designed to answer the following management questions:

1. What is the spatial variability of pathogenic indicator bacteria concentrations in minimally disturbed catchments?
2. What is the temporal variability of pathogenic indicator bacteria concentrations in minimally disturbed catchments?
3. What are the most significant animal sources of pathogenic indicator bacteria draining from minimally disturbed catchments?

E. coli, enterococci and total coliform bacteria concentrations will be measured.

To assess spatial variability, reference catchments have been identified to represent the coastal redwood forest biogeographic regions. Assessment will not be conducted to assess reference catchments representing the interior chaparral of Modoc desert biogeographic regions within the boundaries of the North Coast Regional Water Board jurisdiction.

To assess temporal variability, reference catchments will be samples each season during dry conditions and early fall and late winter wet weather periods.

To assess animal sources, host specific bacterial source markers for universal (AllBac), human (HF183), cow (CowM2), dog (DogBact), and birds (Gull2 Taqman) will be used to quantify the sources of the *Bacteroides* bacteria in the water sample (Griffith et al 2013).

Sample Collection

Assessment of the spatial and temporal variability within the Coastal Redwood Forest and Oak Savanna biogeographic regions will be conducted by collecting water samples at each of the listed location in Table 3. Field crews will find these sampling locations using the road maps found in the Appendix, or by GPS if needed.

Table 7. Sampling Locations for Task 1

SWAMP ID	Stream Name	Sampling Location	Road	Latitude	Longitude
103CD0679	Cedar Creek	Jed Smith State Park	Howard Hill Road off Hwy 199	41.7889	-124.0778
103CK4061	Clarks Creek	Jed Smith State Park	Walker Road off Hwy 199	41.8126	-124.1094
103ML0155	Mill Creek	Jed Smith State Park	Howard Hill Road off Hwy 199 at Stout Memorial Grove	41.7909	-124.0850
107PR7848	Prairie Creek	Prairie Creek Redwoods State Park	Drury Parkway at Edgar C. Wagner Grove	41.4083	-124.0316
107LM1856	Lost Man Creek	Redwood National Park	Lost Man Creek exit	41.3276	-124.0157
107LL0600	Little Lost Man Creek	Redwood National Park	Lost Man Creek exit	41.3281	-124.0261
111CW0458	Cow Creek	Humboldt Redwoods State Park	Bull Creek Flats Road	40.3508	-123.9635
111CF1805	Calf Creek	Humboldt Redwoods State Park	Bull Creek Flats Road	40.3526	-123.9760
111HR0606	Harper Creek	Humboldt Redwoods State Park	Bull Creek Flats Road	40.3514	-123.9884
111AL1359	Albee Creek	Humboldt Redwoods State Park	Bull Creek Flats Road - Albee Creek campground exit	40.3556	-124.0075
111ML0252	Mill Creek	Humboldt Redwoods State Park	Bull Creek Flats Road - Hamilton Barn Environmental campground exit	40.3504	-124.0223
111LM0001	Little Mill Creek	Humboldt Redwoods State Park	Bull Creek Flats Road - Hamilton Barn Environmental campground exit	40.3439	-124.0268
113PG1586	Phillips Gulch	Salt Point State Park	Highway 1	38.5858	-123.3367
113MR1171	Miller Creek	Salt Point State Park	Highway 1	38.5778	-123.3317
113ST0986	Stockhoff Creek	Stillwater Cove Regional County Park	Highway 1 – Day Use Parking	38.5484	-123.2948
114FZ3710	Feezeout Creek	Willow Creek State Park	Freezeout Creek Road	38.4428	-123.0378

Each location in Table 2 will be sampled six (6) times each:

- Four (4) dry weather samples - once each season (e.g., Winter, Spring, Summer, Fall)
- Two (2) wet weather samples – early fall and late winter (e.g., October and March)

To assess sampling variability, triplicate water samples will be collected at one (1) randomly selected location for each of the six (6) sample periods. Triplicate water samples will allow the derivation of the overall variability associated with sampling and analysis. Triplicate samples results in twelve (12) extra water samples.

To assess the potential for sample contamination, travel blank samples will be collected for each day of sampling. Sterile sample water will be poured into a sample container at the first location sampled each day and the travel blank sample stored on ice with the ambient water samples for delivery to the laboratory. Estimate eighteen (18) blank samples will be collected.

The resulting total sample size will be a total of 64 dry season water samples and 32 wet season water samples, including triplicate samples and travel blank samples. Water samples will be collected at each sampling location for the analyses and labs listed below:

- 80 water samples to Sonoma County Public Health Laboratory for the analysis of *E. coli*, enterococci and *Bacteroides* bacteria concentrations. Sample number is based on 10 samples collected during 6 sampling events and includes 8 samples for triplicate and 12 blanks.

- 46 samples to Humboldt County Public Health Laboratory for the analysis of *E. coli*, enterococci and *Bacteroides* bacteria concentrations. Sample number is based on 6 samples collected during 6 sampling events, and includes 4 samples for triplicate and 6 blanks.

Task 2: Listed Ocean Beach Assessment

Task 2 is designed to answer the following management question:

1. What is the temporal variability of pathogenic indicator bacteria concentrations at the ocean beaches listed for impaired SHELL beneficial use?
2. What are the most significant animal sources of pathogenic indicator bacteria at the listed ocean beach?

Currently, each of the twelve (12) listed ocean beaches is actively samples as part of the California Beach Watch Program (i.e., AB411). The Beach Watch Program water sample collection is conducted by county public health agency staff. County staff currently collect water samples weekly during the dry weather season for the analysis of total coliform, *E. coli*, and enterococci bacteria concentrations for comparison to California Ocean Plan (SWRCB 2012) standards.

Task 2 involves collection of additional water samples by county staff concurrently with the sample collection associated with the Beach Watch Program. These water samples are currently analyzed for total coliform, *E. coli* and enterococci bacteria concentrations.

Additional ocean beach water samples will be collected for analysis of *Bacteroides* bacteria concentrations. To assess animal sources, host specific bacterial source markers for human, cow, dog, and gulls will be used to quantify the sources of the *Bacteroides* bacteria in the water sample (Griffith et al 2013).

Sample Collection

Assessment of the temporal variability of pathogenic indicator bacteria will be conducted by collecting additional water samples at each of the listed location in Table 4. Field crews will find these sampling locations using the road maps found in the Appendix, or by GPS if needed.

Table 8. Sampling Locations for Task 2

SWAMP ID	Listed Water Body Name	Sampling Locations	Latitude	Longitude
108ML0001	Trinidad State Beach	Trinidad St. Beach at Mill Creek	41.0616	-124.1487
108HBOHB1	Old Home Beach	Old Home Beach at Scenic Drive	41.0481	-124.1251
108LF0001	Luffenholtz Beach	Luffenholtz Beach at Luffenholtz Creek	41.0415	-124.1200
108LR0001	Moonstone County Park	Moonstone Beach at Little River	41.0275	-124.1115
109SW0001	Clam Beach (near Strawberry Creek)	Clam Beach at Strawberry Creek	40.9964	-124.1167
109MA0001	Clam Beach (near Mad River mouth)	Clam Beach at Mad River	40.9567	-124.1278
113VR0001	MacKerricher State Park (near Virgin Creek)	MacKerricher State Park at Virgin Creek	39.4715	-123.8040
113PD0001	Pudding Beach	Pudding Beach at Pudding Creek	39.4590	-123.8090
113HC0001	Hare Beach	Hare Beach at Hare Creek	39.4172	-123.8129
113CA0001	Caspar Headlands State Beach	Caspar Beach at Caspar Creek	39.3618	-123.8164
113BI0001	Big River Beach	Mendocino Bay at Big River	39.3021	-123.7945
115BBCCB1	Campbell Cove	Campbell Cove State Beach across from Bodega Bay Jetty	38.3130	-123.0608

Each ocean beach location in Table 4 will be sampled once each in June, July, and August.

The resulting total sample size will be a total of 36 water samples. No travel blanks or replicate samples will be collected. Water samples will be collected by County staff at each ocean beach for the analyses and labs listed below:

- 18 water samples to Sonoma County Public Health Laboratory for the analysis of *Bacteroides* bacteria concentrations based on 5 Mendocino County samples collected during 3 sampling events and 1 Sonoma County samples collected during 3 sample events.

- 18 samples to Humboldt County Public Health Laboratory for the analysis of *Bacteroides* bacteria concentrations based on 6 samples collected during 3 sampling events.

Task 3: Listed Stream Assessment

Task 3 is designed to answer the following management question:

3. What is the temporal variability of pathogenic indicator bacteria concentrations at the mouths of stream listed for impaired REC-1 beneficial use?
4. What are the most significant animal sources of pathogenic indicator bacteria draining to mouths of the listed freshwater streams?

Task 3 involves collection of water samples by Regional Water Board staff or Humboldt Baykeeper citizen volunteers. Waters samples will be analyzed for of *E. coli* bacteria concentrations to determine the load reduction needed to support REC-1 beneficial use.

To assess animal sources, host specific bacterial source markers for human, cow, dog, and gulls will be used to quantify the sources of the *Bacteroides* bacteria in the water sample (Griffith et al 2013).

Sample Collection

Assessment of the temporal variability of pathogenic indicator bacteria will be conducted by collecting water samples at each of the listed location in Table 4. Field crews will find these sampling locations using the road maps found in Appendix, or by GPS if needed.

Table 9. Sampling Locations for Task 3

SWAMP ID	Listed Water Body Name	Sampling Location	Latitude	Longitude
108LR0663	Little River	Little River at Hwy 101	41.0153	-124.1070
109NR1488	Norton Creek	Norton Creek at Hwy 101	40.9603	-124.1170
110JG0264	Jolly Giant Creek	Jolly Giant Creek at Samoa Blvd	40.8656	-124.0890
110GS1625	Gannon Slough	Gannon Slough at Hwy 101	40.8497	-124.0810
110MS1481	Lower Elk River and Martin Slough	Martin Slough at Pine Hill Road	40.7523	-124.1820
110EL1278	Lower Elk River and Martin Slough	Elk River at Hwy 101	40.7557	-124.1910

Each location in Table 5 will be sampled six (6) times each:

- Four (4) dry weather samples - once each season (e.g., Winter, Spring, Summer, Fall)
- Two (2) wet weather samples – early fall and late winter (e.g., October and March)

To assess sampling variability, triplicate water samples will be collected at one (1) randomly selected location for each batch of samples delivered to the laboratory. Triplicate water samples will allow the derivation of the overall variability associated with sampling and analysis. Replicate samples results in twelve (12) extra water samples.

To assess the potential for sample contamination, travel blank samples will be collected for each day of sampling. Sterile sample water will be poured into a sample container at the first location sampled each day and the travel blank sample stored on ice with the ambient water samples for delivery to the laboratory. Estimate six (6) blank samples will be collected.

The resulting total sample size will be a total of 36 dry season water samples and 18 wet season water samples, including triplicate samples and travel blank samples. Water samples will be collected at each sampling location for the analyses and labs listed below:

- 54 samples to Humboldt County Public Health Laboratory for the analysis of *E. coli* and *Bacteroides* bacteria concentrations. The Sample number is based on 6 samples collected during 6 sampling events, including 12 replicate samples and 6 travel blanks.

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.

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 field sheets. 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 10. Sample Handling and Preservation Requirements

Analyte	Units	Container	Sample Volume	Preservation	Maximum Holding Time
E. coli Total coliform	MPN/100 mL	125 mL Sterile Plastic container	100 mL	Cool to 6 ◦C in the dark.	6 hours
Enterococcus	MPN/100 mL	125 mL Sterile Plastic container	100 mL	Cool to 6 ◦C in the dark.	6 hours
Bacteroides	cells/mL	110mL irradiated nuclease-free Plastic container	100mL	Cool to 6 ◦C in the dark Samples to be filtered Freeze filters at -80°F	6 hours

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. Water samples to be analyzed for *Bacteroides*, *Enterococcus*, total coliform and *E. coli* bacteria will be delivered to either:

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

or

County of Humboldt
Department of Health & Human Services
529 I Street
Eureka, CA 95501
Phone: 707-565-4711

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.

Samples received by the contract laboratories will be processed immediately upon receipt and within the specified holding time. *Bacteroides* samples will be filtered on a 0.2 um sterile filter and the sample filters stored in a -80ºF freezer until the *Bacteroides* analysis is performed.

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 11. Laboratory Analytical Methods

Analyte	Laboratory / Organization	Project Action Limit	Project Quantitation Limit	Achievable Laboratory Limits	
				Analytical Method/ SOP	MDLs
E. coli	Public Health Laboratories	<126 MPN /100mL	1 MPN /100mL	Colilert®	1 MPN /100mL
Total coliform	Public Health Laboratories	<1000MPN /100mL	1 MPN /100mL	Colilert®	1 MPN /100mL
Enterococcus	Public Health Laboratories	<35 MPN /100mL	1 MPN /100mL	Enterolert®	1 MPN /100mL
Bacteroides	Public Health Laboratories	Reporting Limit	50% detection efficiency	Appendix 7	50% detection efficiency

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 field sheet will be completed at each site. The field sheets 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.

Field Triplicates

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

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.

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.

B7: Instrument/Equipment Calibration and Frequency

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

B8: Inspection/Acceptance of Supplies and Consumables

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

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

Microbiological sample bottles will be provided by the manufacturer. They will be shipped to and stored at the County Public Health Laboratories 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 field sheets, 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 field sheet 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 field sheets. Deficiencies that cannot be immediately corrected will be noted on the field sheets 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 12. Report Due Dates

Report Type	Frequency	Responsible Party	Schedule
Data Report	Per batch analyzed	Sonoma Public Health Lab Director	On-going
Data Report	Per batch analyzed	Humboldt Public Health Lab Manager	On-going
Draft Monitoring Plan Data Report	Once	Project Manager	September 2017
Final Monitoring Plan Data Report	Once	Project Manager	January2018

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 field sheets from the field crews, the Project QA Officer and Project Manager will review all data, field sheets and field notes to verify that the QAPP was followed. Items reviewed will include:

- Comparison of the scheduled sampling plan with field sheets and custody forms to assure that planned samples were collected.
- Review of field sheets 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 field sheets 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. Field sheets 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

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Appendix 2: Field Data Sheet

SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ | *Project Code: RWB1_RuR_FY1011 | Page 1 of 1

*Station ID:	Arr. Time:	*Agency: RB1	*Funding: FY09-10 WDPF (09-1-001)	*Protocol: MPSL-DFG_Field_v1.0	Entered in d-base (Initial/date)
Location ID:	*SampleTime:	*Group:		*PurposeFailure:	
*Date (mm/dd/yyyy):	Dep. Time:	*Personnel:			

Habitat Observations (CollectionMethod = Habitat_generic)

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	DGPS	Lat (dd.dddddd) / Long (ddd.dddddd)
OCCUPATION METHOD:	Walk-In		Target:	
SITE ODOR:	None Sulfides Sewage Petroleum Smoke Other		*Actual:	-
WATER ODOR:	None Sulfides Sewage Petroleum Mixed Other		GPS Device S/N:	Accuracy (ft / m):
WATER CLARITY:	Clear (see bottom) Cloudy (>4" vis) Murky (<4" vis)	BEAUFORT SCALE: (see back)	WIND DIRECTION (from):	PHOTOS - (Rename to : StationCode_yyyy_mm_dd_position) ex: 105KLAMGL_2010_01_12_US
WATER COLOR:	Colorless Green Yellow Brown			(RB)
OTHER PRESENCE:	Vascular Nonvascular OilySheen Foam Trash NONE Other	Duplicate? YES / NO		(LB)
DOM. SUBSTRATE:	Bedrock Concrete Cobble Gravel Sand Mud Unk Other			(US)
SKY CODE:	Clear Partly Cloudy Overcast Fog Smoky Hazy	DupID:		(DS)

OVERLAND RUNOFF (last 24 hours):	None Light Moderate/Heavy Unknown				
PRECIPITATION:	None Fog Drizzle Rain Snow	PRECIPITATION (last 24 hrs):	Unknown <1" >1" None	EVIDENCE OF FIRES:	No
HYDROMODIFICATION:	None			HYDROMOD LOCATION::	NA
*LOCATION:	Bank Thalweg Midchannel	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 / Integrated; LABEL_ID = FieldQA; create collection record upon data entry)

SAMPLE TYPE: GRAB | COLLECTION DEVICE: Individual bottle: by hand

DepthCollection (m)	Bacteria	Salinity									
0.1											

Field Measurements (SampleType = FieldMeasure; Method = Field) | **Turbidity (ntu)**

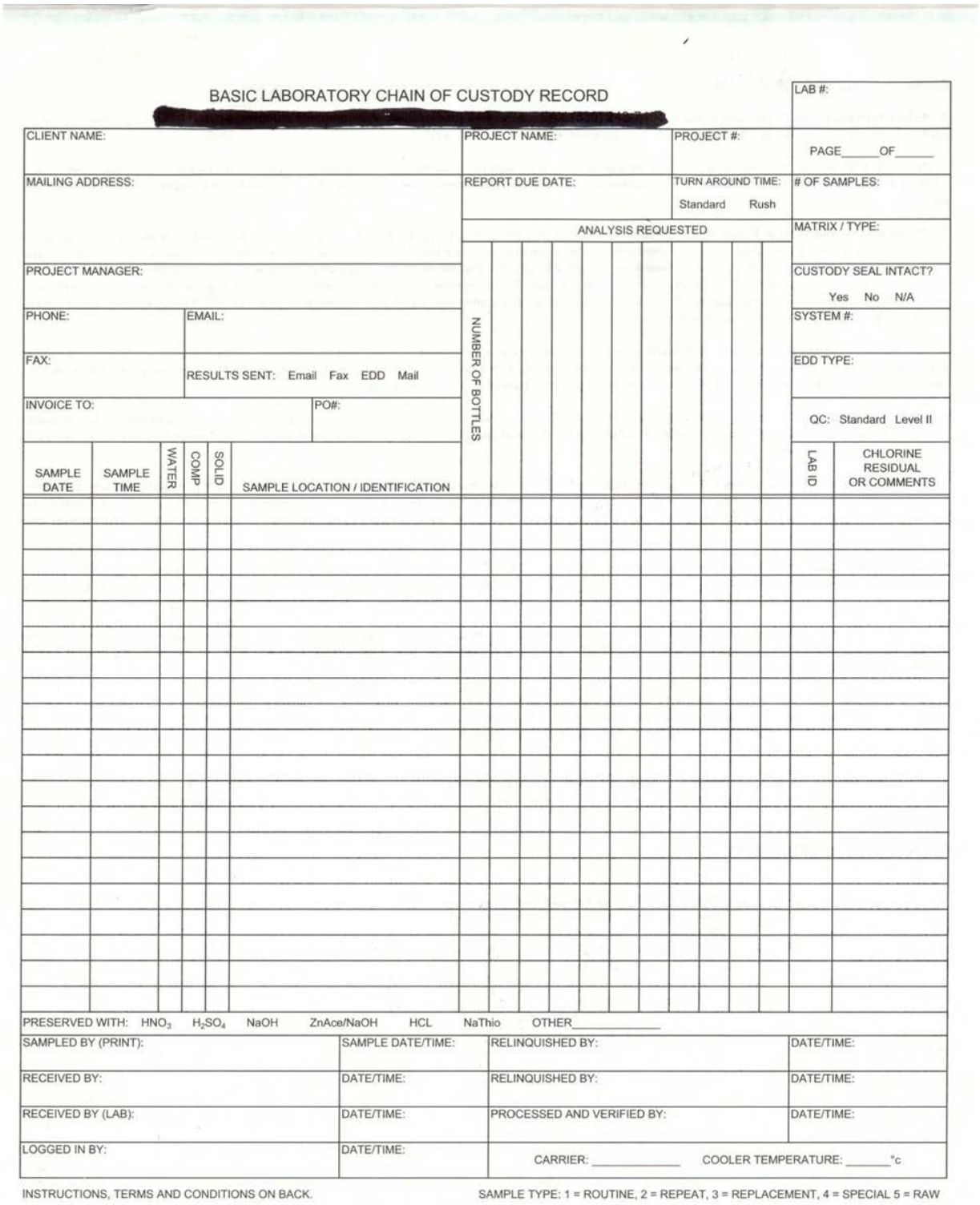
DepthCollection (m)	Air Temp (°C)	STREAM WIDTH (m):	WATER DEPTH (m):	Velocity (fps)	Water Temp (°C)	Specific Conductivity (uS/cm)	pH	pH (mV)	O2 (mg/L)	O2 (%)	Salinity (ppt)	Barometric Pressure
0.1												

DISTANCE FROM BANK (m):		Instrument/Method	VIS	YSI DataSonde: 600XL		S/N:	YSI 650MDS
		Calibration Date			Calibration Sheet No.		

COMMENTS:

*Purpose (circle applicable): (WaterChem) Habitat FieldMeasure Rev. 12/31/2009

Appendix 3: Chain of Custody Form



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

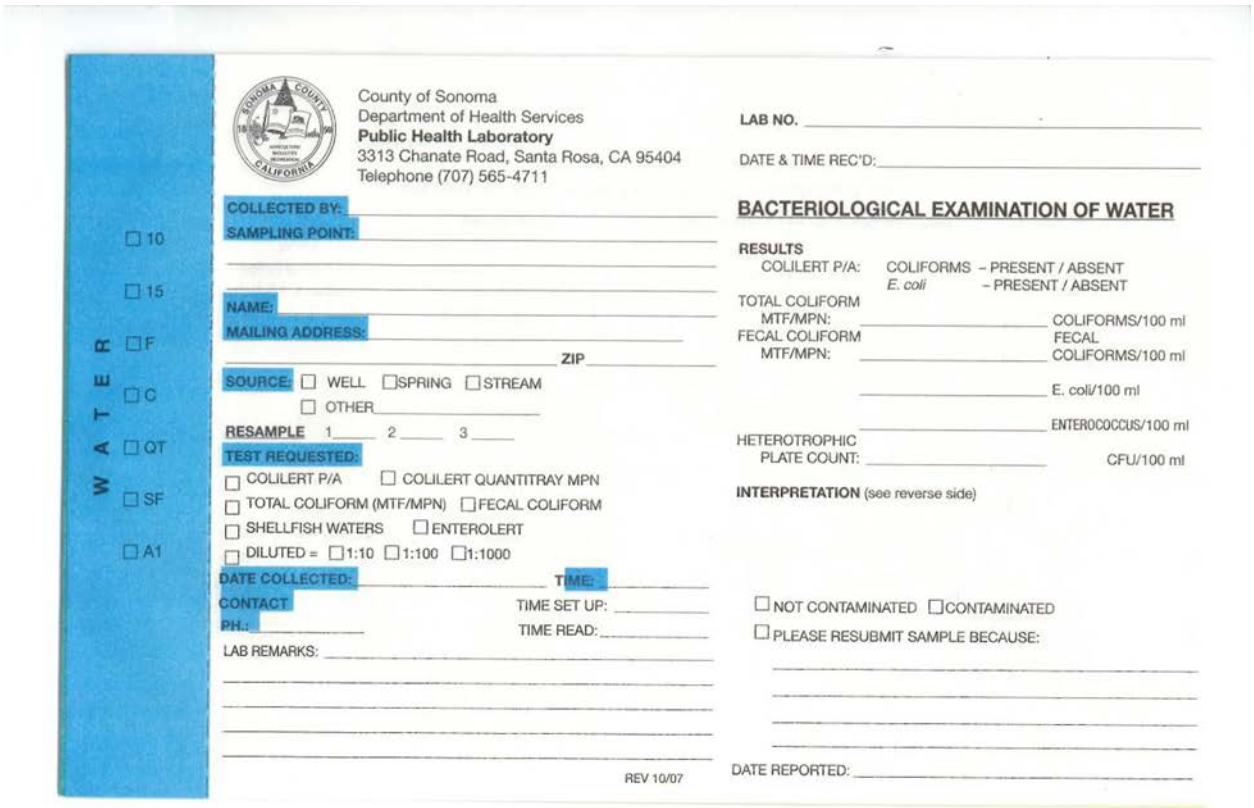
SAMPLE DATE	SAMPLE TIME	WATER	COMP	SOLID	SAMPLE LOCATION / IDENTIFICATION	NUMBER OF BOTTLES											LAB ID	CHLORINE RESIDUAL OR COMMENTS

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: _____
_____
_____
_____
_____

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:
_____
_____
_____
_____

REV 10/07

DATE REPORTED: _____

Appendix 4: Sonoma County Standard Operating Procedure and Quality Assurance Progarm for Analysis of *E. coli*, *Enterococcus*, and Total Coliform Bacteria Concentrations

County of Sonoma
DEPARTMENT OF HEALTH SERVICES
PUBLIC HEALTH LABORATORY
David Yong, Ph.D., Director of Laboratory Services

Colilert®, Colilert 18® and Enterolert® Quality Control

SOP: WA003.00 **Effective Date:** JUN 7 2007

PRINCIPLE:

Quality Control practices must be performed to ensure that the results obtained during testing are correct. This procedure outlines all quality control methods used for IDEXX Laboratories water testing system.

EQUIPMENT AND MATERIALS:

Equipment:

- Quanti-Tray Sealer
- Incubator maintained at 35.0°C ± 0.5°C
- Incubator maintained at 41.0°C ± 0.5°C
- 365 nm UV light (Spectroline Model CM-10)

Materials:

- Quanti-Tray 2000 (IDEXX cat# WQT2K)
- Enterolert® Reagent (IDEXX cat# WENT200)
- Colilert®Reagent (IDEXX cat # WP200)
- Colilert 18® Reagent (IDEXX cat# WP200-18)
- Sterile deionized water 90mL
- Idexx water collection vessels with or without sodium thiosulfate (IDEXX cat# WV120ST-200, WV120SB-200)
- Bromothymol blue dye (Weber Cat# 4324-15)
- 50 mL TSB (made in house)
- Blood Agar Plates (BAP) (Hardy cat# A10)
- 1 µL loops (Hardy cat# HS1F)
- Quanti-Tray 2000 rubber insert (IDEXX cat # WQTSRBR-2K)

Storage Requirements:

All reagents and Quanti-Trays must be stored at 4-25 °C and away from light.

QUALITY CONTROL:

Test each new lot or shipment of Quanti-Trays for auto-fluorescence. Procedure: Fill Quanti-Tray with 100 mLs of sterile water and seal with the Quanti-Tray Sealer. Check for fluorescence with 365 nm UV lamp. If fluorescence is detected, hold Quanti-Trays from use. Contact Idexx and notify the laboratory director. Record results in the Water QC Binder under "Idexx Media QC" (see Form W005).

Test the Quanti-Tray 2000 for any leakage in the wells monthly. Procedure: Fill a water collection vessel with 100 mLs of water. Add 25 drops of bromothymol blue dye or similar dye that will be visible once sealed in the Quanti-Tray. Seal the tray with the Quanti-Tray Sealer and observe for dye outside of wells. If dye is observed outside of wells, there may be a problem with the sealer or with the lot of trays. Hold Quanti-Trays from use and notify the laboratory director . Record results on the "Quanti-Tray Sealer QC" sheet (see Form W006) and file in the Water QC Binder.

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SOP: WA003.00	Title: Colilert®, Colilert 18® and Enterolert® Quality Control	Effective Date: JUN 7 2007

PROCEDURE:

Water collection vessels QC:

1. Fill bottle with sterile water to 100 mL fill line.
2. Observe bottle with water in a darkened room using 365 nm UV lamp for auto- florescence and record result on QC form (see Form W007).
3. If auto-fluorescence is observed, hold bottles from use. Contact Idexx and notify the laboratory director.
4. Use 50 mL of Double Strength Tryptic Soy Broth (DS-TSB), add 50 mL of sterile water, mix and use 50 mL of this diluted solution.
5. Fill bottle with 50 mL of TSB. Invert bottle and swirl contents to contact all surfaces.
6. Incubate bottle at 35°C + 0.5°C for 48 hours. Check for turbidity and record results on QC form (see Form W007).
7. If turbidity is observed, hold bottles from use. Contact Idexx and notify the laboratory director.
8. Record results on QC form and store completed form in Water QC Records binder under "Equipment Sterility QC" (see Form W004). Attach "Certificate of Performance" from Idexx that comes with the new lot of bottles to QC form.

Colilert®, Colilert 18®, and Enterolert® Reagent QC: .

1. Test each new lot or shipment of Colilert®, Colilert 18® or Enterolert® Reagent for appropriate growth reaction and auto-fluorescence.
2. For each reagent being tested, label a water collection vessel (bottle) with QC organism and type of test (Colilert®, Colilert 18®, or Enterolert®).
3. Fill bottles with sterile water to 100 mL fill line.
4. Add appropriate reagent (Colilert®, Colilert 18® or Enterolert® Reagent). Shake bottles to completely dissolve reagent.
5. Observe bottle with reagent in a darkened room using 365 nm UV lamp for auto-florescence and record result on QC form (see Form W008). If auto-fluorescence is observed, hold reagent from use. Contact Idexx and notify the laboratory director.
6. If no auto-fluorescence is observed, use this bottle as the sterility control.
7. Use 18-24 hour old cultures of QC organisms listed in table below.
8. Touch a sterile 1 µL inoculating loop to a single, well isolated colony. Inoculate the colony into 5 ml of sterile water. Vortex.
9. To each test bottle add 1 µL of bacterial dilution using a sterile 1 µL inoculating loop.
10. Shake Colilert®, Colilert 18®, and Enterolert® bottles to completely dissolve reagent.

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Colilert®, Colilert 18®, and Enterolert® Reagent QC (cont.):

11. Incubate Colilert® Reagent bottles for 24 hours at 35° ± 0.5° C. Do not read after 28 hours.

12. Incubate Colilert 18® Reagent bottles for 18 hours at 35° ± 0.5° C. Do not read after 22 hours.

13. Incubate Enterolert® bottle for 24 hours at 41° ± 0.5° C. Do not read after 28 hours.

14. Record results on QC form (see Form W008). If expected growth is not achieved (see table below for expected results), hold reagent from use. Contact Idexx and notify the laboratory director.

15. Update QC forms when new reagents are put into use. Store completed QC forms in Water QC binder under "Idexx Media QC".

Table 1 - Colilert® and Colilert 18® Reagent Expected Results:

Organism	ATCC #	Expected Result
E. coli	25922	Yellow, Fluorescence
Klebsiella pneumoniae	31488	Yellow, No Fluorescence
Pseudomonas aeruginosa	10145	Clear, No Fluorescence
Sterility and auto-fluorescence	Sterile water + reagent	Clear, No Fluorescence

Table 2 - Enterolert® Reagent Expected Results:

Organism	ATCC #	Expected Result
Enterococcus faecium	35667	Fluorescence
Serratia marcescens	43862	No Fluorescence
Aerococcus viridans	10400	No Fluorescence
Sterility and auto-fluorescence	Sterile water + reagent	No Fluorescence

*** Incubate Enterolert® test at 41 ± 0.5°C.

PROCEDURE NOTES:

Long incubation:
If Colilert® sample is inadvertently incubated for over 28 hours, the lack of yellow color is a valid negative test. A yellow color after 28 hours is not valid; repeat the test. Negative Enterolert® results are also valid after 28 hours of incubation.

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REFERENCES:

Colilert® Test Kit package insert. Copyright 2002 Idexx Laboratories Inc.
Enterolert® Test Kit package insert. Copyright 2004 Idexx Laboratories Inc.
Colilert 18® Test Kit package insert. Copyright 2002 Idexx Laboratories Inc.
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DOCUMENT HISTORY:

Revison Number	Effective Date	Changes to Document
0		Orignial Version

Director

6-7-2007
Date

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Appendix 5: Humboldt County Standard Operating Procedure and Quality Assurance Program for Analysis of *E. coli*, *Enterococcus*, and Total Coliform Bacteria Concentrations

County of Humboldt Department of Health and Human Services **Public Health Laboratory** 529 "I" Street Eureka, CA 95501	
Title: **Surface Water Coliform Testing**	
SOP Version Number: 2	Revision Date: 5/3/2011
Effective Date: 11/15/06	

PRINCIPLE:
The purpose of this procedure is to detect indicator organisms such as coliform bacteria, *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium* in raw surface water, such as water collecting on the ground, in a stream, river, lake, wetland, or ocean using Colilert® and Enterolert® test kits.

Colilert® simultaneously detects total coliforms and *Escherichia coli* in water. Two-nutrient indicators, ONPG and MUG, are Colilert's® carbon sources. ONPG can be metabolized by the coliform enzyme β-galactosidase thus turning the sample yellow. MUG can be metabolized by the *Escherichia coli* enzyme β.glucuronidase which causes the sample to fluoresce. Colilert detects these bacteria at 1 cfu/100ml within 24 hours.

Enterolert® detects enterococci such as *Enterococcus faecium* and *Enterococcus faecalis* in fresh and marine water. When enterococci utilize their β-glucosidase enzyme to metabolize Enterolert's nutrient indicator, 4-methyl-umbelliferyl β-D-glucoside, the sample fluoresces. Enterolert detects enterococci at 1 cfu/ 100ml sample within 24 hours.

SPECIMEN:

Type: Greater than 100 mLs of surface water in a 120 mL sterile container to allow adequate headspace.

Handling Conditions: Collect greater than 100 mLs of surface water in a sterile container **without** sodium thiosulfate provided by the laboratory. Sample should be packed in a cooler packed with ice, or freezer packs or placed in a refrigeration system maintained at 1-5°C for regulatory tests. Time between collection of sample and analysis should not exceed 8 hours. If collection time exceeds 8 hours, samples must be recollected.

Disposable gloves, protective goggles and laboratory coat must be worn when handling samples. Autoclave all surface water samples before discarding.

EQUIPMENT AND MATERIALS:

Equipment:
Idexx Quanti-Tray Sealer, Model 2X
Incubator maintained at 35.0°C ± 0.5°C
Incubator maintained at 41.0°C ± 0.5°C

Materials:
Colilert Reagent Pack, IDEXX cat # WP200
Quanti-Tray 2000 Well, IDEXX cat# WQT2K
Enterolert Reagent Pack, IDEXX cat# WENT200
Sterile deionized water 90mL, Hardy cat # D090
10mL pipettes, Fisher cat# 1366850
Quanti-Tray 2000 Well Comparator, IDEXX cat# WQT2KC
365 nm UV light, IDEXX cat# WL200
Idexx water collection vessels w/o sodium thiosulfate, IDEXX cat# WV120SB-200
Quanti-Tray 2000 rubber insert, IDEXX cat # WQTSRBR-2K
Idexx Antifoam Solution, IDEXX cat # WAFDB

Storage Requirements:
Quanti-Tray 2000 and reagents must be stored at 4-25°C and away from light.

QUALITY CONTROL:

For quality control procedures on surface water please see the following procedures Volume Verification Quality Control, and Colilert, Colilert18 and Enterolert Quality Control.

PROCEDURE:

Colilert Procedure:

1. Turn on the Quanti-Tray Sealer; wait for light to turn green. This could take 10-15 minutes.
2. Log sample into APPOLOLIMS system and print out a worksheet (Appendix 2).
    a. Write date of test, analyst's initials and dilution factor on the worksheet
    b. Check all lot numbers and expiration dates
3. Make sure the numbers on the bottle match the bottle numbers on the water requisition.
4. Don gloves and appropriate personal protective equipment. Shake the sample 25 times in 7 seconds in a one foot arc. Pour off excess in designated sink to achieve the appropriate 100 ml sample.
5. Aseptically, pipette 10 ml of sample into 90ml of deionized sterile water.

Note: A 1:10 dilution is routinely used when performing this procedure unless otherwise specified by the submitter.

6. Carefully separate one Colilert® reagent Snap Pack from the strip. Tap the pack of reagent on the counter to ensure the contents are in the bottom of the pack.
7. While holding the Snap Pack at arms length, open the pack by snapping back the top at the score line. Avoid the poof of reagent dust that the pack emits upon opening.
8. Using aseptic technique, empty the contents of Colilert® reagent into the sample/deionized water solution. Cap the bottle and shake to mix the reagent. Let the reagent dissolve.

9. After reagent has dissolved, aseptically pour the sample into a Quanti-Tray 2000 Well by squeezing edges of tray inward to obtain an opening for easy pouring; then seal the Well using the Quanti-Tray Sealer. **Note:** Avoid touching the foil tab on the tray.
10. On the back of the tray, place accessioning number and write location of sample, date, time, and dilution factor.
11. Place tray in an incubator set at 35.0 ± 0.5°C for 24-28 hours.
12. After the incubation period, count the number of large and small yellow wells. Write the numbers of positive small and large wells on the back of the tray and the APOLLOLIMS worksheet. Use the Colilert Comparator to see an example of a positive result. See Result Interpretation chart below for Colilert.
13. Shine 365nm UV light on tray in a dark room. Count the number of large and small wells that fluoresce. Use the Colilert Comparator to see an example of a positive result. See Result Interpretation chart below for Colilert.

Result Interpretation for Colilert

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*

Enterolert Procedure:

1. Repeat steps 1 through 10 above. Use Enterolert reagent instead of Colilert reagent with sample.
2. Place tray in an incubator set at 41.0 ±0.5°C for 24-28 hours.
3. After incubation, shine UV light on trays in dark room. Count the number of small and large wells that fluoresce. Write the numbers of positive small and large wells on the back of the tray and the APOLLOLIMS worksheet. See Result Interpretation chart below for Enterolert.

Result Interpretation for Enterolert

Appearance	Result
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

CALCULATIONS:

Colilert Trays:

-To determine total coliform, count the number of large and small yellow wells, and use the Idexx Quanti-Tray 2000 MPN Table (see Appendix 1) to determine MPN. Multiply the

amount on the chart by the dilution factor (routinely 10) to calculate correct MPN for the dilution factor used. Write results on the APOLLO LIMS worksheet.
-To determine *E. coli* (fecal coliform), count the number of large and small wells that fluoresce under 365 nm UV lamp. Use the Idexx Quanti-Tray 2000 MPN Table to determine MPN. Multiply the amount on the chart by the dilution factor (routinely 10) to calculate correct MPN for the dilution factor used. Write results on the APOLLOLIMS worksheet.

Enterolert Trays:
-To determine the amount of enterococcus present, count the number of large and small wells that fluoresce under the 365 nm UV light. Use the Idexx Quanti-Tray 2000 MPN Table (Appendix 1) to determine MPN. Multiply the amount on the chart by the dilution factor (routinely 10) to calculate correct MPN for the dilution factor used. Write results on the APOLLOLIMS worksheet.

REPORTING RESULTS:

Procedures for Abnormal Results:
-Wells that produce a greenish-black supernatant with or without a black precipitate: This reaction is infrequent and occurs in well water samples. If this occurs, the sample should be invalidated on the basis of this atypical reaction. The client can recollect and use a Multiple Tube Fermentation (MTF) Method to retest the water.

-Wells that produce a whitish or grayish-white fluorescence: This reaction is atypical and would not be counted as positive for *Enterococci* with Enterolert.

-Wells that produce a yellow fluorescence: A well that exhibits this kind of reaction is interpreted as negative with Enterolert.

Reporting Format: Total coliform, *E. coli* and *Enterococci* are reported as absent or enumerated using MPN/ 100 mL water sample.

PROCEDURE NOTES:

Long incubation: If Colilert sample is inadvertently incubated for over 28 hours, the lack of yellow color is a valid negative test. A yellow color after 28 hours is not valid; repeat the test. Negative enterolert results are also valid after 28 hours of incubation. For Colilert 18 the same applies, but the maximum number of hours is 22 hours.

Dilutions: Use only sterile, non- buffered, oxidant free water for making dilutions. Multiply the MPN by the dilution factor to obtain the proper quantitative result. Marine samples must be diluted at least tenfold with sterile, fresh water.

Background color: If a water sample has some background color, compare inoculated colilert sample to a control blank of the same water (if sample volume permits). Document any abnormalities (i.e. color, odor) about the water sample on the worksheet.

Excess foam: If excess foam causes problems when pouring into Quanti-Tray, you may choose to use Idexx Antifoam Solution. The Antifoam Solution is used to eliminate foaming when sample is mixed with Colilert or Enterolert. Add two drops of Antifoam to a 100mL sample if necessary.

LIMITATIONS:

Colilert is a primary water test. Performance characteristics do not apply to samples altered by any pre-enrichment or concentration.

REFERENCES:

Colilert Test Kit package insert. Copyright 2002 Idexx Laboratories Inc.

Enterolert Test Kit package insert. Copyright 2004 Idexx Laboratories Inc.

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DOCUMENT HISTORY:

Version Number	Date	Author	Change Comments
1	11/07/2006	T. Yost/ J. Quirk	original
2	5/03/2011	K. Edwards	Minor Revisions

Appendix 1

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

Small Wells Positive

# Large Wells Positive	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.9	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
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24	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
25	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
26	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
27	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
28	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
29	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
30	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
31	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
32	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
33	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
34	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
35	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
36	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
37	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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39	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
40	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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45	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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47	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
48	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
49	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

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Confidential Page 6 of 8 Surface Water Coliform Testing

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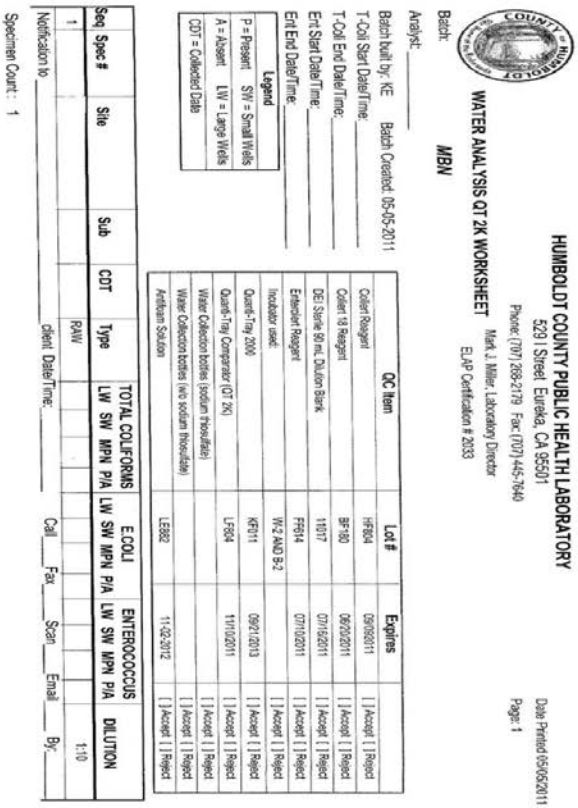
Appendix 1 (continued)

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																						
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.6
1	26.6	27.7	28.7	29.8	30.8	31.9	33.0	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.8	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.8	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.8	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.8	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.8	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.2	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.9	67.2	68.6	69.9	71.3	72.8	74.2	75.6	77.0	78.4	79.8
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.8	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.9	69.4	71.0	72.6	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.8	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.9	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.8	79.6	81.3	83.0	84.8	86.6	88.3	90.2	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	81.6	83.5	85.4	87.3	89.1	91.0	92.9	94.7	96.6	98.5	100.4	102.3	104.3	106.2	108.2	110.2	112.2	114.2	116.2	118.3	120.3	122.4	124.4	126.5
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.6	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.0	89.0	91.0	93.0	95.0	97.0	99.0	101.0	103.1	105.2	107.3	109.5	111.6	113.8	116.0	118.2	120.5	122.8	125.1	127.3	129.6	131.9	134.1	136.4
31	91.0	93.0	95.0	97.1	99.1	101.1	103.2	105.3	107.4	109.5	111.7	113.9	116.1	118.4	120.6	122.9	125.2	127.5	129.8	132.2	134.5	136.9	139.3	141.7
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.9	103.2	105.4	107.6	109.9	112.2	114.5	116.8	119.2	121.5	123.9	126.4	128.9	131.4	133.9	136.4	139.0	141.6	144.3	146.9	149.6	152.3	155.1	157.8
34	106.7	109.1	111.4	113.8	116.2	118.6	121.0	123.5	126.0	128.5	131.1	133.7	136.3	139.0	141.7	144.4	147.2	150.0	152.9	155.8	158.7	161.6	164.6	167.6
35	113.7	116.2	118.6	121.1	123.6	126.2	128.8	131.4	134.1	136.8	139.5	142.3	145.1	148.0	150.9	153.9	156.9	160.0	163.1	166.3	169.5	172.8	176.1	179.5
36	121.3	123.9	126.5	129.1	131.8	134.5	137.3	140.1	143.0	145.9	148.9	151.9	155.0	158.1	161.3	164.6	167.9	171.3	174.8	178.3	181.9	185.6	189.4	193.2
37	129.4	132.1	134.8	137.6	140.4	143.3	146.2	149.2	152.3	155.4	158.6	161.9	165.2	168.6	172.1	175.6	179.3	183.0	186.8	190.7	194.7	198.8	202.9	207.2
38	138.3	141.1	144.0	146.9	149.9	153.0	156.1	159.4	162.7	166.1	169.6	173.2	176.9	180.7	184.7	188.7	192.9	197.2	201.6	206.2	210.8	215.6	220.5	225.6
39	148.7	151.8	155.0	158.2	161.5	165.0	168.5	172.0	175.8	179.6	183.6	187.7	191.9	196.3	200.8	205.5	210.3	215.3	220.5	225.8	231.3	237.0	242.9	249.0
40	160.7	164.1	167.6	171.2	174.9	178.8	182.8	186.9	191.2	195.6	200.2	204.9	209.9	215.0	220.3	225.8	231.5	237.5	243.7	250.1	256.8	263.7	270.9	278.3
41	173.2	177.0	180.9	184.9	189.0	193.3	197.7	202.3	207.1	212.0	217.2	222.5	228.1	233.9	240.0	246.3	252.9	259.8	267.0	274.5	282.3	290.5	299.0	307.8
42	187.4	191.8	196.3	201.0	205.8	210.8	216.0	221.4	227.0	232.8	238.9	245.2	251.8	258.7	265.9	273.4	281.3	289.5	298.1	307.1	316.5	326.3	336.5	347.2
43	203.5	208.5	213.7	219.1	224.7	230.5	236.6	243.0	249.6	256.6	263.9	271.5	279.5	287.9	296.7	305.9	315.5	325.6	336.2	347.3	358.9	371.1	383.8	397.1
44	222.0	227.5	233.3	239.3	245.6	252.2	259.1	266.4	274.0	282.0	290.4	299.2	308.5	318.2	328.4	339.1	350.3	362.0	374.3	387.1	400.5	414.5	429.2	444.5
45	243.7	249.8	256.2	262.9	270.0	277.4	285.2	293.4	302.0	311.1	320.6	330.6	341.1	352.1	363.7	375.8	388.5	401.8	415.7	430.3	445.5	461.5	478.2	495.8
46	270.1	276.9	284.1	291.7	299.7	308.2	317.1	326.5	336.4	346.8	357.8	369.3	381.4	394.1	407.4	421.4	436.0	451.3	467.3	484.1	501.7	520.1	539.4	559.6
47	303.0	310.6	318.6	327.0	335.9	345.3	355.2	365.7	376.8	388.5	400.8	413.8	427.5	441.9	457.1	473.1	489.9	507.6	526.2	545.7	566.2	587.7	610.3	634.0
48	344.1	352.9	362.1	371.8	382.0	392.8	404.1	416.0	428.5	441.7	455.6	470.2	485.6	501.8	518.9	536.9	555.8	575.7	596.6	618.5	641.5	665.6	690.9	717.4
49	397.1	407.1	417.5	428.5	440.0	452.1	464.8	478.2	492.2	506.9	522.4	538.6	555.7	573.6	592.4	612.1	632.8	654.5	677.3	701.2	726.3	752.6	780.2	809.2
50	467.1	478.9	491.2	504.0	517.4	531.3	545.9	561.1	577.0	593.6	610.9	629.0	647.9	667.6	688.2	709.7	732.2	755.7	780.3	806.1	833.1	861.4	891.1	922.2

(0.02215.01)

Appendix 2



HUMBOLDT COUNTY PUBLIC HEALTH LABORATORY
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Date Printed:05/05/2011
Page: 1

WATER ANALYSIS QT 2K WORKSHEET
Mark J. Miller, Laboratory Director
ELAP Certification # 2033

Batch: **MBN**

Analyst: _____

Batch built by: KE Batch Created: 05-05-2011

T-Coli Start Date/Time: _____

T-Coli End Date/Time: _____

Ent Start Date/Time: _____

Ent End Date/Time: _____

QC Item	Lot #	Expires	
Colilert Reagent	HF804	09/09/2011	[ ] Accept [ ] Reject
Colilert-18 Reagent	BF180	06/30/2011	[ ] Accept [ ] Reject
DEI Sterile 99 mL Dilution Blank	11017	07/16/2011	[ ] Accept [ ] Reject
Enterolert Reagent	FF614	07/1/2011	[ ] Accept [ ] Reject
Incubator used:	WJ-2 AND B-2		[ ] Accept [ ] Reject
Quanti-Tray 2000	KF011	09/21/2013	[ ] Accept [ ] Reject
Quanti-Tray Comparator (QT 2K)	LF624	11/1/2011	[ ] Accept [ ] Reject
Water Collection bottles (sodium thiosulfate)			[ ] Accept [ ] Reject
Water Collection bottles (w/o sodium thiosulfate)			[ ] Accept [ ] Reject
Antifoam Solution	LE882	11-02-2012	[ ] Accept [ ] Reject

Legend
P = Present SW = Small Wells
A = Absent LW = Large Wells
CDT = Collected Date

Seq	Spec #	Site	Sub	CDT	Type	TOTAL COLIFORMS LW	SW	MPN	P/A	E.COLI LW	SW	MPN	P/A	ENTEROCOCCUS LW	SW	MPN	P/A	DILUTION
1				RAW														1:10

Notification to _____ Client Date/Time: _____ Call___ Fax___ Scan___ Email___ By_____

Specimen Count: 1

County of Humboldt Department of Health and Human Services **Public Health Laboratory** 529 "I" Street Eureka, CA 95501
Title: **Bathing Beach Water Coliform Testing using IDEXX Colilert -18 and Enterolert Quanti-tray 2K Method.**

SOP Version Number: 4	Revision Date: 6/23/2008, 04/14/10
Effective Date: 09/29/2005	10/23/2012

PRINCIPLE:

The Colilert-18 Test Kit method simultaneously detects total coliforms and E.coli in water. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert-18's nutrient-indicator, MUG, the sample fluoresces. Colilert-18 can simultaneously detect these bacteria at 1 cfu/ 100mL within 18 hours even with as many as 2 million heterotrophic bacteria per 100 mL present.

Enterolert Test Kit is used for the detection and confirmation of enterococci such as *E. faecium* and *E. faecalis* in fresh and marine water. Enterolert utilizes a nutrient indicator that fluoresces when metabolized by enterococci. This procedure can detect these bacteria at 1 CFU/100mL within 24 hours.

Humboldt County has been funded by the U.S. Environmental Protection Agency Beach Environmental Assessment and Coastal Health Act (BEACH ACT), through the California Department of Health Services, to monitor for the presence of bacteria in ocean water areas near creek mouths that deliver storm water into the ocean. Humboldt County Health and Human Services Division of Environmental Health currently has six Humboldt County beaches chosen for monitoring based on their relatively high visitor use and proximity to creeks with summer flow. The Trinidad Rancheria is also funded through the California DHS to test five locations in the Trinidad State Beach recreational area.

SPECIMEN:

Type: Marine water, ≥ 100mLs

Handling Conditions: Collect ≥100mLs in a sterile container not containing sodium thiosulfate, provided by the laboratory. In order to insure accurate results, the samples must be packed in crushed or cubed ice or placed in a refrigeration system maintained equal to or below 10 ° C. Time between collection of sample and analysis should not exceed 24 hours. If collection time exceeds 24 hours, samples must be recollected.

EQUIPMENT AND MATERIALS:

Equipment:
IDEXX Quanti-Tray Sealer Model 2X
Incubator maintained @ 35° ± 0.5°C
Incubator maintained @ 41° ± 0.5 °C
Quanti-Tray 2000 Rubber Insert
Pipetboy or Pipette Bulb
6 watt, 365 nm UV light

Materials:
IDEXX water collection vessels without sodium thiosulfate, Catalogue number WV120SB-200, IDEXX
90 mL sterile water blanks (2 per sample site), Catalogue number D090, Hardy
Quanti-Tray 2000 tray, Catalogue number WQT2K, IDEXX
Colilert 18 reagent pack, Catalogue number WP200I-18, IDEXX
Enterolert reagent pack, Catalogue number WENT200, IDEXX
Quanti-Tray 2000 comparator, Catalogue number WQT2KC, IDEXX
10 mL pipettes, Catalogue number 53300-750, VWR (Becton Dickinson)

Storage Requirements: Store Quanti-Tray 2000 and IDEXX reagents at 2-25°C away from light.

PROCEDURE:

Start analysis of water samples as soon as possible to avoid unpredictable changes in the bacterial sample collected.

1. Turn on the Quanti-Tray Sealer Model 2X (it takes approximately 15 minutes to warm up).
2. Verify the bottle number for each water sample matches the water requisition or Chain of Custody (COC) form. Log samples into APOLLOLIMS and make a worklist. Completely fill out the QC portion of the worklist. Write in dilution factor in the section indicated. See Appendix 2 for a worksheet example and Appendix 3 for an example of a completed worksheet.
3. Label two 90ml sterile water dilution blanks with the lab accession number assigned to that water sample.
4. Shake water sample 25 times within 7 seconds at a 1' arc. Aseptically open the sample and carefully pour off excess water to the 100ml line.
5. Immediately open the coordinating sterile water blanks and aseptically pipette 10 mL of test water into each sterile 90ml blank. . This will yield a 1:10 dilution.
6. Carefully separate one Snap Pack (of reagent) from the strip. Be careful not to accidentally open an adjacent pack. Tap the Snap Pack to ensure that all the Colilert 18 powder is in the bottom part of the pack. Open one pack by snapping back the top at the scoreline. **Caution: Do not touch the opening of the pack. Hold pack away from face and point side with scoreline away from you (A "poof" of dust is produced once the Snap Pack is opened**)

7. Add contents of one Colilert 18 reagent pack to the sterile water blank containing test water.
8. Aseptically cap and seal the 1:10 dilution. Shake until dissolved.
9. Repeat steps #5 through #7 using an Enterolert reagent Snap pack for the second 1:10 dilution.
10. Aseptically open Quanti-Tray 2000 using one hand to hold tray upright with the well side facing palm. Squeeze the upper part of Quanti-Tray 2000 so that tray bends toward palm. *Gently* pull foil tab to separate the foil from the tray. **AVOID TOUCHING INSIDE OF THE FOIL OR TRAY**.
11. Pour sample/reagent mixture directly into a Quanti-Tray 2000. Avoid contact with the foil tab.
12. Tap small wells 2-3 times to release any air bubbles. Allow foam to settle.
13. Place Quanti-Tray 2000 Rubber Insert onto Quanti-Tray sealer input shelf on left hand side with the large catchment area (for sample overflow) facing away from Sealer.
14. Place the sample filled Quanti-Tray 2000 (well side face down) onto Rubber Insert making sure that the Tray is properly seated in the Rubber Insert, and with each well of the tray in its corresponding Rubber Insert hole.
15. Slide the Rubber Insert into the Sealer until the motor grabs the Rubber Insert and begins to draw it into the Sealer.
16. After 15 seconds, remove the now sealed tray and Rubber Insert from the ejection port on the right hand side of the Sealer.
17. Label the tray with lab accession number, site location, date and time sealed and initials of person performing the test.

Colilert 18 reagent:
Place the sealed tray in a **35° +/- 0.5°C** incubator (Water 1 or Water 2) for **18-22 hours**. Count the number of positive wells (see result interpretation table below) and refer to the MPN table provided with the trays (see Appendix 1). Refer to *Calculations* below for determining positive results.

Enterolert reagent:
Place the sealed tray in a **41° ± 0.5°C** incubator (B-2) for **24 hours.**
Count the number of positive wells (see result interpretation table below) and refer to the MPN table provided with the trays (see Appendix 1). Refer to *Calculations* below for determining positive results.

Looking for Fluorescence:
Use a 6 watt, 365 nm, UV light located in the microscope room. Wear UV protecting goggles. Hold the light away from your eyes and within 5 inches of the/towards the Quanti-tray sample, in a dark environment (microscope room).

Result Interpretation for Colilert 18
Colilert-18 results are definitive at 18-22 hours. In addition, positives for both total coliforms and E.coli observed before 18 hours and negatives observed after 22 hours are also valid.

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*

Result Interpretation for Enterolert

Enterolert results are definitive at 24-28 hours. In addition, positives for enterococci observed before 24 hours and negatives observed after 28 hours are also valid.

Appearance	Result
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

CALCULATIONS:

Colilert 18 Trays: To determine total coliform, count the number of yellow wells and use the IDEXX Quanti-Tray 2000 MPN Table (see Appendix 1) to determine the MPN. To determine *E. coli* result count the number of wells that fluoresces. Use the IDEXX Quanti-Tray 2000 MPN Table to determine the MPN. If a 1:10 dilution was used multiply the MPN number by the dilution factor, i.e. 10.

Enterolert Trays: To determine enterococcus result, count the number of wells that fluoresce. Use the IDEXX Quanti-Tray 2000 MPN Table to determine the MPN. If a 1:10 dilution was used multiply the MPN number by the dilution factor, i.e. 10.

Note: In Apollo LIMS the computer calculates the dilution factor so verify that the final MPN provided by Apollo and which is printed on the report matches the calculated MPN.

REPORTING RESULTS:

Procedures for Abnormal Results:

Wells that produce a greenish-black supernatant with or without a black precipitate: This reaction is infrequent and occurs in well water samples. If this occurs, the sample should be invalidated on the basis of this atypical reaction. The client can recollect a new sample and use a Multiple Tube Fermentation (MTF) Method to retest the water.

Wells that produce a whitish or grayish-white fluorescence: This reaction is atypical and would not be counted as positive for enterococci with Enterolert.

Wells that produce a yellow fluorescence: A well that exhibits this kind of reaction is interpreted as negative with Enterolert.

Reporting Format: Total coliform, *E. coli* and Enterococci are reported as either present or absent and MPN/ 100 mL water sample.

PROCEDURE NOTES:

A slight tinge may be observed when Colilert-18 is added to the sample.

Dilutions: Use only sterile, non-buffered, oxidant free water for making dilutions. Multiply the MPN by the dilution factor to obtain the proper quantitative result. Marine samples must be diluted at least tenfold with sterile, fresh water.

Background color: If a water sample has some background color, compare inoculated colilert sample to a control blank of the same water (if sample volume permits). Document any abnormalities (i.e. color, odor) about the water sample on the worksheet.

REFERENCES:

Colilert -18 Test Kit package insert. Copyright 2007 IDEXX Laboratories Inc.
Enterolert Test Kit package insert. Copyright 2007 IDEXX Laboratories Inc.
IDEXX Quanti-Tray Sealer Model 2X User Manual
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Oral communication August 2, 2005. Sharon Muhilly, Technical Support at IDEXX Laboratories
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Standard Methods for the Examination of Water and Wastewater. 18th Edition, 1992. American Public Health Association.
Standard Methods for the Examination of Water and Wastewater. 20th Edition, 1998. American Public Health Association.
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DOCUMENT HISTORY:

Version Number	Date	Author	Change Comments
1	08/31/05	Tiffany Yost	original
2	06/23/2008	Kylee McMahan	Delete Colilert QC and according appendixes (it is a separate SOP), added several details for testing of bathing beaches.
3	04-14-10	Mark Miller	Change BTB to LIMS, tighten collection storage requirements.
4	10/23/2012	Kelsey McMahan	Updated LIMS information, updated changes in Bathing Beach procedure, and added new Appendixes

Appendix 1 - MPN Table

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.3	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.3	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	15.9	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	23.0	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.8	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.9	29.1	30.3	31.6	32.8	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.8	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	38.9	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.9	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.8
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	48.9	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.9	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.8	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.8	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.9	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.6	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.9	58.8	60.8	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.8	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.8	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	79.0	81.3	83.6	86.0	88.4	90.8	93.3	95.8	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.9	76.2	78.5	80.9	83.3	85.7	88.2	90.6	93.2	95.8	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.9	142.3	145.9	149.5
42	82.5	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	98.9	101.9	105.0	108.1	111.3	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.8	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.2	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.8	325.5	344.8	365.4	387.3	410.6	435.2

09-83215.01

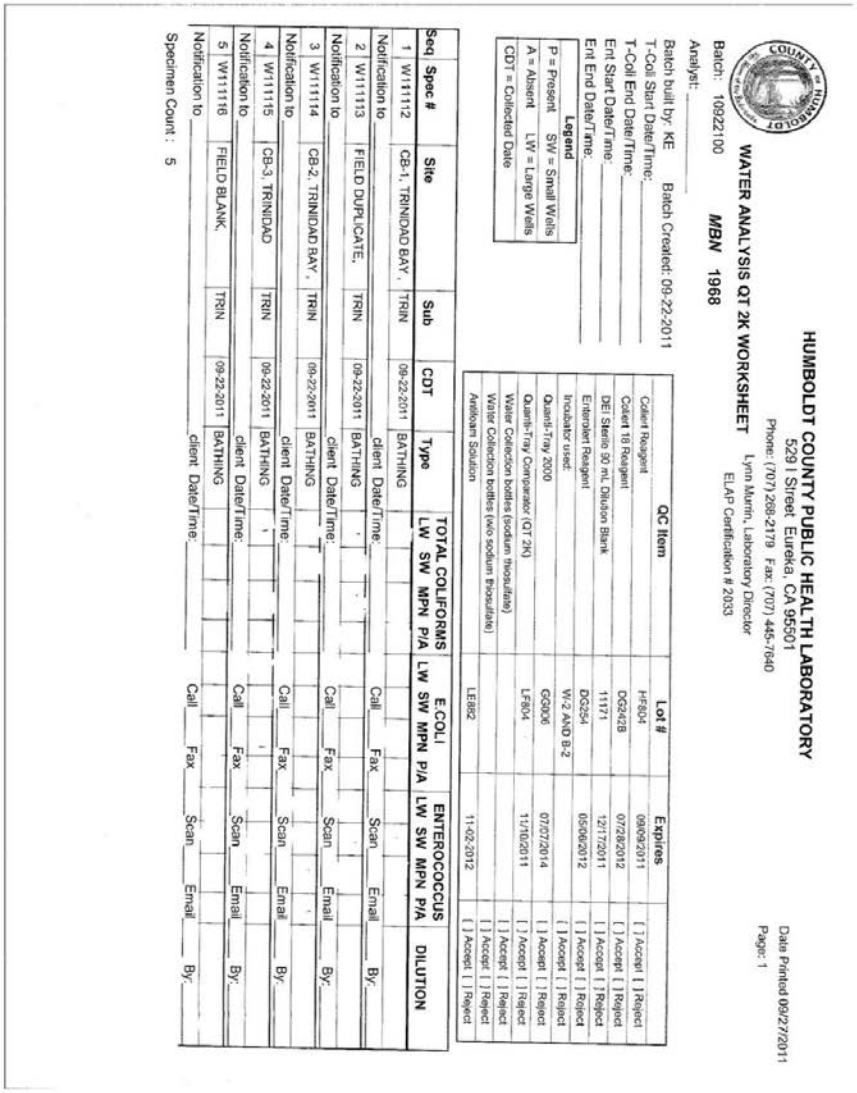
Appendix 1 – MPN Table (continued)

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.6
1	26.5	27.7	28.7	29.8	30.9	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.8	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.8	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.5	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.9	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.9
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.3	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.6	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.9	84.4	86.0	87.5	89.1	90.7	92.3	93.9
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.6	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.5	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.3	229.6	235.0	240.5	246.1	251.9	257.8	263.8
42	164.3	168.6	172.6	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.8	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.3	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.8	268.2	277.8	287.8	298.1	308.8	319.8	331.4	343.3	355.6	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

06-02135-01

Appendix 2 – Example Worksheet



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ELAP Certification # 2033

Date Printed 09/27/2011
Page: 1

WATER ANALYSIS QT 2K WORKSHEET

Batch: 10922100 ***MBN*** **1968**

Analyst:_____

Batch built by: KE Batch Created: 09-22-2011
T-Coli Start Date/Time:_____
T-Coli End Date/Time:_____
Ent Start Date/Time:_____
Ent End Date/Time:_____

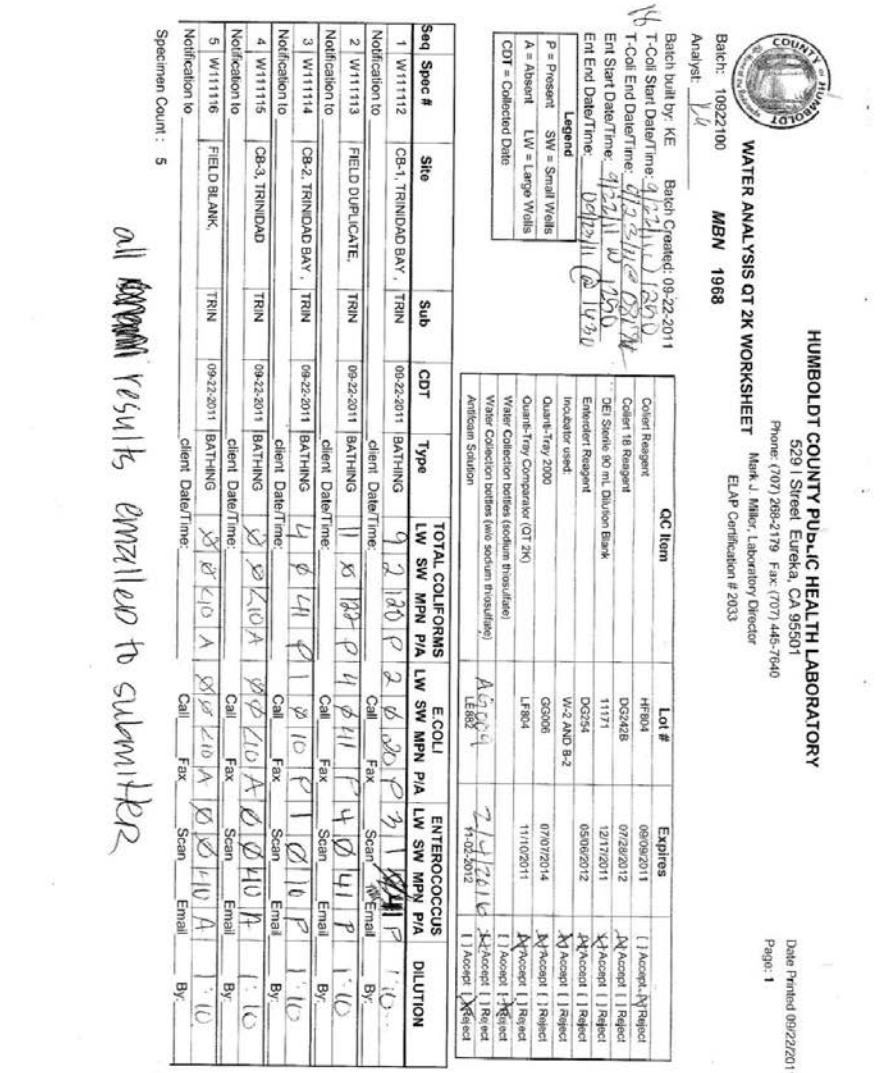
Legend
P = Present SW = Small Wells
A = Absent LW = Large Wells
CDT = Collected Date

QC Item	Lot #	Expires	
Colert Reagent	HF804	09/09/2011	[ ] Accept [ ] Reject
Colert 18 Reagent	DG242B	07/28/2012	[ ] Accept [ ] Reject
DEI Sterile 99 mL Dilution Blank	11171	12/17/2011	[ ] Accept [ ] Reject
Enterolert Reagent	DG254	05/06/2012	[ ] Accept [ ] Reject
Incubator used:	W-2 AND B-2		[ ] Accept [ ] Reject
Quanti-Tray 2000	G3006	07/07/2014	[ ] Accept [ ] Reject
Quanti-Tray Comparator (QT 2K)	LF804	11/10/2011	[ ] Accept [ ] Reject
Water Collection bottles (sodium thiosulfate)			[ ] Accept [ ] Reject
Water Collection bottles (w/o sodium thiosulfate)			[ ] Accept [ ] Reject
Antifoam Solution	LE882	11-02-2012	[ ] Accept [ ] Reject

Seq	Spec #	Site	Sub	CDT	Type	TOTAL COLIFORMS LW	TOTAL COLIFORMS SW	TOTAL COLIFORMS MPN	TOTAL COLIFORMS P/A	E.COLI LW	E.COLI SW	E.COLI MPN	E.COLI P/A	ENTEROCOCCUS LW	ENTEROCOCCUS SW	ENTEROCOCCUS MPN	ENTEROCOCCUS P/A	DILUTION
1	W111112	CB-1, TRINIDAD BAY,	TRIN	09-22-2011	BATHING													
Notification to_____ client Date/Time:_____ Call___ Fax___ Scan___ Email___ By:_____																		
2	W111113	FIELD DUPLICATE,	TRIN	09-22-2011	BATHING													
Notification to_____ client Date/Time:_____ Call___ Fax___ Scan___ Email___ By:_____																		
3	W111114	CB-2, TRINIDAD BAY,	TRIN	09-22-2011	BATHING													
Notification to_____ client Date/Time:_____ Call___ Fax___ Scan___ Email___ By:_____																		
4	W111115	CB-3, TRINIDAD	TRIN	09-22-2011	BATHING													
Notification to_____ client Date/Time:_____ Call___ Fax___ Scan___ Email___ By:_____																		
5	W111116	FIELD BLANK,	TRIN	09-22-2011	BATHING													
Notification to_____ client Date/Time:_____ Call___ Fax___ Scan___ Email___ By:_____																		

Specimen Count: 5

Appendix 3 – Example Worksheet (Filled out)



HUMBOLDT COUNTY PUBLIC HEALTH LABORATORY
529 I Street Eureka, CA 95501
Phone: (707) 268-2179 Fax: (707) 445-7640
Mark J. Miller, Laboratory Director
ELAP Certification # 2033

Date Printed 09/22/2011
Page: 1

WATER ANALYSIS QT 2K WORKSHEET

Batch: 10922100 *MBN* 1968

Analyst: KE

Batch built by: KE Batch Created: 09-22-2011

T-Coli Start Date/Time: 9/22/2011 1200

T-Coli End Date/Time: 9/23/2011 0200 PM

Ent Start Date/Time: 9/22/11 @ 1200

Ent End Date/Time: 09/23/11 @ 1430

Legend	
P = Present	SW = Small Wells
A = Absent	LW = Large Wells
CDT = Collected Date	

QC Item	Lot #	Expires	
Colilert Reagent	HF804	08/08/2011	[ ] Accept [X] Reject
Colilert 18 Reagent	DG242B	07/28/2012	[X] Accept [ ] Reject
DEI Sterile 90 mL Dilution Blank	11171	12/17/2011	[X] Accept [ ] Reject
Enterolert Reagent	DG254	05/06/2012	[X] Accept [ ] Reject
Incubator used:	W-2 AND B-2		[X] Accept [ ] Reject
Quanti-Tray 2000	G3006	07/07/2014	[X] Accept [ ] Reject
Quanti-Tray Comparator (QT 2K)	LF804	11/10/2011	[X] Accept [ ] Reject
Water Collection bottles (sodium thiosulfate)			[ ] Accept [X] Reject
Water Collection bottles (w/o sodium thiosulfate)			[X] Accept [ ] Reject
Antifoam Solution	A120254 LE803	11-02-2012	[X] Accept [ ] Reject

Seq	Spec #	Site	Sub	CDT	Type	TOTAL COLIFORMS LW	SW	MPN	P/A	E.COLI LW	SW	MPN	P/A	ENTEROCOCCUS LW	SW	MPN	P/A	DILUTION
1	W111112	CB-1, TRINIDAD BAY,	TRIN	09-22-2011	BATHING	9	2	120	P	2	0	120	P	3	1	20	P	1:10
Notification to					client Date/Time:					Call		Fax		Scan		Email		By:
2	W111113	FIELD DUPLICATE:	TRIN	09-22-2011	BATHING	1	1	8	P	1	0	4	P	1	0	4	P	1:10
Notification to					client Date/Time:					Call		Fax		Scan		Email		By:
3	W111114	CB-2, TRINIDAD BAY,	TRIN	09-22-2011	BATHING	4	0	41	P	1	0	10	P	1	0	10	P	1:10
Notification to					client Date/Time:					Call		Fax		Scan		Email		By:
4	W111115	CB-3, TRINIDAD	TRIN	09-22-2011	BATHING	2	0	10	A	0	0	10	A	0	0	10	A	1:10
Notification to					client Date/Time:					Call		Fax		Scan		Email		By:
5	W111116	FIELD BLANK:	TRIN	09-22-2011	BATHING	3	0	10	A	0	0	10	A	0	0	10	A	1:10
Notification to					client Date/Time:					Call		Fax		Scan		Email		By:

Specimen Count: 5

all [redacted] results emailed to submitter

Appendix 6: Standard Operating Procedure for Analysis of Bacteroides Bacteria Concentrations

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 7: Quality Assurance Program for Analysis of Bacteroides Bacteria Concentrations

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.

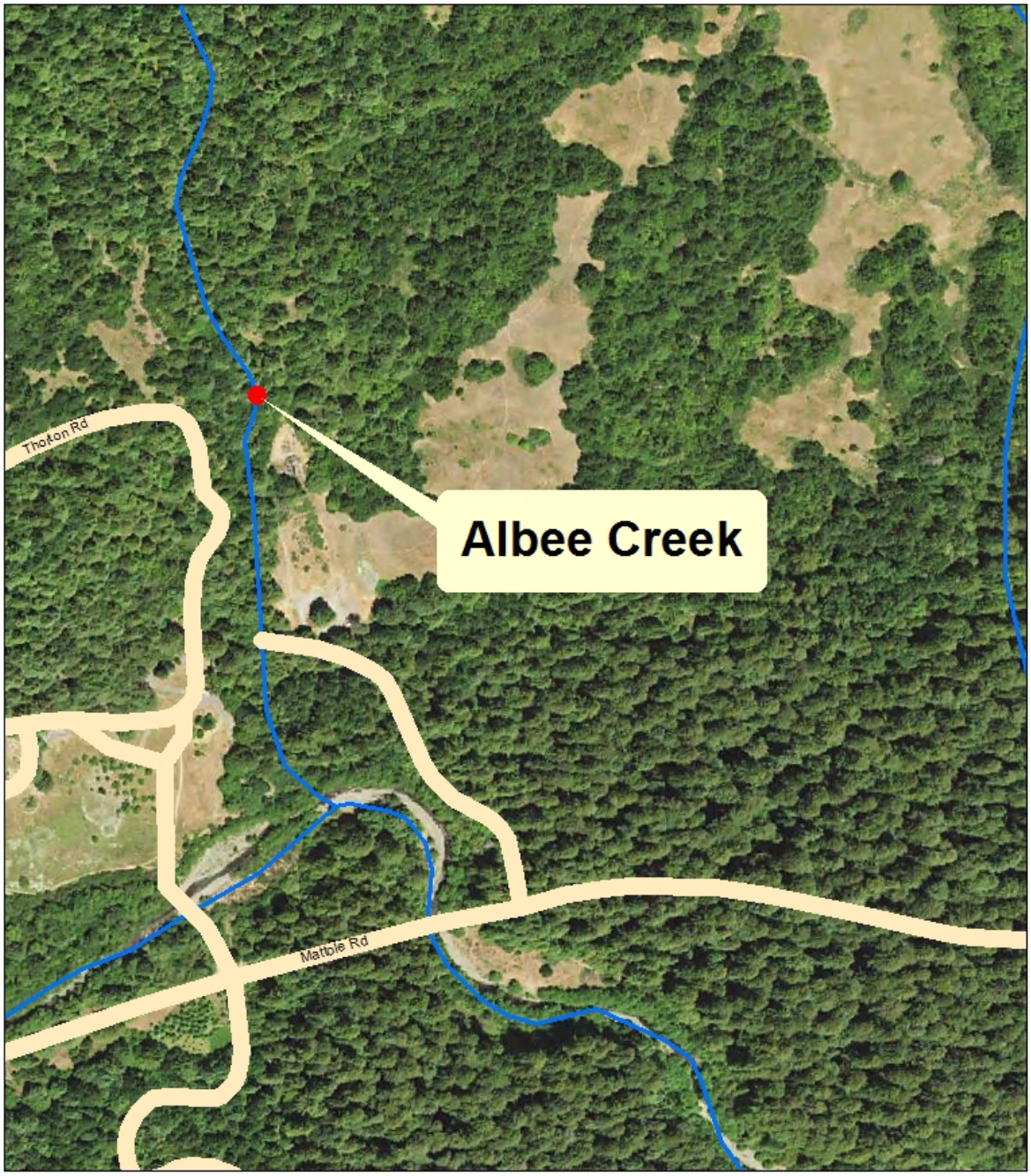
With quantitative assays, the standards are run in duplicate to ensure reasonable reproducibility. Each sample is run in duplicate to also demonstrate reasonable reproducibility.

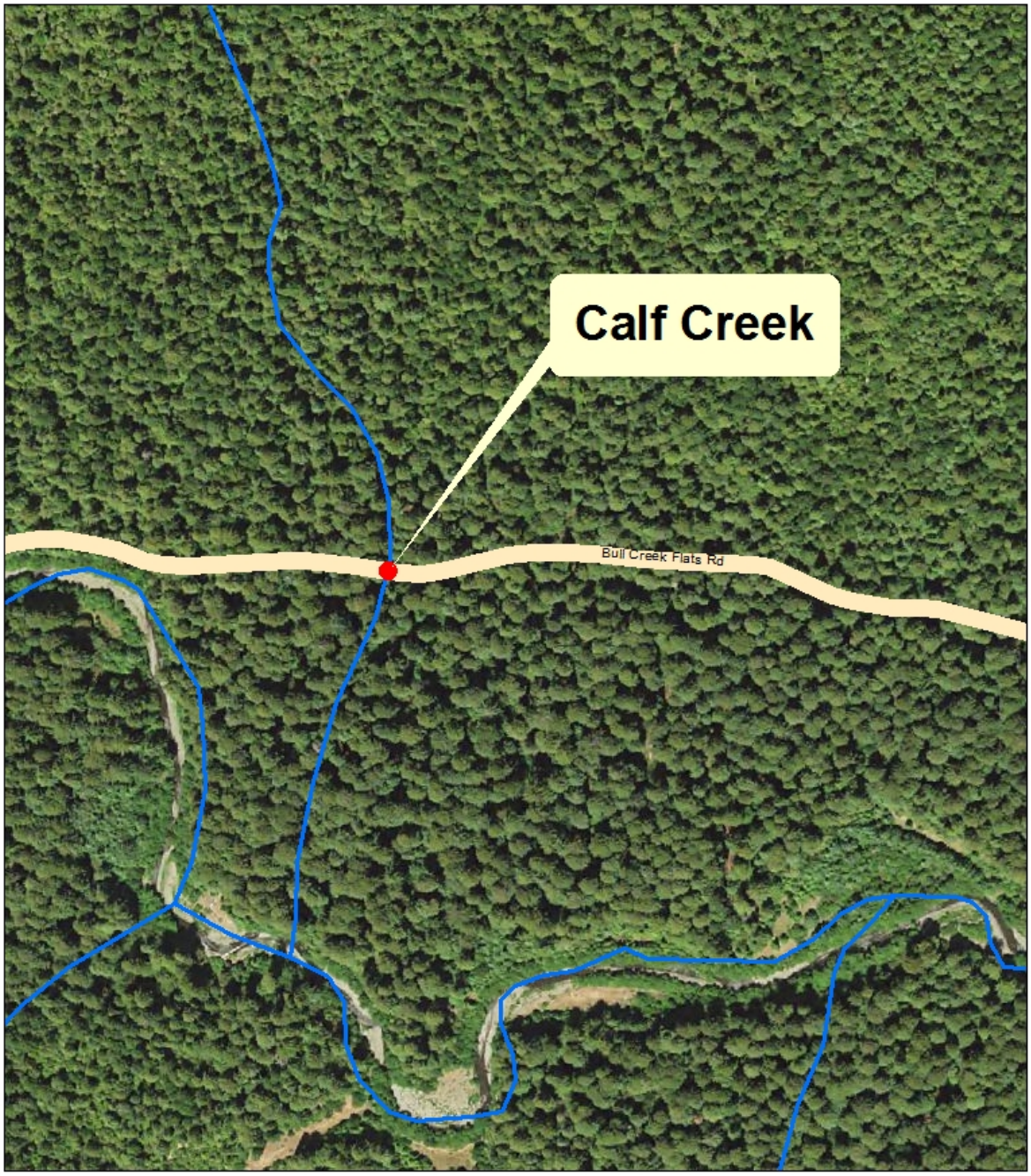
If applicable outdates and lot numbers of molecular reagents are checked each day of use and recorded.

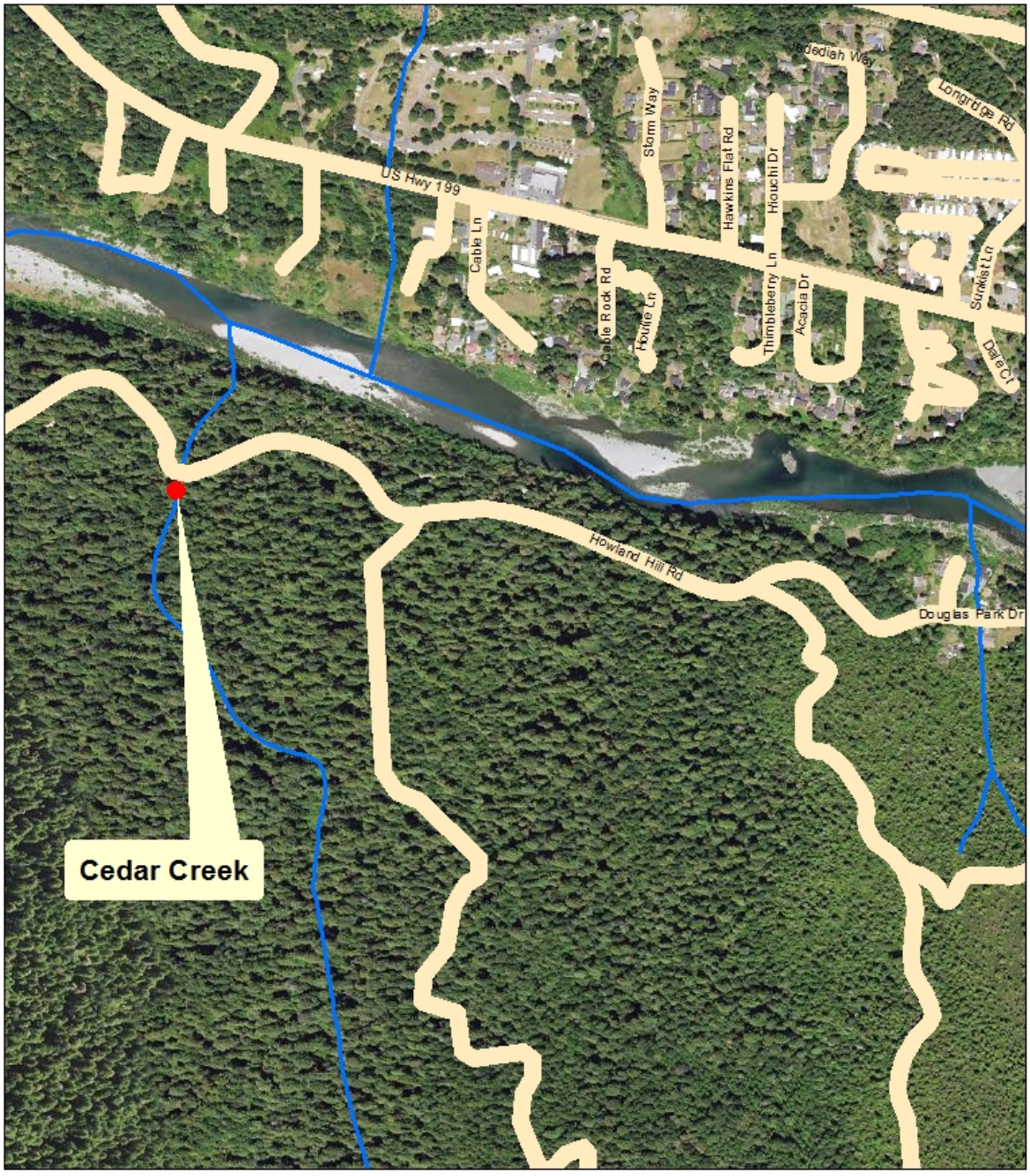
Assessment of Data Precision, Accuracy, Validation and Reporting:

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.

Appendix 8: Sampling Location Maps


Albee Creek
Thoton Rd
Mattole Rd
CALIFORNIA
Water Boards
Albee Creek
SWAMP ID 111AL1359
Sampling Location
Miles
0
0.03
0.06
0.12


Calf Creek
Bull Creek Flats Rd
CALIFORNIA
Water Boards
Calf Creek
SWAMP ID 111CF1805
Sampling Location
Miles
0
0.03
0.06
0.12



CALIFORNIA Water Boards

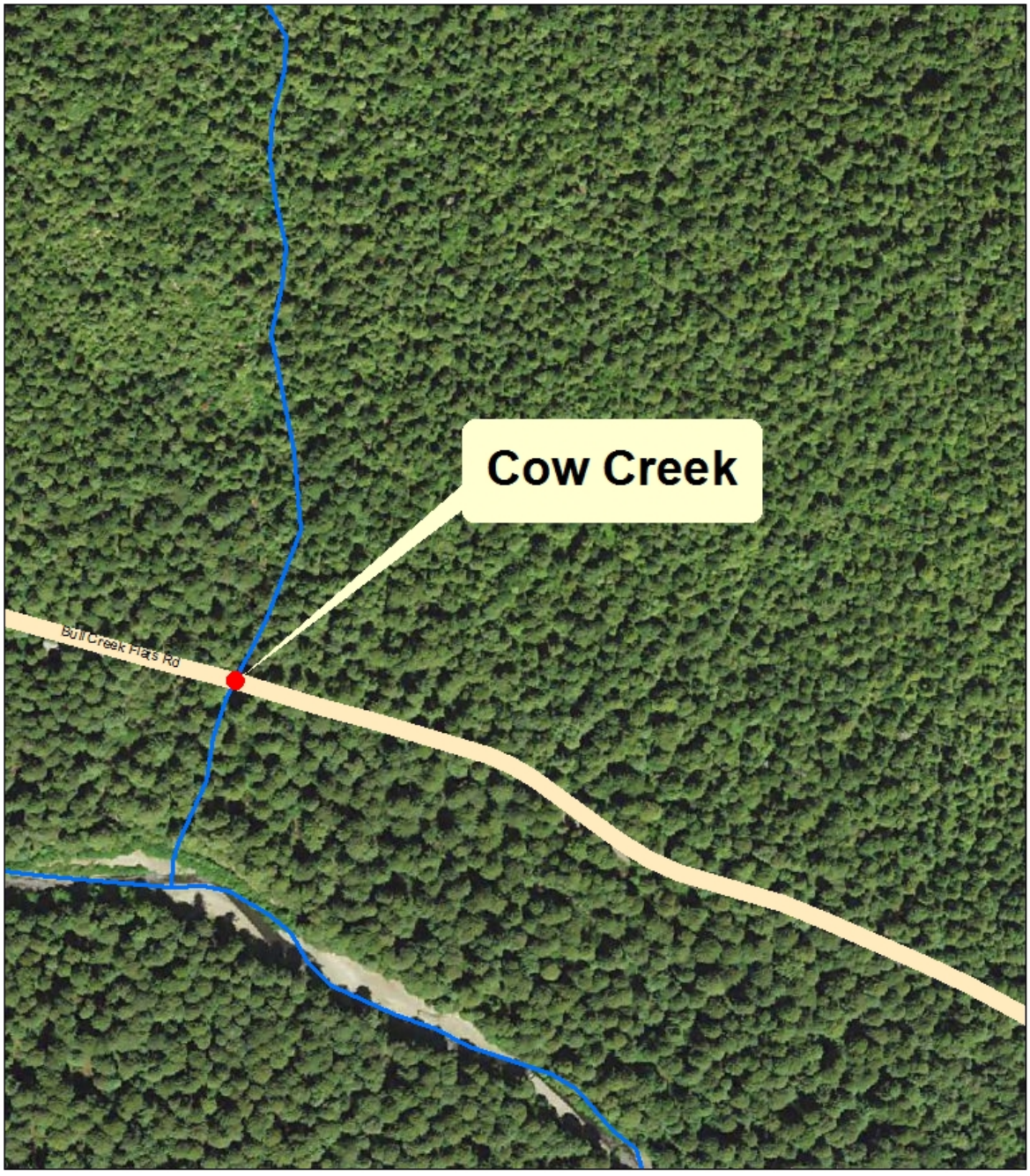
Cedar Creek
SWAMP ID 103CD0679
Sampling Location

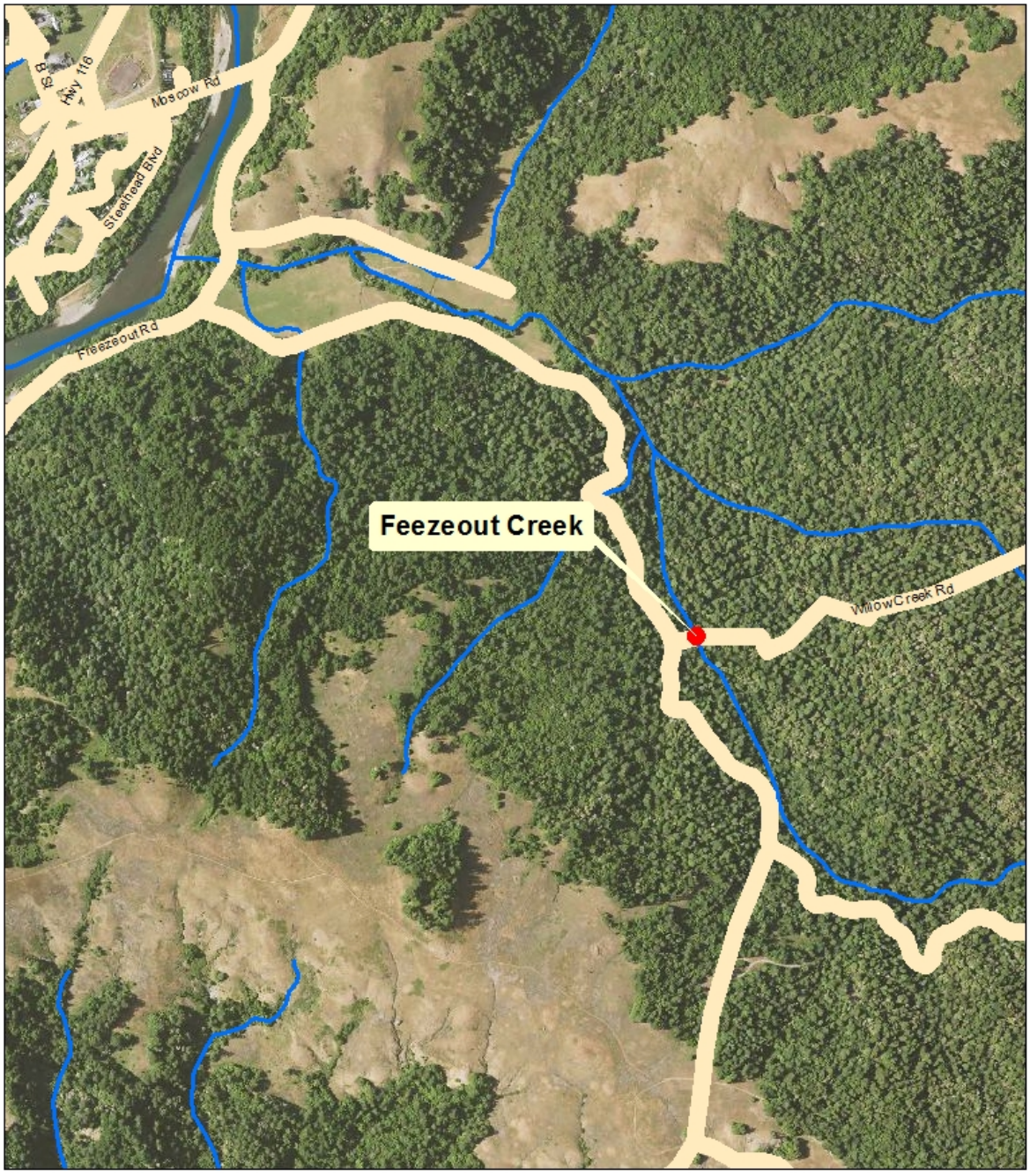
0 0.05 0.1 0.2 Miles

CALIFORNIA Water Boards

Clarks Creek
SWAMP ID 103CK4061
Sampling Location

0 0.05 0.1 0.2 Miles

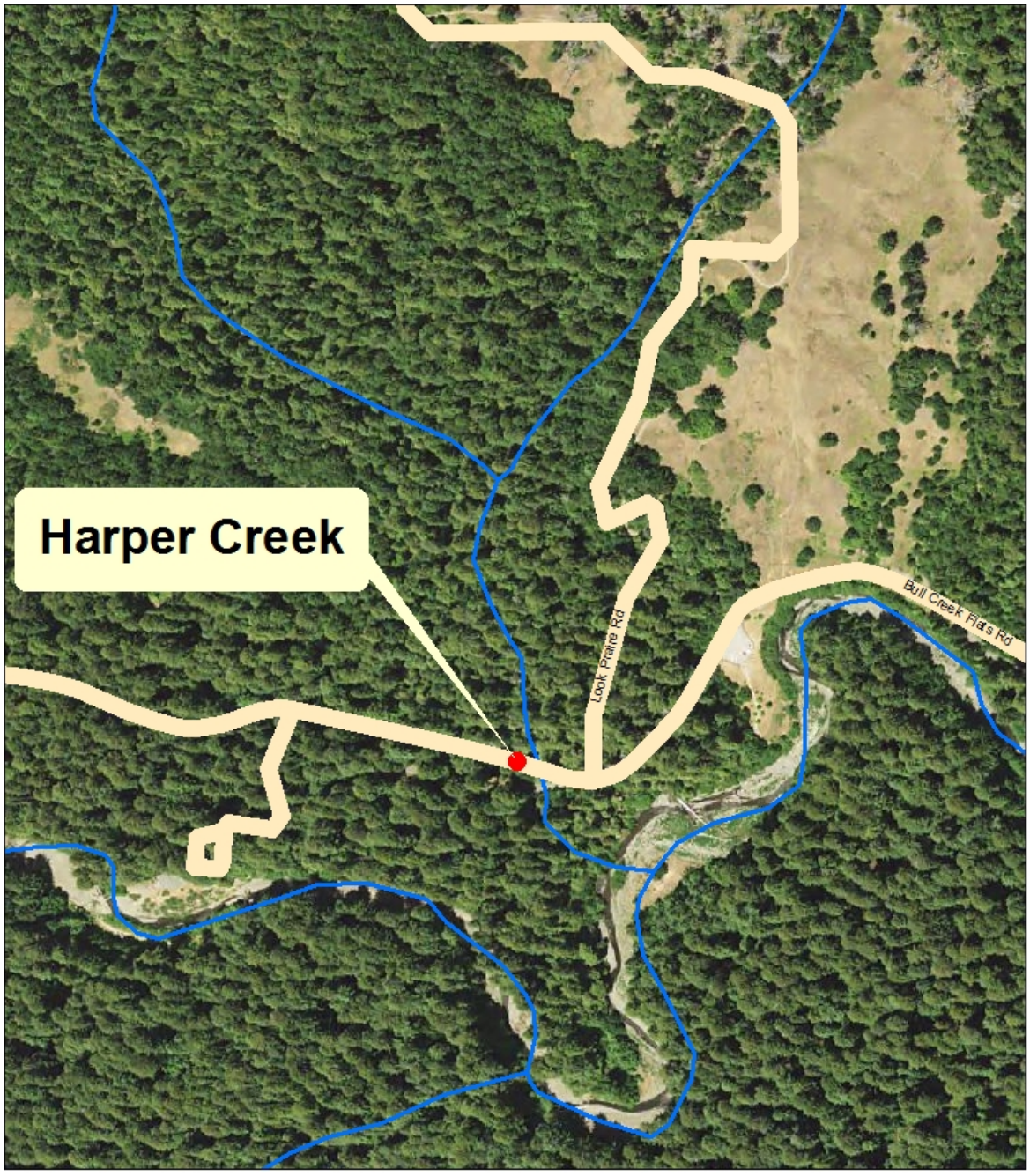

Cow Creek
Bull Creek Flats Rd
CALIFORNIA
Water Boards
Cow Creek
SWAMP ID 111CW0458
Sampling Location
Miles
0
0.03
0.06
0.12



CALIFORNIA Water Boards

Freezeout Creek
SWAMP ID 114FZ3710
Sampling Location

0 0.075 0.15 0.3 Miles


Harper Creek
Look Prairie Rd
Bull Creek Flats Rd
CALIFORNIA
Water Boards
Harper Creek
SWAMP ID 111HR0606
Sampling Location
Miles
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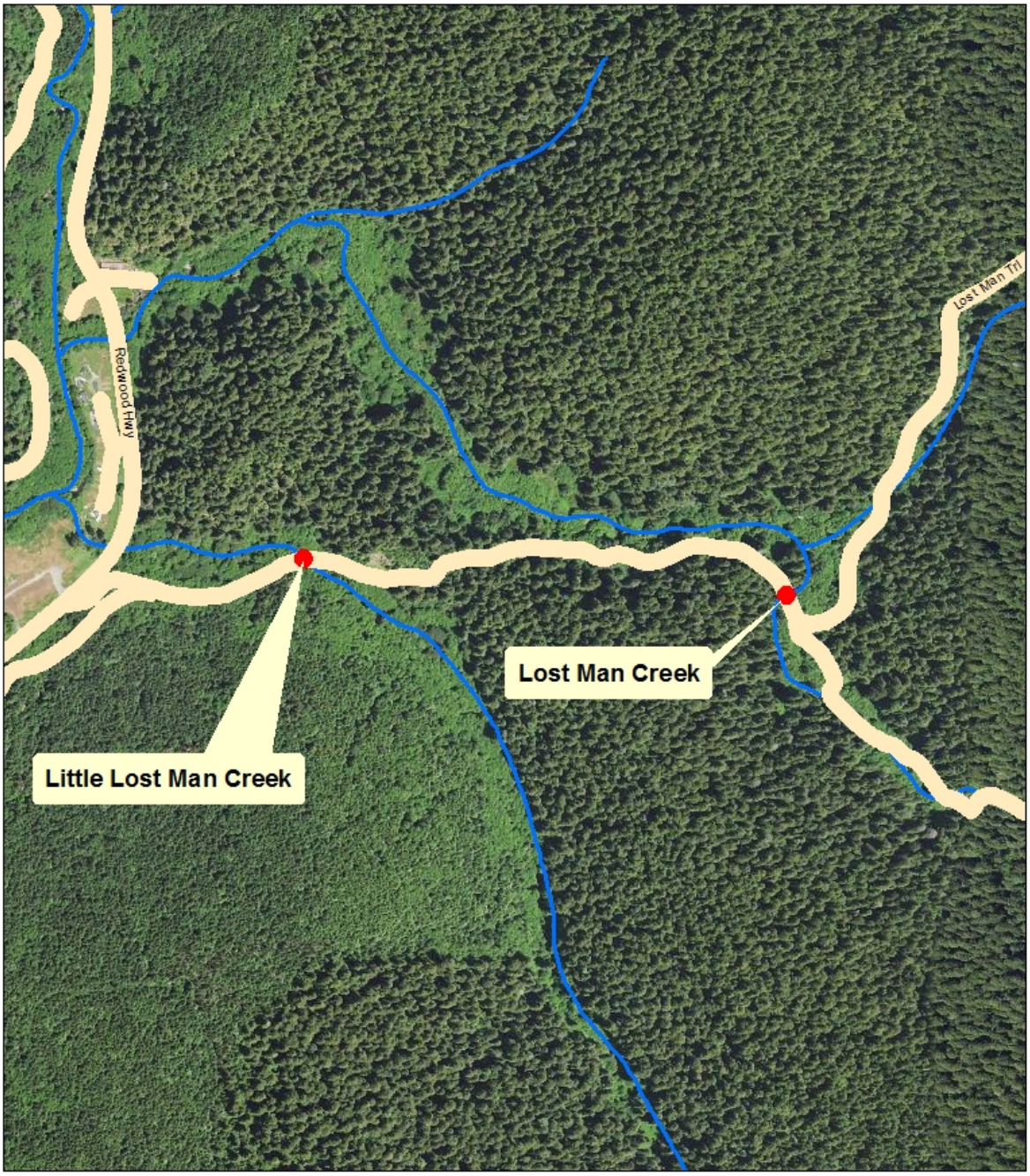
0.03
0.06
0.12



CALIFORNIA
Water Boards

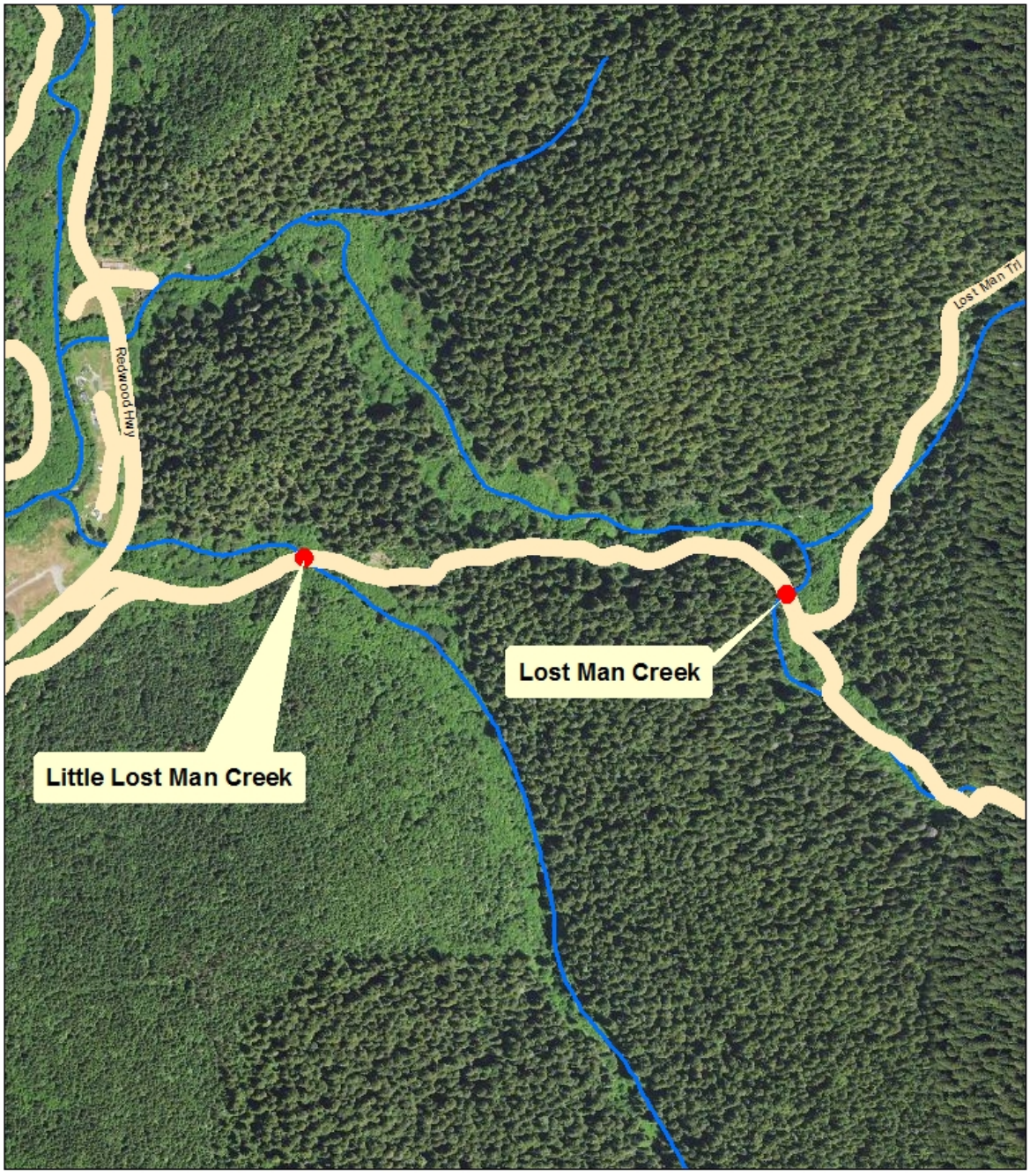
Little Lost Man Creek
SWAMP ID 107LL0600
Sampling Location

Miles
0 0.05 0.1 0.2

CALIFORNIA Water Boards

Little Mill Creek
SWAMP ID 111LM0001
Sampling Location

0 0.03 0.06 0.12 Miles



CALIFORNIA Water Boards

Lost Man Creek
SWAMP ID 107LM1856
Sampling Location

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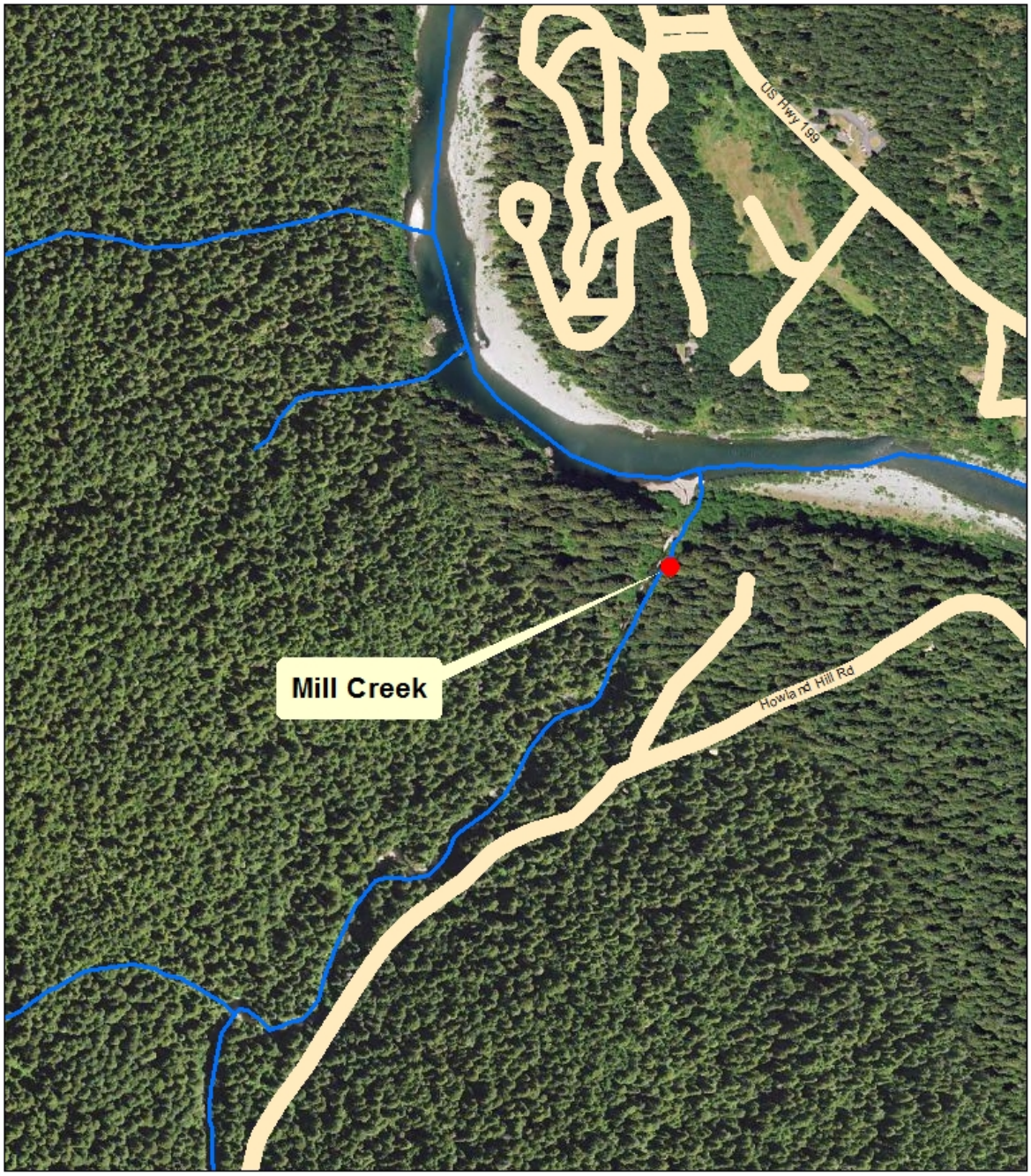
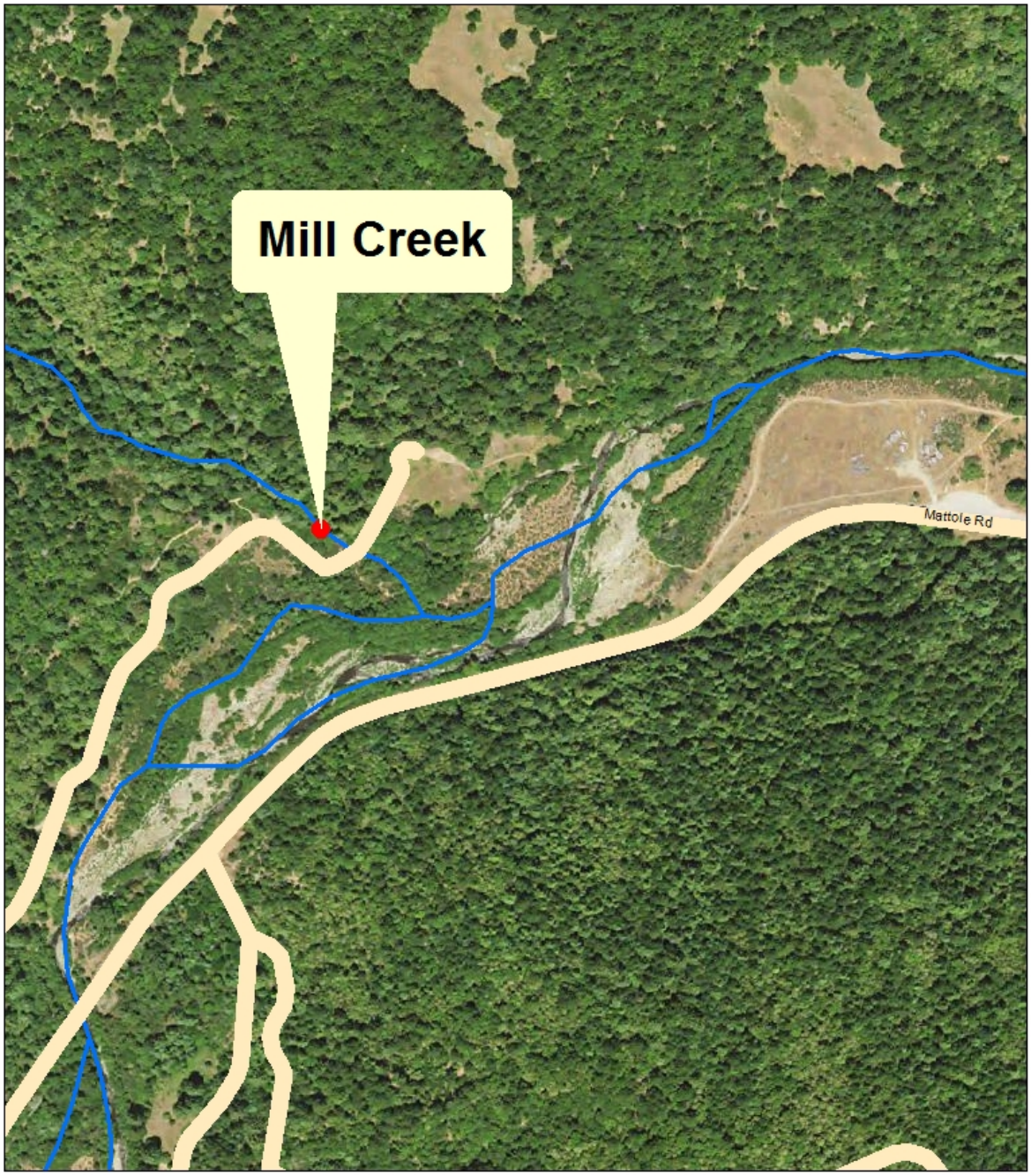


CALIFORNIA Water Boards

Mill Creek
SWAMP ID 103ML0155
Sampling Location

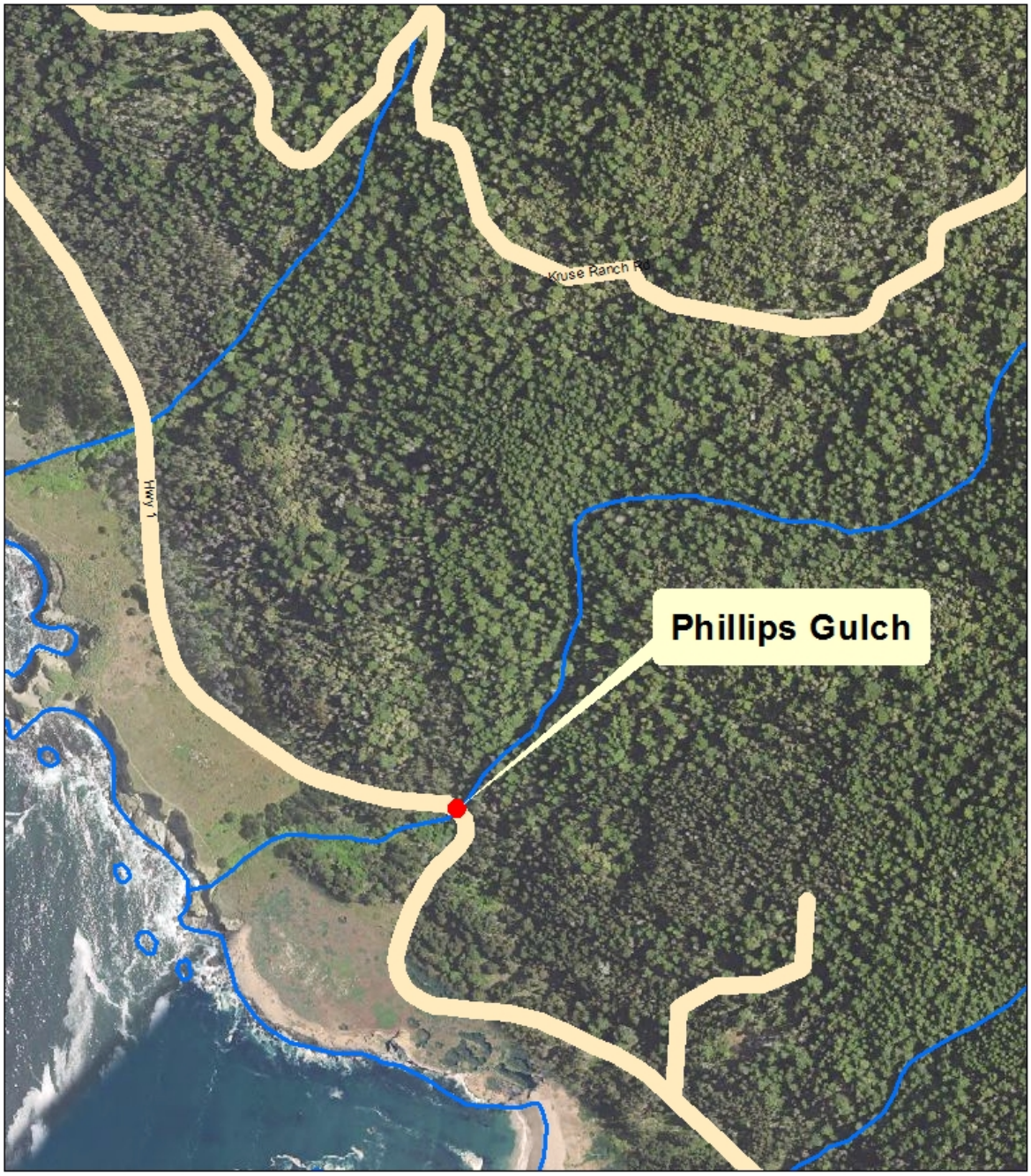
0 0.05 0.1 0.2 Miles


Mill Creek
Mattole Rd
CALIFORNIA
Water Boards
Mill Creek
SWAMP ID 111ML0252
Sampling Location
Miles
0
0.03
0.06
0.12

CALIFORNIA
Water Boards

Miller Creek
SWAMP ID 113MR1171
Sampling Location

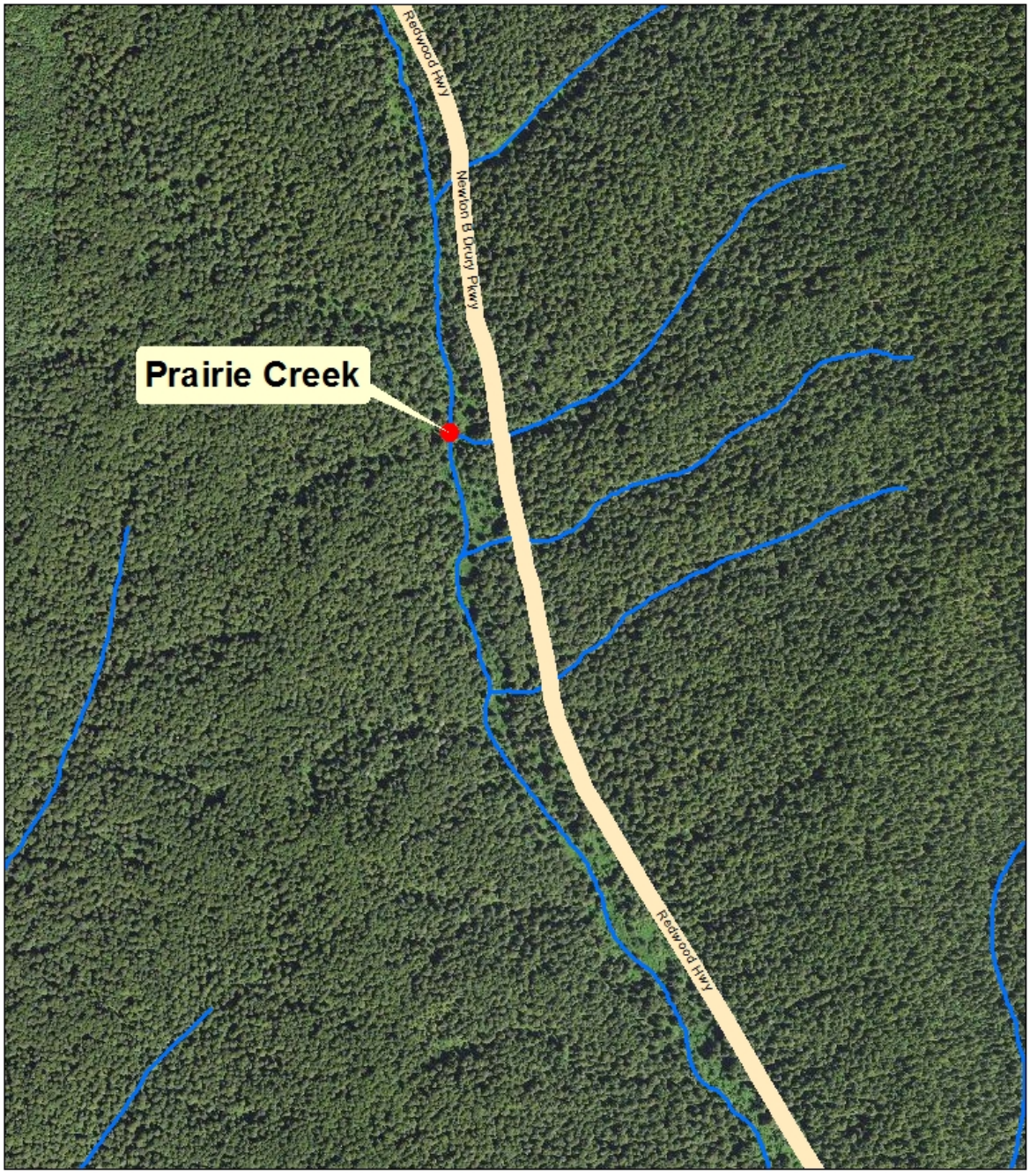
Miles
0 0.04 0.08 0.16



CALIFORNIA Water Boards

Phillips Gulch
SWAMP ID 113PG1586
Sampling Location

0 0.04 0.08 0.16 Miles



CALIFORNIA Water Boards

Prairie Creek
SWAMP ID 107PR7848
Sampling Location

0 0.075 0.15 0.3 Miles

CALIFORNIA Water Boards

Stockhoff Creek
SWAMP ID 113ST0986
Sampling Location

0 0.04 0.08 0.16 Miles

CALIFORNIA Water Boards

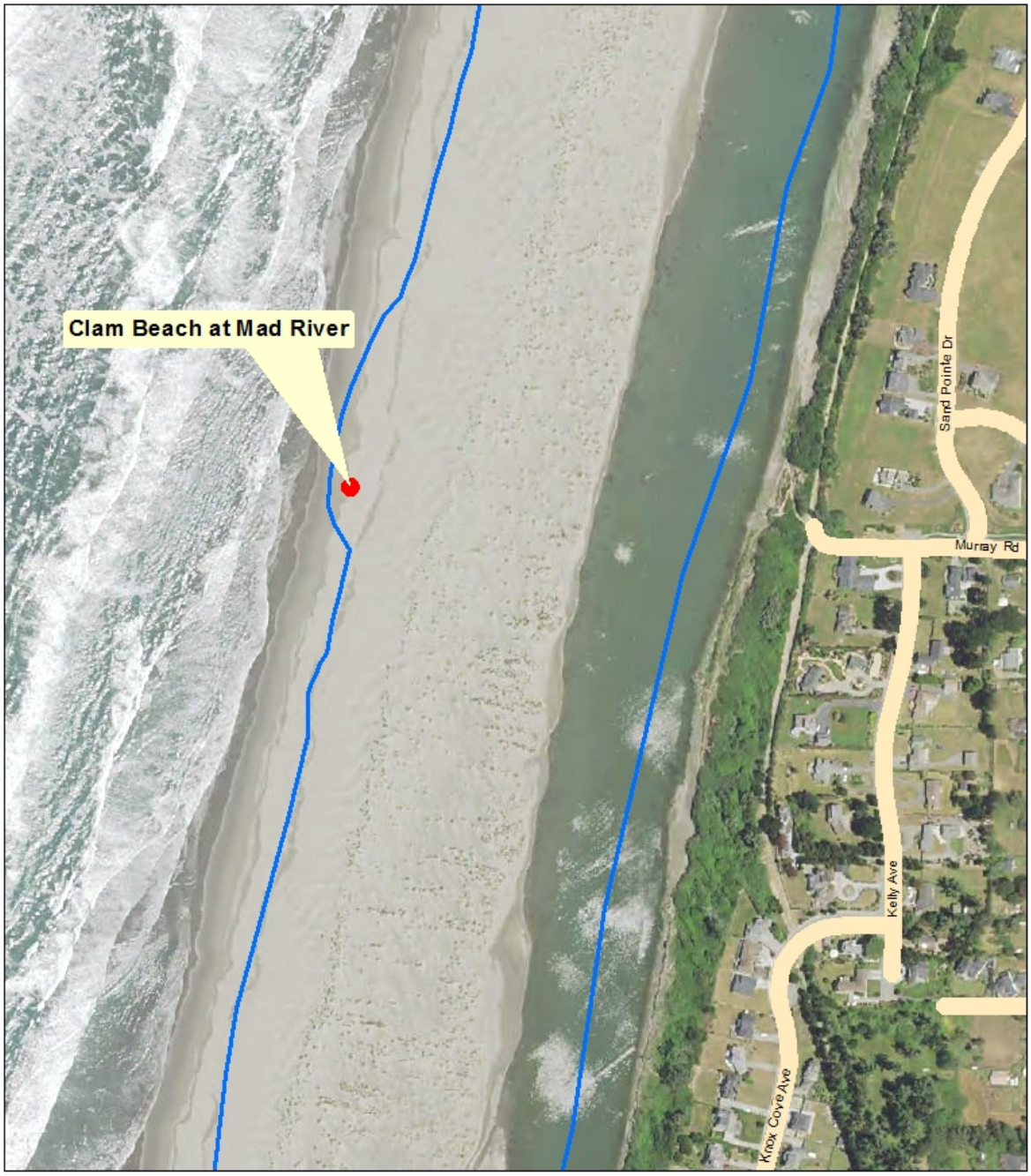
Campbell Cove at Bodega Bay
SWAMP ID 115BBCCB1
Sampling Location

0 0.0225 0.045 0.09 Miles

CALIFORNIA Water Boards

Caspar Beach at Caspar Creek
SWAMP ID 113CA0001
Sampling Location

0 0.015 0.03 0.06 Miles



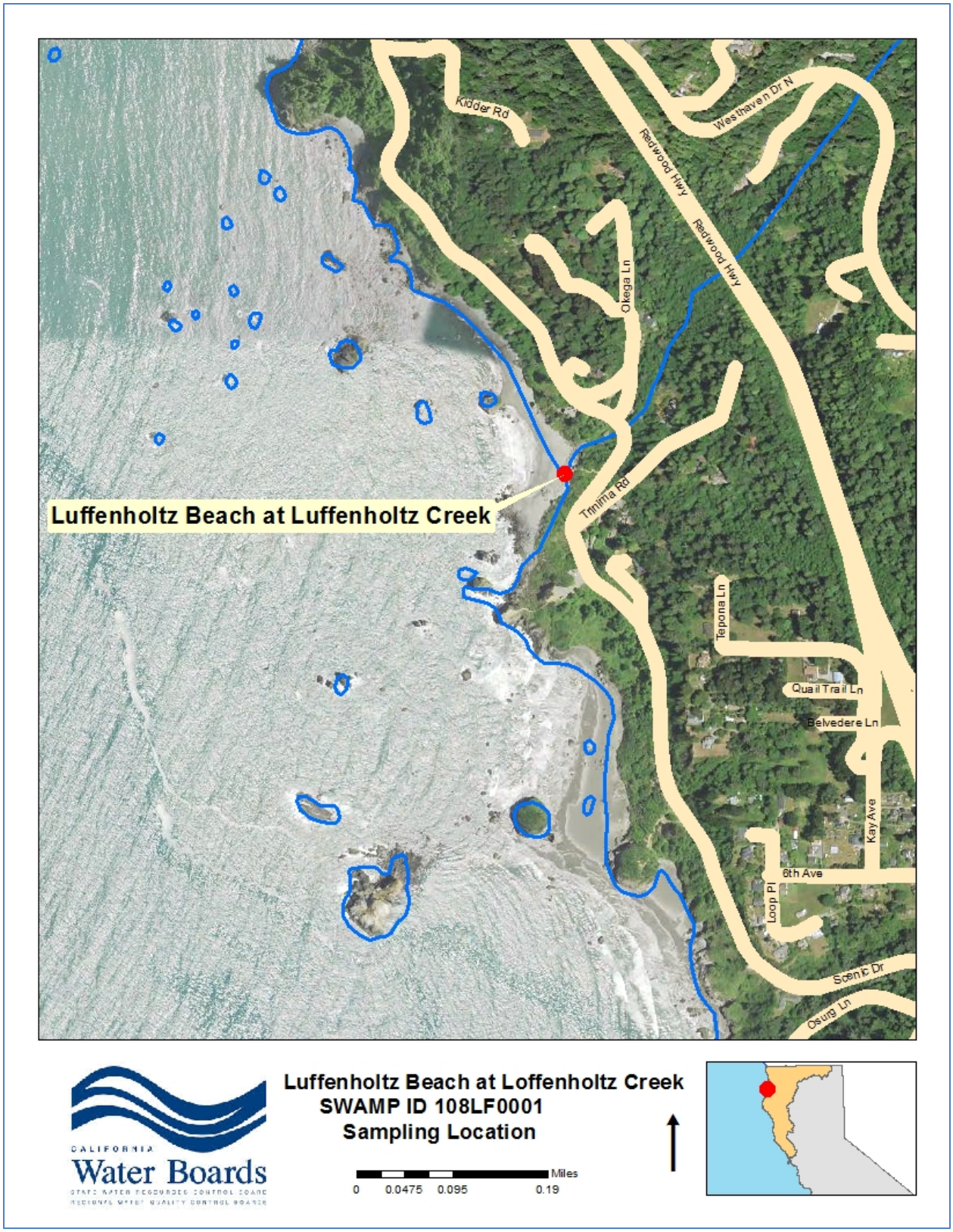
CALIFORNIA Water Boards

Clam Beach at Mad River
SWAMP ID 109MA0001
Sampling Location

0 0.03 0.06 0.12 Miles


Clam Beach at Strawberry Creek
Clam Beach Dr
Redwood Hwy
Redwood Hwy
Little River Dr
Little River
Central Ave
CALIFORNIA
Water Boards
Clam Beach at Strawberry Creek
SWAMP ID 109SW0001
Sampling Location
Miles
0
0.0275
0.055
0.11


Pacific Dr
Bay Vw
del Mar Dr
Hare Beach at Hare Creek
Shoreview Ln
Cypress Rd
Old Hwy 1
CALIFORNIA
Water Boards
Hare Beach at Hare Creek
SWAMP ID 113HC0001
Sampling Location
Miles
0
0.02
0.04
0.08


Luffenholtz Beach at Luffenholtz Creek
Kidder Rd
Redwood Hwy
Redwood Hwy
Westhaven Dr N
Okega Ln
Trinidad Rd
Tepona Ln
Quail Trail Ln
Belvedere Ln
Kay Ave
6th Ave
Loop Pl
Scenic Dr
Osurg Ln
CALIFORNIA
Water Boards
Luffenholtz Beach at Loffenholtz Creek
SWAMP ID 108LF0001
Sampling Location
Miles
0 0.0475 0.095 0.19

CALIFORNIA Water Boards

Makerricher State Park at Virgin Creek
SWAMP ID 113VR0001
Sampling Location

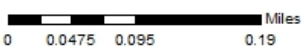
0 0.02 0.04 0.08 Miles

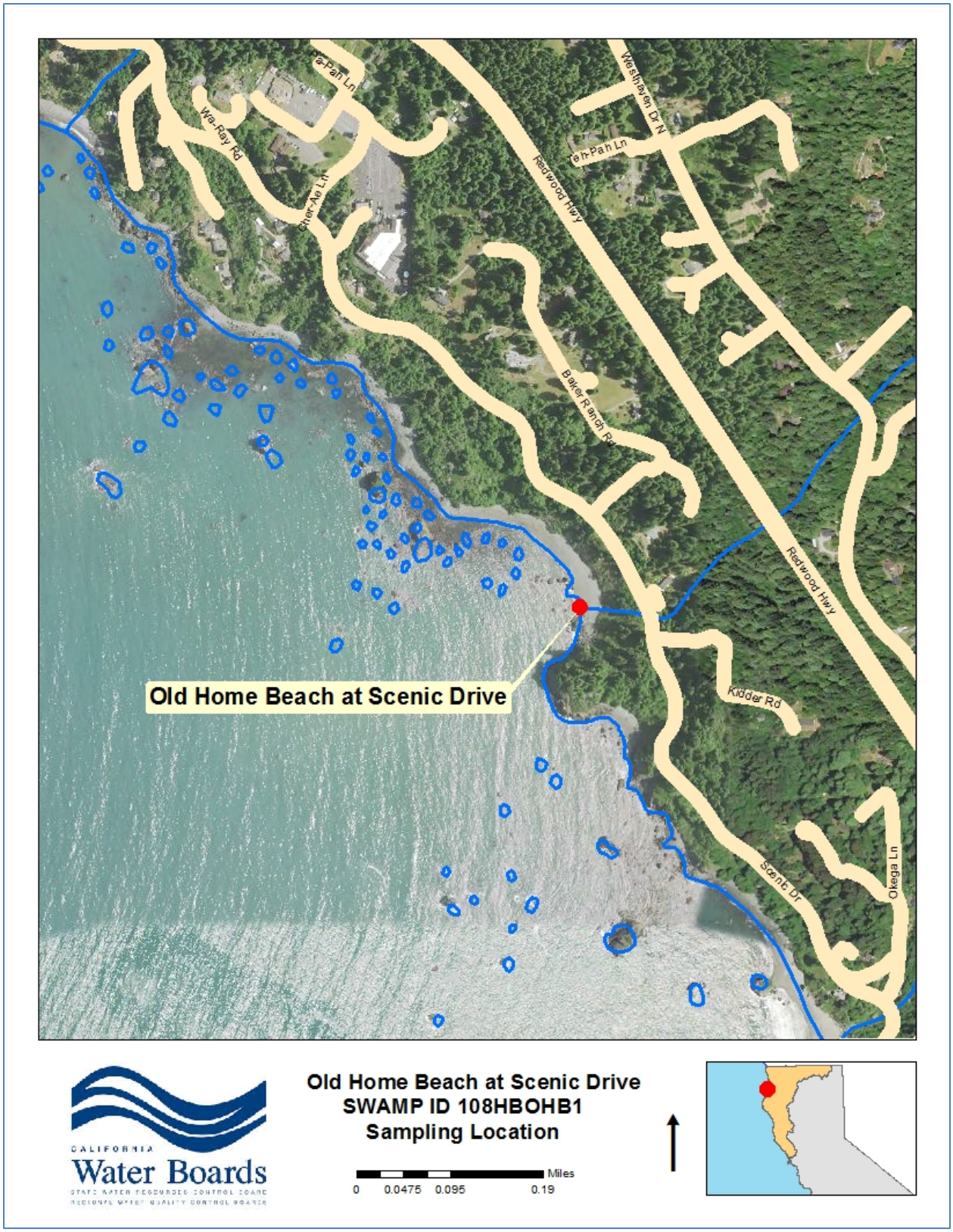
CALIFORNIA Water Boards

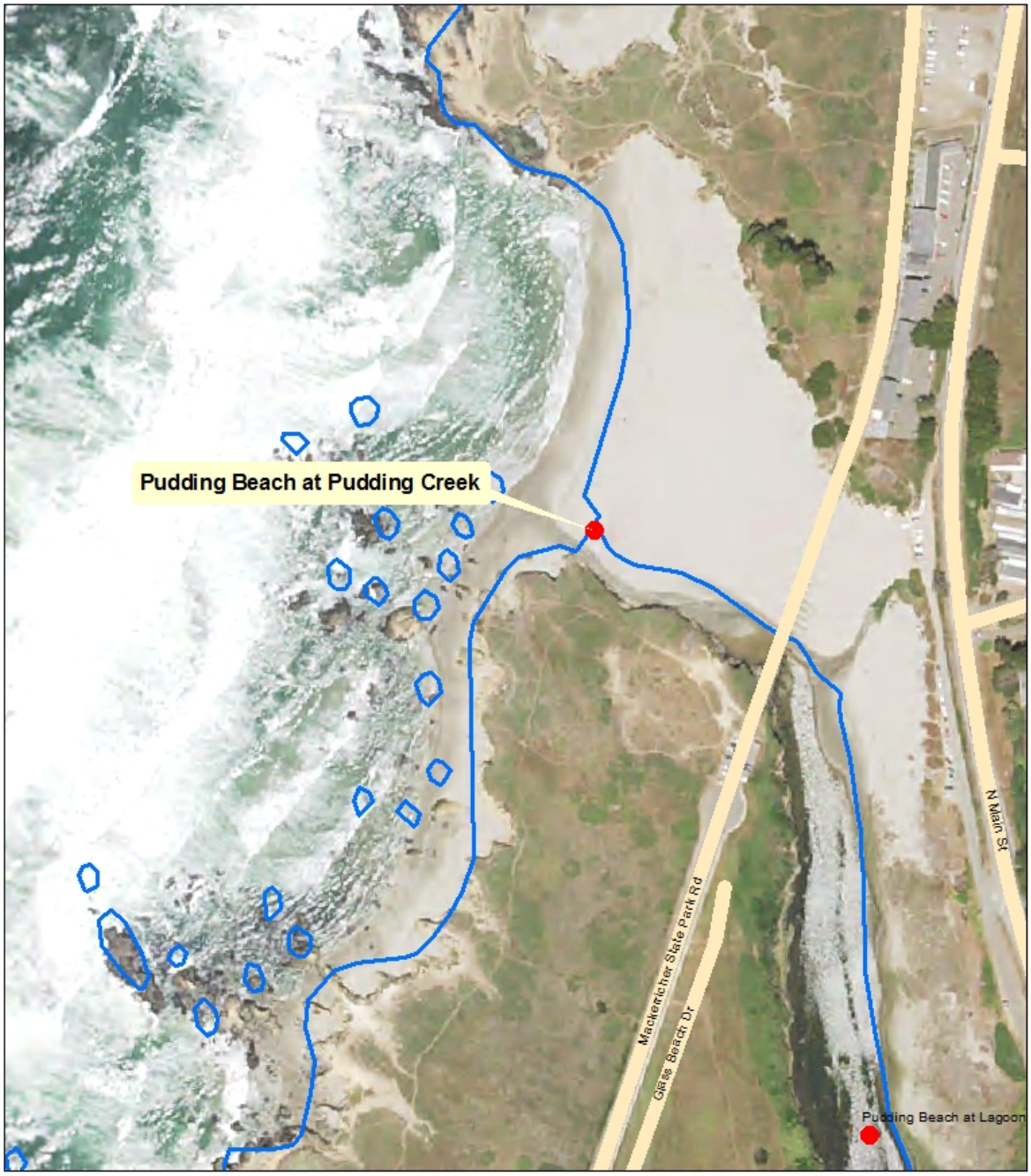
Caspar Beach at Caspar Creek
SWAMP ID 113CA0001
Sampling Location

Miles
0 0.0225 0.045 0.09

Moonstone Beach at Little River
SWAMP ID 108LR0001
Sampling Location




Pa-Pah Ln
Wa-Ray Rd
Sher-Ae Ln
Redwood Hwy
Weshpeyen Dr N
Teh-Pah Ln
Baker Ranch Rd
Redwood Hwy
Old Home Beach at Scenic Drive
Kidder Rd
Scenic Dr
Okega Ln
CALIFORNIA
Water Boards
Old Home Beach at Scenic Drive
SWAMP ID 108HBOHB1
Sampling Location
Miles
0 0.0475 0.095 0.19



CALIFORNIA Water Boards

Pudding Beach at Pudding Creek
SWAMP ID 113PD0001
Sampling Location

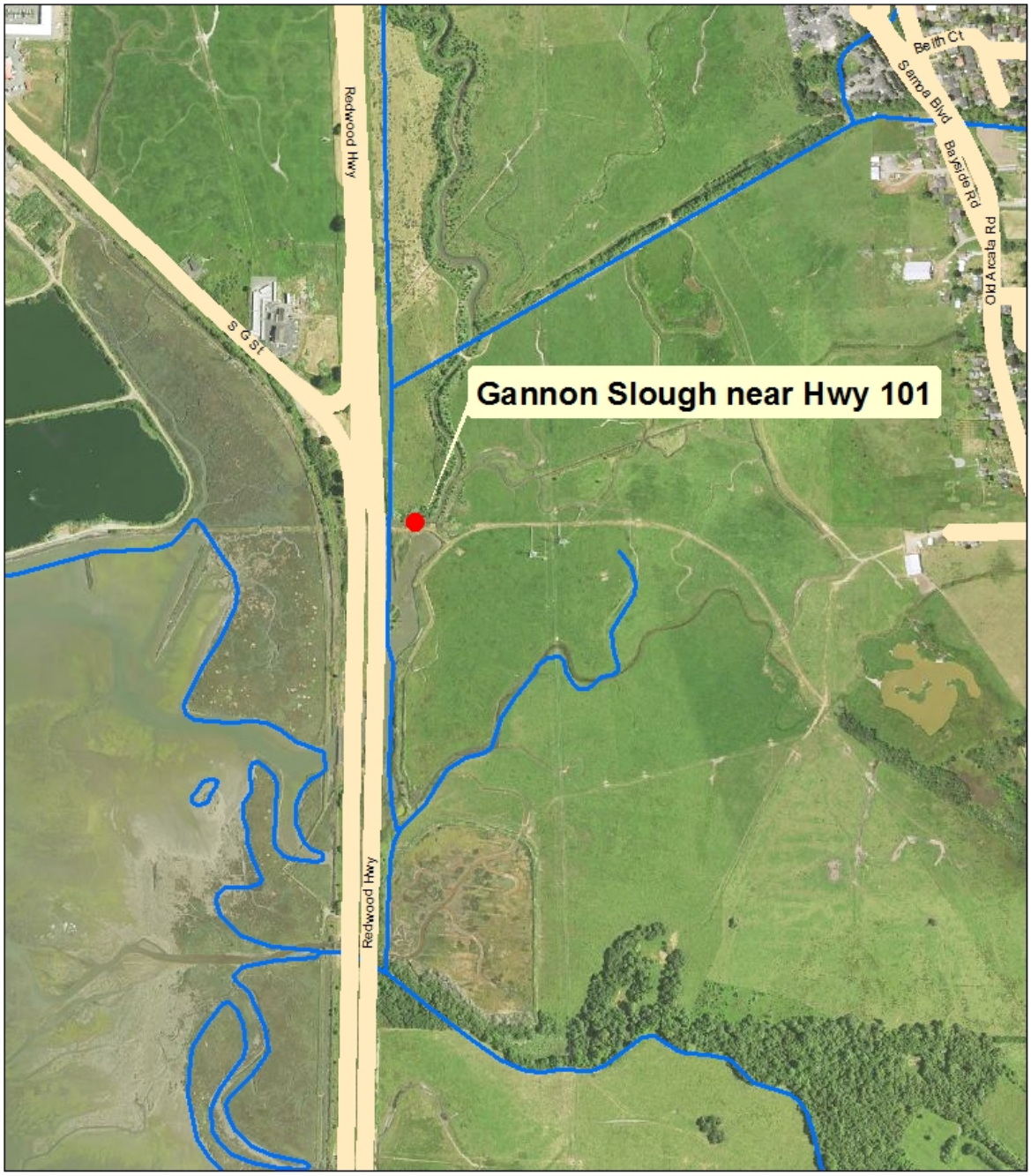
0 0.02 0.04 0.08 Miles

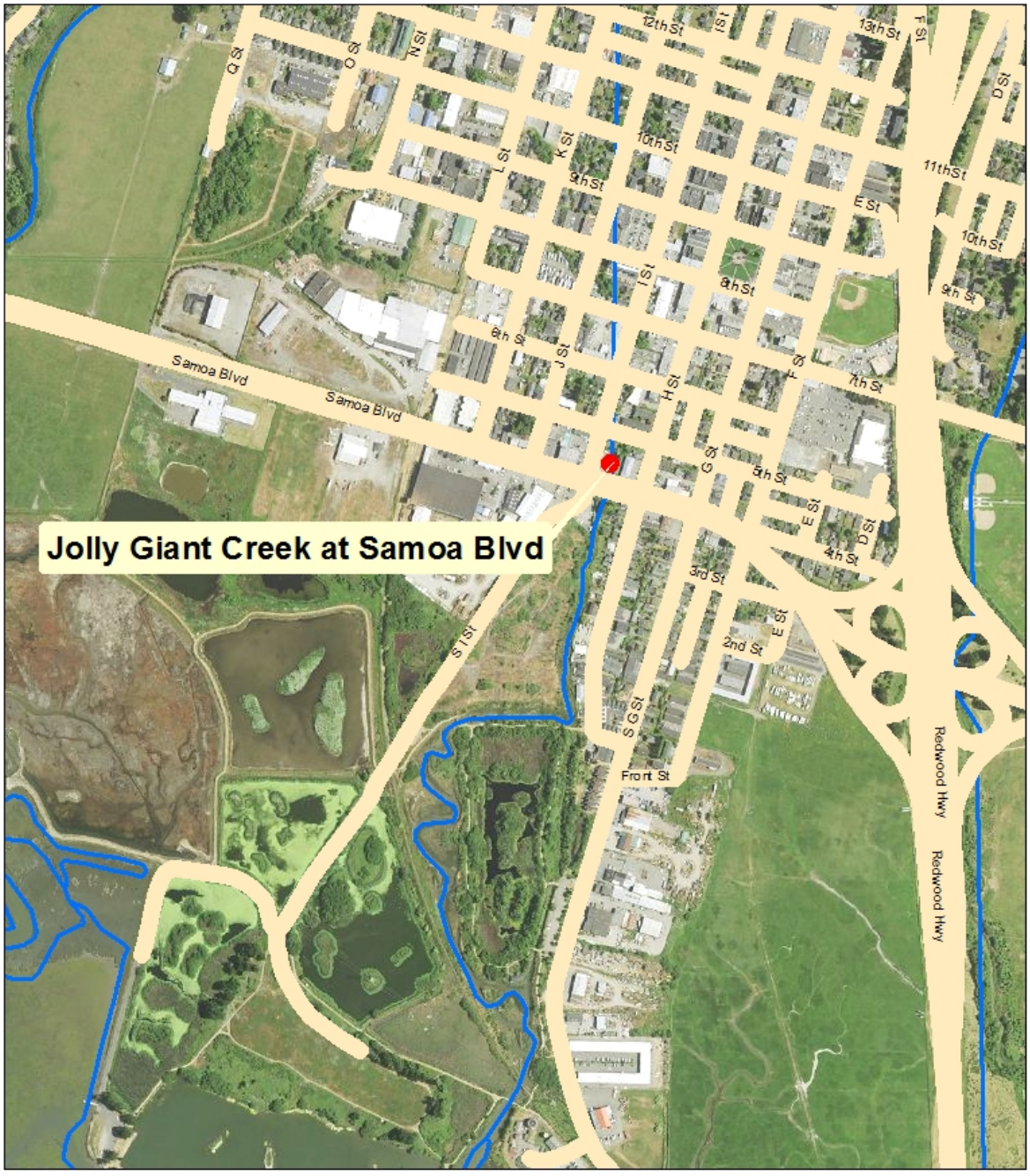

Trinidad State Beach at Mill Creek
Stagecoach Rd
Anderson Ln
Patricks Point Dr
Redwood Hwy
State Park Rd
Underwood Dr
Main St
Ocean
Ocean Ave
Trinity St
West St
Ewing St
Hector St
Edwards St
Galindo St
Bay St
Lighthouse Rd
CALIFORNIA
Water Boards
Trinidad State Beach at Mill Creek
SWAMP ID 108LML0001
Sampling Location
Miles
0
0.0475
0.095
0.19

CALIFORNIA Water Boards

Elk River at Hwy 101
SWAMP ID 110EL1278
Sampling Location

0 0.04 0.08 0.16 Miles

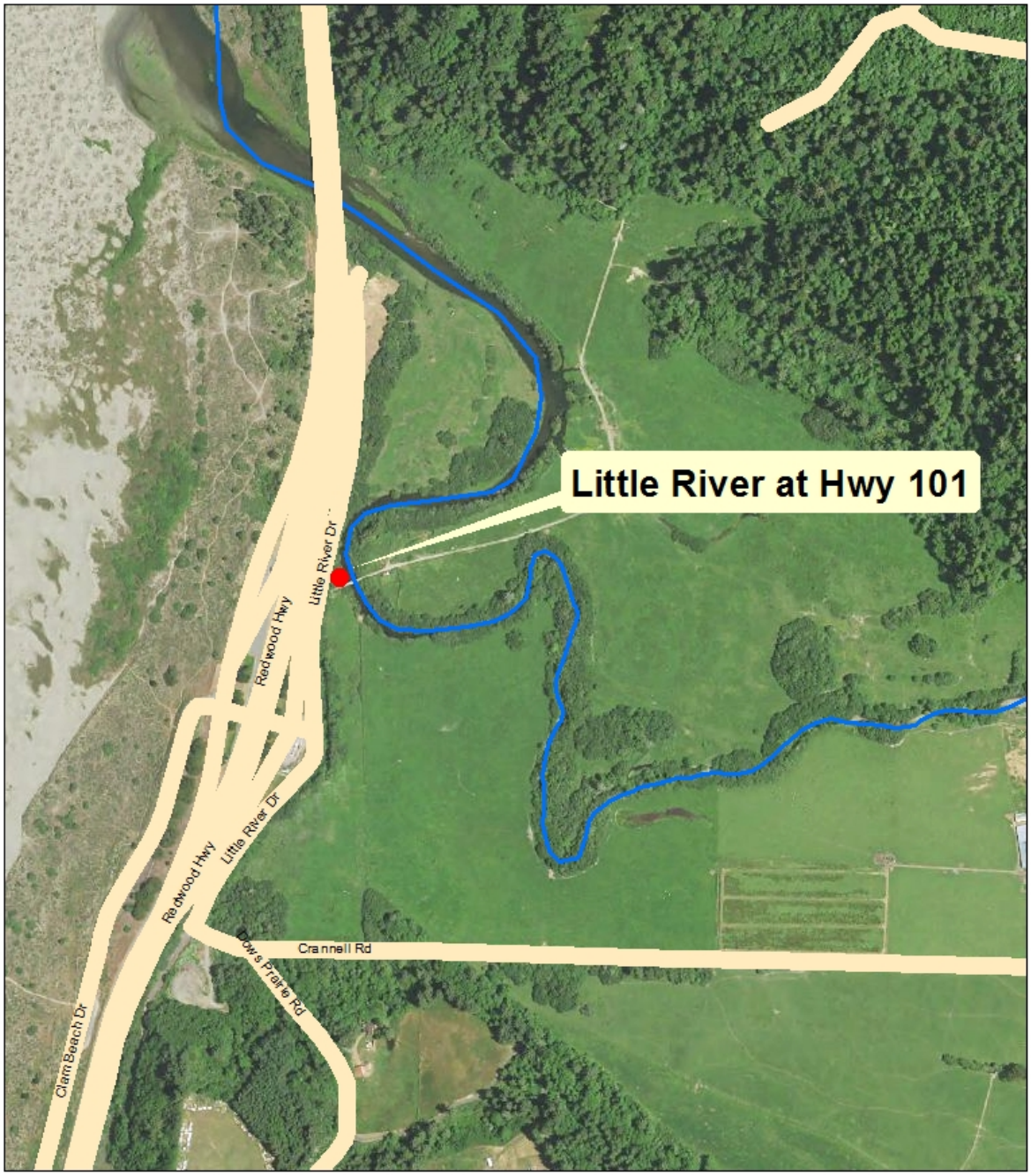

Gannon Slough near Hwy 101
Redwood Hwy
S G St
Redwood Hwy
Samoa Blvd
Bayside Rd
Old Arcata Rd
Beith Ct
CALIFORNIA
Water Boards
Gannon Slough near Hwy 101
SWAMP ID 110GS1625
Sampling Location
Miles
0
0.05
0.1
0.2



CALIFORNIA Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS

Jolly Giant Creek at Samoa Blvd
SWAMP ID 110JG0264
Sampling Location

Miles
0 0.05 0.1 0.2



CALIFORNIA Water Boards

Little River at Hwy 101
SWAMP ID 108LR0663
Sampling Location

0 0.0475 0.095 0.19 Miles


Bay Pointe Ct
Herrick Ave
Carolyn Ct
Pinecrest Ct
Nancy Ct
Alpine Ct
Myers Ave
Gladding Ave
Ryan Ct
Zeck Ln
Kirkby Ln
Noe Ave
Gatliff Ave
Fredricson Ln
Country Ln
Valley View Dr
Pine Hill Rd
Martin Slough at Pine Hill Road
Elk River Rd
CALIFORNIA
Water Boards
Martin Slough at Pine Hill Road
SWAMP ID 110MS1481
Sampling Location
Miles
0
0.04
0.08
0.16


Norton Creek at Hwy 101
Redwood Hwy
Redwood Hwy
Leiz Ave
Lily Rd
Boeing Ct
Boeing Ave
Marius Ct
Dylan Ct
Perini Rd
Meyers Rd
Hallway Ave
Silverado Ave
Killdeer Rd
Kringlet Ave
Shamberger Ct
Coach Way
Hazel Ave
Gassaway Rd
McKinleyville Ave
Wilbur Ave
Springer Ave
Fortune St
Ladru Ave
Sand Pointe Dr
Kelly Ave
Boller Ave
Annie Ln
Daffodil Ave
Pine Ave
Murray Rd
Norton Creek at Hwy 101
SWAMP ID 109NR1488
Sampling Location
Miles
0
0.0425
0.085
0.17
CALIFORNIA
Water Boards

Appendix 9: Approval Signatures

Appendix 9: Title and Approval Sheet

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

Steve Butkus 26 Oct 2015

Signature Date

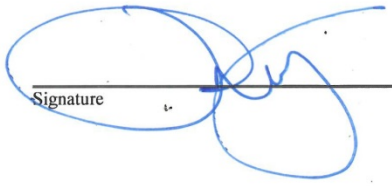
Appendix 9: Title and Approval Sheet

Bryan McFaden, Contract Manager
North Coast Regional Water Quality Control Board

Signature 10/26/15
Date

Appendix 9: Title and Approval Sheet

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



Signature

10-26-15

Date

Appendix 9: Title and Approval Sheet

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

Renée Spears — 10.19.2015

Signature — Date

Appendix 9: Title and Approval Sheet

Contract Laboratory Director

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

Signature 10/27/2015
Date

Appendix 9: Title and Approval Sheet

Contract Laboratory Director

Jeremy Corrigan, Humboldt County Public Health Laboratory, Lab Manager
County of Humboldt

Signature 10-26-15 Date