TOTAL MAXIMUM DAILY LOAD FOR BACTERIA IN BALLONA CREEK, BALLONA ESTUARY, AND SEPULVEDA CHANNEL

COORDINATED MONITORING PLAN FOR DEL REY LAGOON

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APPENDICES

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LIST OF ACRONYMS

ALERT	Automatic Local Evaluation in Real-Time
APHA	American Public Health Association
AWWA	American Water Works Association
BCB	Ballona Creek Bacteria
CA DOHS	California Department of Health Services
Cal/OSHA	California Office of Occupational Safety and Health Administration
CalTrans	California Department of Transportation
CA DOHS	California Department of Health Services
CFU	Colony Forming Unit
CHP	Chemical Hygiene Plan
CMP	Coordinated Monitoring Plan
COC	Chain of Custody
CSV	Comma Separated Value
CWA	Clean Water Act
DMR	Discharge Monitoring Report
ELAP	Environmental Laboratory Accreditation Program
EMD	Environmental Monitoring Division (LA City)
EPA	Environmental Protection Agency
GIS	Geographic Information System
ICSD	Information and Control System Division (LA City)
LREC-1	Limited Water Contact Recreation
LACDPW	Los Angeles County Department of Public Works
LACSD	Los Angeles County Sanitation Districts
LARWQCB	Los Angeles Regional Water Quality Control Board
LIMS	Laboratory Information Management System
MPN	Most Probable Number
NIST	National Institute of Standards and Technology
NPDES	National Pollutant Discharge Elimination System
PE	Performance Evaluation
QA/QC	Quality Assurance/Quality Control
REC-1	Water Contact Recreation
REC-2	Non Contact Recreation
RPD	Relative Percentage Difference
SOP	Standard Operating Procedure
S & T	Status and Trends Monitoring Program (LA City)
TMDL	Total Maximum Daily Loads
USC	University of Southern California
WLA	Waste Load Allocation
WP	Water Pollution
WPCF	Water Pollution Control Federation
WPD	Watershed Protection Division (LA City)

1.0 EXECUTIVE SUMMARY

The Ballona Creek, Sepulveda Channel, and Ballona Estuary were listed on the State's 1998 303(d) list as impaired due to exceedances of total and/or fecal coliform water quality standards. To address the high bacteria concentrations in the Creek and its tributaries, the Los Angeles Regional Water Quality Control Board (Regional Board) on June 8, 2006, adopted the Total Maximum Daily Loads for Bacterial Indicator Densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel (BC Bacteria TMDL). The Bacteria TMDL subsequently became effective on April 27, 2007 after approval by the U.S. Environmental Protection Agency (US EPA). This TMDL has multi-part numeric targets based on the updated bacteria objectives for marine and fresh waters designated for contact recreation (REC-1), and fresh waters with Limited REC-1 and REC-2 beneficial use designations.

The City of Los Angeles (CLA) is required to submit a comprehensive bacteria water quality monitoring plan for Del Rey Lagoon (Coordinated Monitoring Plan or CMP) to the Regional Board for approval on April 27, 2008, 12 months after the effective date of the Bacteria TMDL. This document serves to satisfy this requirement.

1.1 Coordinated Monitoring Plan Development

The CMP was developed by the City of Los Angeles (CLA) Watershed Protection Division (WPD) and Environmental Monitoring Division (EMD). The CMP is designed to assess compliance with the load allocations for Del Rey Lagoon in the Bacteria TMDL to provide data to support re-evaluations that will be made when the Bacteria TMDL is scheduled for reconsideration four years after the effective date of the Bacteria TMDL.

The CLA began meeting in late 2007 to compile data and information necessary for completing the CMP.

1.2 Requirements of the Bacteria TMDL Coordinated Monitoring Plan

a) Ambient Monitoring

Ambient monitoring of water quality conditions will begin six months after the Regional Board's approval of the CMP and conclude at the first compliance deadline in each impaired reach and at the confluence of each tributary. As stated in the TMDL, ongoing monitoring efforts by the City of Los Angeles at Del Rey Lagoon may fulfill this requirement.

b) TMDL Effectiveness Monitoring

The TMDL effectiveness monitoring program shall be conducted to assess attainment of the allowable exceedances for Ballona Estuary at the connecting tide gate of Del Rey Lagoon.

If the number of exceedance days is greater than the allowable number of exceedance days the responsible agency shall be considered not to be attaining the TMDLs and/or assigned allocations (non-attaining). The CLA shall be deemed attaining if an investigation which includes accelerated sampling demonstrates that bacterial sources originating within the jurisdiction of the responsible agency have not caused or contributed to the exceedance. Del Rey Lagoon may also be considered for natural source exclusion if it's contributing bacteria loads are determined to be as a result of wild life in the area.

1.3 Sampling Location

The implementation of the Del Rey Lagoon CMP will begin six months following its formal approval by the Regional Board. Six months will allow time for the preparation of field sampling and data analysis as described in the CMP. To conform to the BC Bacteria TMDL requirements, one monitoring location, designated BCB-9 in this CMP, was selected at the connecting tide gate with Ballona Estuary (Table 1.1). Map of the sampling location for Bacteria TMDL CMP is shown in Figure 1. Monitoring stations BCB-1 to BCB-8 on the map are discussed and proposed in the Ballona Creek Bacteria CMP.

Table 1.1

Station ID	**BCB-9**
Station Name	DEL REY
Location	Del Rey Lagoon tide gate (at creek)
Historical ID & Agency	N/A
Creek Section	Estuary (tide gate)
Sampling Frequency	Weekly

1.4 Accelerated Sampling following Elevated Bacterial Levels (Exceedances)

For the summer dry-weather period and the winter dry-weather periods during effectiveness monitoring, accelerated sampling will be conducted as a result of single-sample exceedances. Locations monitored weekly will be subject accelerated

monitoring, at 48 hours after the initial bacterial exceedances and, if the 48-hr sample exceeds, sampling also will occur at 96 hours following the initial bacterial exceedances. All required indicator bacteria, not just the exceeding indicator, will be analyzed during accelerated testing.

1.5 Analytical Methodology

Samples will be tested for total coliforms, *E. coli*, and enterococcus whose presence indicates that enteric pathogenic microorganisms may also be present. Approved sampling and analytical procedures will be used as described in Appendices D and G. Sampling and analytical procedures are to be followed as specified in "Standard Methods for Examination of Water and Wastewater", 18^{th} – 20^{th} edition, 1992 – 1998 respectively, APHA, AWWA, WPCF, Washington, DC, and Microbiological Methods for Monitoring the Environment, Water and Wastes", EPA-600/8-78-017.

1.6 Data Management and Reporting

The City of Los Angeles will ensure compliance is met for data management and reporting. Electronically-formatted data will be archived and submitted to the Regional Board promptly after the data become available. CLA will also submit electronic copies of the monthly reports to the Regional Board.

2.0 INTRODUCTION

This monitoring proposal is submitted to fulfill the 12-month requirement for developing a Coordinated Monitoring Plan (CMP) for Del Rey Lagoon, which is a part of the Total Maximum Daily Loads for Bacterial Indicator Densities in Ballona creek, Ballona Estuary, & Sepulveda Channel (BC Bacteria TMDL). For reference, the Bacteria TMDL amendments to the Water Quality Control Plan, Los Angeles Region (Basin Plan) amendments can be found in Appendix K of this document. All TMDL documents, including the staff report, can also be found on the Los Angeles Regional Water Quality Control Board's (LARWQCB) website at http://www.waterboards.ca.gov/losangeles/.

2.1 Background

Federal regulations under the Clean Water Act (CWA) require states to develop a list of impaired waters and pollutants for which they are impaired, also known as the 303(d) List. The states must then establish the capacity of the water body to assimilate the impairing pollutants. This is done in the form of the pollutant TMDL, which defines that the water body can still receive pollutant loads up to the water quality objectives necessary to protect the designated beneficial uses. Waste Load Allocations (WLA) from point sources and load allocations (LA) from non-point sources must be reduced as needed according to the schedule set in the TMDL to meet TMDL compliance for the water body. The TMDL is incorporated as an amendment to the regional Basin Plan.

Ballona Creek, Ballona Estuary, and Sepulveda Channel were designated as impaired by the State of California and included on California's 2002 and 1998 CWA §303(d) List of Impaired Waters. The Del Rey Lagoon is considered to be potential a non-point source of high coliform count in Ballona Estuary. The potentially high bacterial loading of the Ballona Creek may result in impairments of the marine water contact recreation (REC-1) beneficial use of Ballona Estuary[1]. Recreating in waters with elevated bacterial indicator densities has long been associated with adverse human health effects.

Del Rey Lagoon is part of the Ballona Creek Watershed, which is the largest sub-watershed within the Santa Monica Bay watershed management area. The size of Del Rey Lagoon sub-watershed is approximately 24.5 acres and the responsible agency is the City of Los Angeles. Del Rey Lagoon Park, in which the lagoon is located, is managed by CLA Department of Recreation and Parks. Del Rey Lagoon is connected

[1] Ballona Estuary is from Centinela Avenue to the Pacific Ocean for 3.5 miles and its lower portion runs parallel to the main channel of Marina del Rey.

to Ballona Estuary by a tidal gate. The drainage area for Del Rey Lagoon is shown in ***Figure 2***.

2.2 Waste Load Allocation Targets

The Ballona Creek Bacteria TMDL has a multi-part numeric target based on four bacteriological parameters: total coliform density, enterococcus density, fecal coliform density, and *Escherichia coli* (*E. coli)* density. The density shall be reported as the number of bacteria counted in 100 milliliters of water sampled. These numerical targets and the corresponding waste load allocations have been set based on the Los Angeles Basin Plan objectives for water contact recreation (REC-1), limited water contact recreation (LREC-1), and water non-contact recreation (REC-2) uses.

The Ballona Creek Bacteria TMDL monitoring is divided into three separate periods for compliance purposes, each with specific requirements. The periods are summer dry-weather (April 1 to October 31), winter dry-weather (November 1 to March 31), and wet-weather days (defined as days of $\geq$ 0.1 inches of precipitation and the three days following the rain event).

2.2.1 Single Sample Limits

Del Rey Lagoon is a non-point source to the Ballona Estuary and must meet the REC-1 water quality objectives for marine waters at the confluence. The applicable single sample objectives are:

- Total coliform density shall not exceed 10,000/100ml
- Fecal coliform density shall not exceed 400/100ml
- Enterococcus density shall not exceed 104/100ml
- Total coliform density shall not exceed 1,000/100ml, if the ratio of fecal-to-total coliform exceeds 0.1

Single sample limits apply throughout the year. However, there are a certain number of exceedance days allowed for single sample targets for summer dry-weather, winter dry-weather, and wet-weather days (Table 2-1). The number of allowed exceedance days is established using a "reference system/anti-degradation approach," which is based on historical exceedance levels at existing monitoring locations, including a local reference beach within the Santa Monica Bay coastal watershed (Leo Carrillo State Beach):

Table 2.1. Summary of water quality objectives and allowable exceedences for Ballona Creek Tributaries.

Tributary	**Point of Application**	**Water Quality Objectives**	**Waste Load Allocation** (No**.** exceedance days)
Del Rey Lagoon	At confluence with Ballona Estuary	REC-1 Marine water	For single sample objectives: *(0) summer dry-weather, (3)winter dry-weather, (17) wet-weather* For geometric mean objectives: *(0) for all periods*

2.2.2 Rolling 30-day Geometric Mean Limits

The applicable Del Rey Lagoon geometric mean limits (REC-1 marine water) at the confluence with Ballona Estuary are:

- Total coliform density shall not exceed 1,000/100ml
- Fecal coliform density shall not exceed 200/100ml
- Enterococcus density shall not exceed 35/100ml

The 30-day geometric mean is defined as the 30^{th} root of the product of thirty data points over the most recent thirty days.

The Geometric Mean Limits may not be exceeded at any time. The rolling 30-day geometric means will be calculated on each day, regardless of whether a weekly or daily schedule is selected. If weekly sampling is conducted each test result will supersede the previous result and be assigned to the remaining days of the week until the next sample is collected.

The Rolling 30-Day Geometric Mean calculation may change. Based on recent communication with the Regional Board, we understand the method of calculating the daily Rolling 30-Day Geometric Mean proposed above has been deemed inappropriate and may be revised.

The TMDL will be implemented in two phases over a ten-year period. For the first phase, within six years of the effective date of the TMDL, the allowable number of summer dry-weather, winter dry-weather exceedance days, and the dry-weather rolling 30-day geometric mean targets must be achieved. For the second phase, within 10 years of the effective date of the TMDL, the allowable number of wet-weather exceedance days and rolling 30-day geometric mean targets must be achieved.

2.3 Coordinated Monitoring Plan Development

The CMP for Del Rey Lagoon was developed in parallel with the CMP for Ballona Creek, Ballona Estuary and Sepulveda Channel with the exception that the CLA was the sole responsible agency. This CMP was prepared by the City of Los Angeles staff from the Watershed Protection Division (WPD) and Environmental Monitoring Division (EMD). Contact information of responsible personnel for the CMP is shown in Appendix M.

2.4 Requirements of Coordinated Monitoring Plan

The Ballona Creek, Ballona Estuary, and Sepulveda Channel Bacteria TMDL require that within 12 months of the effective date:

"Responsible jurisdictions and responsible agencies must submit, for Regional Board approval, a comprehensive bacteria water quality monitoring plan for the Ballona Creek Watershed. The plan must be approved by the Executive Officer before the monitoring data can be considered during the implementation of the TMDL. The plan must provide for analyses of all applicable bacteria indicators for which the Basin Plan and subsequent amendments have established objectives. The plan must also include a minimum of two sampling locations (mid-stream and downstream) in Ballona Estuary, Ballona Creek (Reach 1 and 2) and their tributaries."

Resolution 2006-11 does not specify requirements for monitoring at Del Rey Lagoon other than that *"Similar monitoring at the connecting tide gates of Del Rey Lagoon is also required."* The Resolution also states that *"The responsible agency must submit, for Regional Board approval, a comprehensive bacteria water quality monitoring plan for inputs from Del Rey Lagoon to the Ballona Estuary."*

3.0 MONITORING SITE

Since Del Rey Lagoon is a non-point source to Ballona Estuary and the point of application of the Load Allocation is at the confluence (Resolution 2006-011), this CMP proposes one (1) monitoring location at the connecting tide gate in Ballona Estuary (outside of Del Rey Lagoon).

Further information about the proposed monitoring is provided in Table 3.1, Figures 1 and 2 and Appendix B.

Table 3.1 Summary of monitoring site

Station ID	**BCB-9**
Station Name	DEL REY
Location	Del Rey Lagoon tide gate (at creek)
Historical ID & Agency	N/A
Creek Section	Estuary (tide gate/confluence)
Sampling Frequency	Weekly
Status	New
Comments: This is a new sampling site located at the tide gates connecting Del Rey Lagoon and Ballona Estuary. The nearest street is Pacific Avenue. Refer to Thomas Guide coordinates, 702:A3	

Figure 1. Ballona Creek Watershed and monitoring site locations.

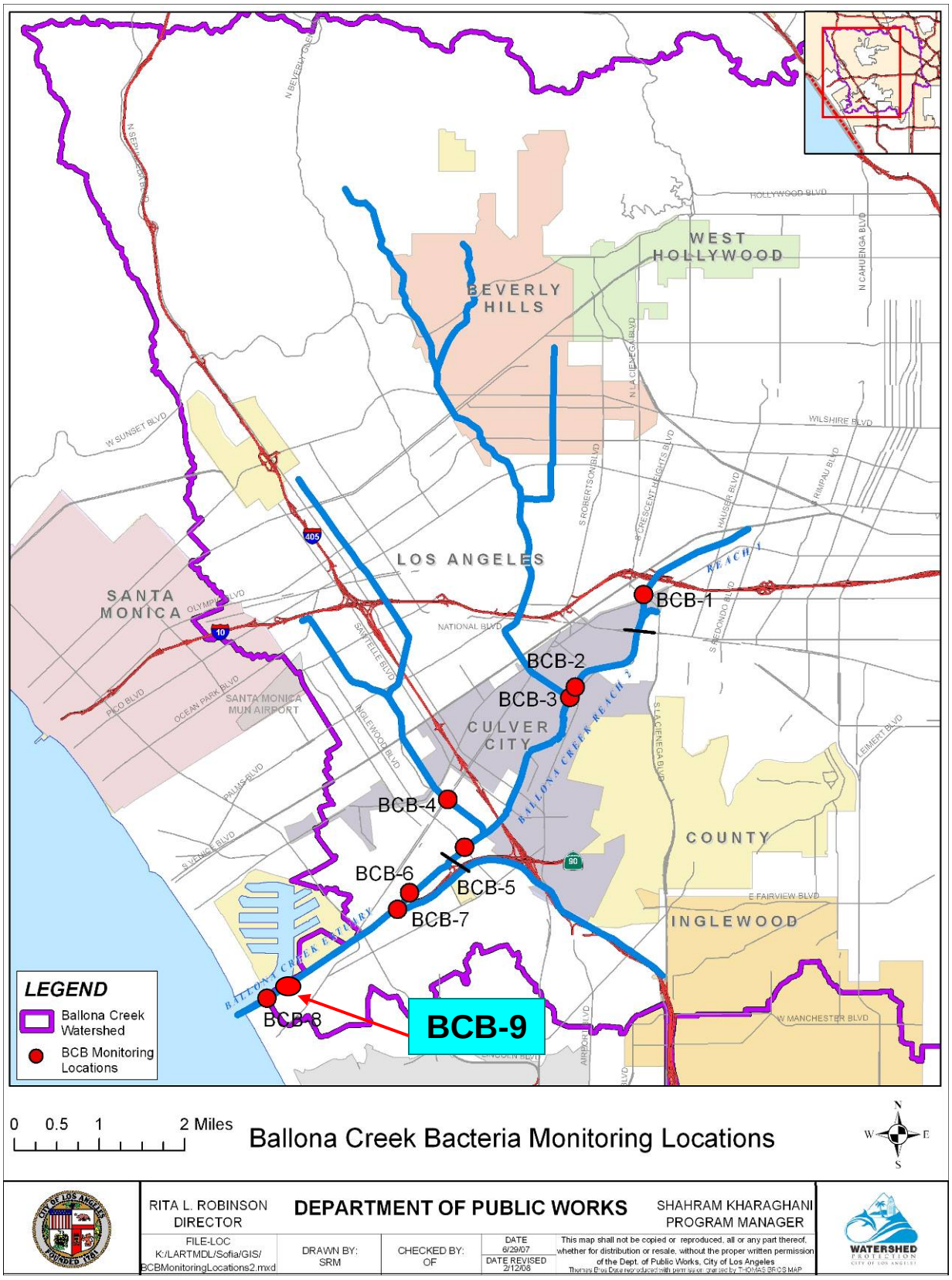

Figure 2. Del Rey Lagoon drainage area.

4.0 MATERIALS AND METHODS

This section provides methodologies for conducting field sampling, procedures for bacterial analyses of the samples and data reporting procedures. Sampling of Del Rey Lagoon at the confluence with the Ballona Creek Estuary will be done concurrently with sampling of Ballona Creek and Estuary as described in the Ballona Creek, Ballona Estuary, and Sepulveda Channel TMDL Coordinated Monitoring Plan.

4.1 Sampling Schedule

During the ambient monitoring period, proposed monitoring location will be monitored weekly with sampling to be conducted on Mondays. When effectiveness monitoring begins, if there are exceedances in the weekly sampling during summer and winter dry weather, accelerated monitoring will be conducted Accelerated samples will be collected 48 hours after the original bacterial exceedances, and if the 48-hour sample also exceeds, 96 hours after the initial bacterial exceedances. Sample collection and analysis will be done by the City of Los Angeles Environmental Monitoring Division (EMD).

4.2 Sampling Procedures

The objective of the sampling program is to provide a representative water sample for bacterial analyses, while following defined safety and quality assurance guidelines. The quality assurance guidelines shall include sampling protocol as well as sample documentation, preservation, and holding time requirements. All contracted samplers or agencies (EMD and WPD) shall submit Standard Operating Procedures (SOPs) for review by Regional Board staff, which is done here in appendices C, D, and L. The SOPs shall specify safety considerations, sampling protocol, and quality assurance guidelines. Appendix C (Field Sampling Equipment and Supply List), Appendix D (Field Sampling SOP), and Appendix L (Laboratory Safety) are EMD's and WPD's protocols for this TMDL.

Each sampling event shall be associated with recorded observations of site conditions, which should minimally include sample ID, collection date and time, air and water temperature weather conditions including rain measurement, sample characteristics (water color and turbidity), and sampler's name (refer to Appendix E).

4.2.1 Procedures for missed samples

For missed samples due to inaccessibility, when sample integrity is compromised, or the scheduled sampling day falls on a holiday, the site should be revisited and sampled on the earliest convenient day, except Sunday, within the week of the originally scheduled sampling date.

4.2.2 Procedures during Rainfall Events

During any rain event, field staff must use his/her best judgment to determine if sampling can be performed safely. In the 24 hours following a rain event resulting in greater than 0.5" of precipitation the safety of the sample collector is paramount and sampling may need to be rescheduled to a different time and/or day.

4.2.3 Procedures following Elevated Bacterial Levels (Exceedances)

During effectiveness monitoring, the City of Los Angeles (EMD) will conduct accelerated sampling and testing 48 hours after the initial bacterial exceedances and, if necessary, additional accelerated testing at 96 hours for those sites that still exceed the single sample limits after 48 hours. All three indicator bacteria will be analyzed during accelerated monitoring, (total coliform, fecal coliform and enterococcus).

The purpose of the accelerated monitoring is to identify the persistence of an exceedance, especially during dry weather when source identification is a priority. Accelerated monitoring may not be as critical during wet weather when the source of the exceedance is known to be stormwater runoff, therefore, accelerated testing during wet weather will not be considered until the 4th year during the re-opener.

4.2.4 Equipment

Equipment and supplies needed for sample collection are listed in Appendix C.

4.2.5 Safety

In an effort to improve employee safety and health awareness, and to prevent occupational related injury and illness, EMD and other participating laboratories have developed a safety program with the intention of satisfying the applicable federal, state, and local regulations. For example, EMD's Safety and Health Program is composed of specific elements required by Cal/OSHA General Industry Safety Order Section 5191: Occupational Exposure to Hazardous Chemicals in Laboratories, and section 3203: The Injury and Illness Prevention Program, and any other applicable regulations. The written safety plan, titled ***The Chemical Hygiene Plan***, is available to all employees for review, and should be recognized as management's commitment to ensure that all employees carry out their work in the safest and most efficient manner possible. EMD employees will be kept familiar with the Division's written Chemical Hygiene Plan (CHP) through training, annual review and monthly staff safety meetings.

It is the City's policy to provide a safe working environment for all of their employees. In addition, all field and laboratory work is to be performed in a manner that provides the highest level of safety for the protection of every employee. See Appendix L for detailed safety protocols.

4.3 Analytical Methodology

Marine/brackish samples collected from Del Rey Lagoon will be tested for the presence of total coliform, E. coli, and enterococcus bacteria. All indicator groups will be quantified from a single sample collected at the designated monitoring site. Necessary dilutions or aliquot volumes shall be processed to ensure that compliance with water quality objectives can be determined. Bacterial results will be reported as the number of organisms per 100 mL of sample for each bacterial indicator. When selecting analytical bacterial methods for TMDL monitoring, the importance of fast recovery times (24 hours or less) should be emphasized. The presence of total coliform, E. coli, and enterococcus bacteria shall be detected and quantified using the defined chromogenic substrate (CS) method (SM 9223B, APHA 1992-98). Fecal coliforms shall be detected and quantified using the membrane filtration (MF) method (SM9221C APHA 1992-98).

The CS method will be used to convert the E. coli result to fecal coliform using a 1:1 translator. The application of a 1:1 translator was approved by the Regional Board in October 2002 after review of the Chromogenic Substrate and Membrane Filtration Comparison Study conducted by the City of Los Angeles (approval letter dated October 16, 2002, from Dennis Dickerson, Executive Officer).

All laboratories performing analyses for TMDL bacterial monitoring shall maintain Environmental Laboratory Accreditation Program certification (ELAP administered by California Department of Health Services) for specified methods from ELAP's "Field of Testing 126: Microbiology of Recreational Water". Additionally, all laboratories shall submit detailed SOPs for review by Regional Board staff. Appendix G provides an example of an SOP developed by the City of Los Angeles-EMD. Each analytical method used for the TMDL monitoring program shall be an approved EPA or Standard Methods for the Examination of Water and Wastewater, 18^{th}-20^{th} edition (APHA 1992-98) method. Laboratories receiving Regional Board approval may use other analytical bacterial methods for marine recreational and TMDL monitoring.

4.3.1 Quality Assurance/Quality Control

The EMD Microbiology Laboratory must employ a program that associates quality assurance with the laboratory facility, staff, instrumentation and equipment, materials and methods, media and reagents, and data validation. These QA/QC measures may be included in the submitted SOPs or defined in a separate QA/QC document such as Appendix I. The quality assurance procedures shall be in accordance with Standard Methods for the Examination of Water and Wastewater, 18-20^{th} Editions (APHA 1992-98). The laboratory must maintain ELAP certification, provide QA/QC documentation as required by the Regional Board, and participate in periodic inter-calibration exercises.

Data from the EMD Microbiology Laboratory will be utilized to comply with the monitoring requirements of the Ballona Creek Bacterial TMDL. The EMD and WPD

divisions will participate in this monitoring program as processing and sampling agencies, respectively.

4.4 Data Management and Reporting

4.4.1 Data Tabulation

Results will be entered into Excel spreadsheets that automatically compute results (MPN/100 mL for CS analysis). All entered data will be given secondary review and corrected as needed, to ensure error-free data entry. Examples of EMD's Microbiology Laboratory data worksheets can be found in Appendix E. Data acquisition, validation, reduction, and reporting procedures can be found in Appendix H.

4.4.2 Data Format and Archive

All data collected will be archived within the City of Los Angeles' **Laboratory Information Management System** (LIMS) database or comparable database. The City of Los Angeles' **Information & Control System Division** (ICSD) staff will ensure electronic submissions of data are parsed and stored correctly into the LIMS database.

4.4.3 "Wet Weather" Determination

The Bacteria TMDL defines "wet weather" as "days with 0.1 inch of rain or greater and the three days following the rain event" (Attachment A to Resolution No. 2006-011, Page 4); however, the TMDL does not specify where the 0.1-inch of rain is to be measured. For clarification, the City of Los Angeles proposes in Table 4.1 the rainfall gage to be used for this monitoring program to determine wet weather days.

Table 4.1. Rainfall gage to be used for the proposed monitoring program.

Jurisdictional Group	Rainfall Gage	Comment
Ballona Creek Watershed	University of Southern California (USC) (375)	LACDPW "ALERT" Station

The proposed gage is a Manually Observed Non-Mechanical Rain Gauge station owned and operated by the County of Los Angeles. Data can be obtained at http://www.ladpw.org/wrd/precip/ under "Station Information." The web page displays 148 Manually Observed Non-Mechanical Rain Gages; these gages are measured once daily by volunteers and reported monthly.

4.4.4 Exceedance Determination and Accelerated Sampling

Bacteriological data will be summarized in tabular form on a daily basis by EMD's Microbiology Unit. Exceedances will be clearly notated and triggers indicating "accelerated monitoring needed" will be programmed into the report. Summer dry-

weather, winter dry and wet weather spreadsheets with triggers will be created, but as mentioned in section 4.2.3 wet weather accelerated sampling will not be conducted until the 4th year reopener. When bacterial levels no longer exceed listed standards, a trigger to return to weekly sampling will be programmed, except for wet weather exceedences which will continue regular sampling regardless of exceedences until the 4th year reopener.

The monitoring agency, the City of Los Angeles, will be responsible for performing accelerated monitoring as required. For the purpose of compliance, this CMP proposes that for single-sample limits, accelerated sampling results should not be counted towards compliance.

4.4.5 Data Reporting

Electronically-formatted data will be archived and submitted to the Regional Board promptly after the data becomes available. Monthly data summary reports will be submitted to the LARWQCB by the last day of each month for data collected during the previous month.

For EMD, laboratory results will be entered into Microsoft Excel spreadsheets that automatically compute results (MPN/100 mL). The City of Los Angeles will archive their own data within LIMS or a comparable database. See Appendix H, Data Acquisition, Reduction, Validation, and Reporting Procedures.

REFERENCES

American Public Health Association. 1992. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington, DC, pp. 9-1 to 9-115.

California Coastal Commission. Staff Report TH 17b. May 16, 2007.

Noble, R. T., J. H. Dorsey, M. K. Leecaster, M. Mazur, C. D. cGee, D. Moore, V. Orozco-Borbón, D. Reid, K. Schiff, P. M. Vainik, and S. B. Weisberg. 1999. Southern California Bight 1998 Regional Monitoring Program. I. Summer Shoreline Microbiology. Appendix C, comparison of Bacterial Indicator Measurements among Southern California Marine Monitoring Laboratories. Southern California Coastal Water Research Project. Westminster, CA, 54-67.

TMDL Draft. The following TMDL drafts are cited in this report:

Total Maximum Daily Load to Reduce Bacterial Indicator Densities during Dry Weather at Santa Monica Bay Beaches—January 14, 2002.

Santa Monica Bay Beaches Wet-weather Bacteria TMDL Draft—Version 4. 11/07/02

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APPENDICES

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Appendix A

Development History of the Ballona Creek Bacteria TMDL

The Ballona Creek Bacteria TMDL was developed by the Los Angeles Regional Water Quality Control Board (RWQCB) to protect beneficial uses associated with water quality in Ballona Creek. Elevated concentrations of bacteria at Ballona Creek have prompted the development of a TMDL. The effective date of the TMDL is April 27, 2007.

Ballona Creek is a ten-mile long channel that begins in Hancock Park and drains into the Pacific Ocean just south of Marina del Rey. The Ballona Creek Watershed totals about 130 square miles. Ballona Creek has 3 segments (or reaches) divided based on the physical characteristics and beneficial use designations: Reach 1 is REC-2, Reach 2 is Limited REC-1, and the Estuary is REC-1. These three segments and Sepulveda Channel are listed as impaired and require a TMDL.

This bacteria TMDL addresses the impaired bacterial water quality of the creek and sets waste load allocations (WLAs) or enforceable bacteria concentration limits in the creek. Affected agencies will be required to reduce the amount of bacteria flowing in Ballona Creek, the Estuary, and Sepulveda Channel.

The watershed is highly developed, consisting of residential, recreational/open space, commercial and industrial land uses. The watershed also includes all or parts of the Cities of Beverly Hills, Culver City, Inglewood, Santa Monica, West Hollywood, and unincorporated Los Angeles County.

The TMDL contains a 14-year compliance schedule to correspond with the Santa Monica Bay Beaches Bacteria TMDL schedule for meeting 100% compliance of waste load allocations (WLAs). The TMDL will be re-evaluated 4 years after the effective date for WLAs based on results of required special studies from year 1 to 3.

A comprehensive bacteria water quality monitoring plan for inputs from Del Rey Lagoon to the Ballona Estuary is also required one year after the effective date.

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APPENDIX B

Ambient/Effectiveness Monitoring Sites

Table B.1 Ambient/Effectiveness monitoring sites for Ballona Creek, Ballona Estuary, and Sepulveda Channel Bacteria TMDL

Station ID	**BCB-9**
Station Name	**Del Rey Lagoon**
Location	**Tide gate**
Historical ID & Agency	**N/A**
Creek Section	**Estuary (confluence)**
Sampling Frequency	**Weekly**

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APPENDIX C

Field Sampling Equipment and Supply List

The following equipment is needed for dry weather water quality sample collection.

1. First Aid kit
2. Portable eyewash bottle with saline solution
3. Ice chest (with ice)
4. Sampling pole with reel
5. Weighted bottle holder (attaches to fishing line/reel)
6. Sterilized polypropylene 125 mL bottles with 1% Sodium Thiosulfate (necessary for freshwater/stormwater samples)
7. Wash bottle with de-ionized water
8. Foaming disinfectant hand cleanser
9. Waterproof labels
10. Paper towels
11. Water-safe pen and Lab marker
12. Field log sheet
13. Chain-of-Custody (COC) sheet
14. Thomas Guide (street map)/Electronic Navigation System
15. Portable camera
16. Trash bag
17. Cell phones (1 for each person)
18. Watch
19. Personal protective equipment:
    i. Safety vest (ANSI 107 Class 2 compliant, high visibility)
    ii. Protective gloves (latex, nitrile, etc.)
    iii. Slip-resistant shoes/boots
    iv. Protective eyewear: UV protection; impact resistant
    v. Foul weather gear (when necessary)
    vi. Rain boots (when necessary)
    vii. Life vest (if entering the flood channel).
    viii. Rope (to be used if the sampler accidentally slips or falls from the slope)
    ix. Flash light (to be used during foul weather)

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APPENDIX D

Field Sampling Standard Operating Procedures

Water Quality Sampling

Overview of Procedure

At the beginning of each month, Watershed Protection Division (WPD) Field Sampling staff provides concern Laboratory Staff section at the Environmental Monitoring Division (EMD) with a sampling schedule detailing when sampling will be conducted, and the number of samples to be delivered. As requested by WPD/PAS, EMD(Environmental Management Division) Sample Receiving Section personnel provides The Laboratory supplies Field Sampling staff with clean, sterile polypropylene bottles with the necessary additives in advance of the sampling schedule. Sample bottles are labeled and Chain-of-Custody (COC) sheets are prepared prior to going out to the field. All necessary gear, including personal protective equipment, must be brought to the field. Grab samples are collected at designated sampling stations. , and the time and date of collection, sampling locations, project name, analysis required, sampler's name or any observation comments or remarks are recorded on the COC and Field Log sheets. Samples are then stored on ice while at the sampling site to keep the sample temperature at 4ºC. , Sample temperature are checked and recorded by EMD/Sample Receiving Personnel staff before receiving. The quantity, sample label and other detailed information are also being checked for completeness against the Chain of Custody (COC). A copy of COC with EMD/Sample Receiving staff signature will then be given to WPD field sampling staff before leaving the Receiving section after all the requirements are completed. and temperature readings are then taken and recorded at EMD laboratory staff, EMD/ Sample Receiving is located at 5th floor, Pregerson Building at Hyperion Treatment Plant. The samples are and then delivered to EMD/Microbiology Laboratory section at the Environmental Monitoring Division at the 4th floor of Pregerson Building at Hyperion Treatment Plant. Laboratory staff will need to make sure the samples are received within the maximum allowable holding time of six hours, from the time of collection to the time of receipt by EMD/DSM Laboratory staff. The original COC sheet is signed, dated, and noted as to the time the samples are received and given to EMD staff. The Field Sampling crew retains a copy of the COC.

NOTE: Do we need to mention the detail handling of samples while at EMD. I notice the title says "Field Sampling" SOP. I think we can just stop after WPD/PAS field sampling staff received the COC from EMD/Sample Receiving. Please comment on this. Thank you.

II. Sampling Procedure:

A. Coordination with Laboratory

At the beginning of each month, the monthly sampling schedule is sent via email to the supervisor of the Microbiology Section at EMD. If unforeseen changes are made to the schedule, EMD staff is notified immediately. Contact name is listed below:

Microbiology Section:

Water Microbiologist III

Supervisor: Ioannice Lee

Phone: (310) 648-5196

Email: Ioannice.Lee@lacity.org

B. Gather the necessary equipment:

See Appendix C

C. Sampling Locations/Frequency:

Station ID	BCB-9
Station Name	Del Rey Lagoon
Location	Tide gate
Historical ID & Agency	N/A
Creek Section	Estuary (confluence)
Sampling Frequency	Weekly

* Nearest major cross street to sample site. See provided GPS coordinate for actual sampling site.

D. Field Log sheet.
A field log sheet is provided in Appendix E. This form is for recording details about each sampling event (including date, time, locations, samplers, comments), and is retained by the sampling staff. The form is to be prepared before leaving to the field, and the appropriate information is filled out after each sample is collected.

E. Chain of Custody (COC) form.
A COC form is to be completed for each sampling event. The form should be prepared prior to leaving to the field. At each sampling station, the sampler enters his/her initials, along with time of collection. The original COC is to follow the samples at all times. The sampler must sign and date the COC when relinquishing the sample to Laboratory Staff (Sample Receiving, EMD) who in turn, signs the form to indicate receipt of the sample. A photocopy is given to the sampling staff, and the laboratory retains the original COC along with the samples to be analyzed. A blank COC is provided in Appendix E.

F. Collecting Samples
When sampling from a bridge, a fishing pole/reel is used to lower the sample bottle into the stream.
   a. Obtain clean and sterile polypropylene bottles with Sodium Thiosulfate (necessary for freshwater/stormwater samples). Confirm that the bottle has the appropriate pre-printed label.
   b. Note the sample collection time on the Field Log sheet, COC, and sample label.

c. Be very careful to avoid contamination of the sample bottle. Wear clean gloves and avoid touching the mouth of the bottle and the inside of the cap.
d. Attach the bottle-holder to the fishing line, and secure the bottle. Unscrew the bottle lid, and set it aside. Release the drag on the reel, and lower the bottle into the stream. Allow the bottle to fill with water (leave 0.5 – 1 inch of headspace for later mixing of the sample in the laboratory), and then reel it in. Replace the lid securely, and place the sample into the ice chest.
e. Rinse bottle holder with de-ionized water after each station.
f. Fill in appropriate information on the COC and field log sheet.
g. Samples should be delivered to the laboratory as soon as possible. When relinquishing custody of the samples, inform Laboratory staff of the start time of the six hour holding time limit for the samples. Laboratory staff will read the sample temperature, sign and date the COC, and make a copy of the COC for field staff to keep.
h. Upon returning from the field, file the COC (copy) and field log sheet in the appropriate binder. Rinse all field equipment with de-ionized water.

III. Contact Information:

Laboratory:
Environmental Monitoring Division
Microbiology Section
Supervisor: Ioannice Lee
Phone: (323) 648-5196

Email: Ioannice.Lee@lacity.org

TMDL Implementation Section:
Senior Engineer: Reza Iranpour, Ph.D., P.E.
Phone: (213) 485-0577
Email: Reza.Iranpour@lacity.org

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APPENDIX E

Field and Data Entry Worksheets

Examples of worksheets for Chain of Custody sheets (2 pages) and recording analytical results used by the City of Los Angeles' Watershed Protection Division and Environmental Monitoring Division are provided herein. They include field observations, Chromogenic Substrate data entry, and Membrane Filtration data entry. Once completed, data are then entered into the LIMS database.

Field and data entry worksheet includes: Watershed Protection Division Field Collection Sheet, Environmental Monitoring Division Chain of Custody, and Environmental Monitoring Division/Microbiology Group Data Entry Sheet : Data Sheet for Quantitray Counts using the Chromogenic Substrate Method and Data Sheet for Bacterial Densities using Membrane Filtration Method.

As soon as all these documents are completely filled up, bacterial counts from the data worksheets are entered into LIMS database. The final bacterial densities of each samples are automatically calculated through the system. A report is generated after the results are validated and the final results are sent to L.A. County Health Department electronically.

CITY OF LOS ANGELES – DEPARTMENT OF PUBLIC WORKS – BUREAU OF SANITATION

WATERSHED PROTECTION DIVISION – SECTION

FIELD COLLECTION SHEET

Program:_____

Sampling Date: _____

STATIONS	STATION CODE	STAFF INITIALS	COLLECTION TIME	TEST REQUESTED	SAMPLE ID	COMMENTS (Weather, Water condition,…)

CHAIN-OF-CUSTODY

Relinquished by: (signature)	Print Name	Date/Time	Accepted by: (signature)	Print Name	Date/Time

Email results to:

REVISED DATE

Date:

Department of Public Works
Bureau of Sanitation
Environmental Monitoring Division

Sample Chain of Custody

EMD
LIMS #: _____

EMD Sample ID: _____
Project Name: _____

Sampling Information:
Sampling Agency: _____
Agency Sample ID#: _____
Phone Number: _____
Fax Number: _____
Contact Person: _____
email address: _____

Sampler's Name: _____
Sampler's Title _____

Sampler's Signature: _____

Witness: Name _____
Title _____

Name _____
Title _____

Sample Location: _____

Sampling Program: _____

Purpose of program: _____

Report Time Frame: _____

Sample Date: _____

Sampling Time: _____

Sampling Address: _____

Requested Analysis:
Metals: ☐
Organics: ☐
Conventional Chemistry: ☐
Micro Biological: ☐
Toxicity: ☐
Air Testing: ☐
See back of page for specifics analyses

Sample Notification:

PC: _____ Date: _____

Wet: _____ Date: _____

Micro: _____ Date: _____

Toxicity: _____ Date: _____

Metals: _____ Date: _____

Semi-Vol: _____ Date: _____

Volatile: _____ Date: _____

Current Holder Name	Signature	Title	Received Date	Received Time	Released Date

Analysis to be performed on the Sample(s):

EMD
LIMS #: _____

	Locator:	Collection Time:		Locator:	Collection Time:
-1			-6		
-2			-7		
-3			-8		
-4			-9		
-5			-10		

Sample Information: Liquid: ☐ Solid: ☐ Other: ☐ Temperature _____
Grab ☐ Composite: ☐
Start time: _____ Finish time: _____ pH _____
Container: Glass Size: _____ Color: _____ Number: _____
Plastic Size: _____ Color: _____ Number: _____ Residual Cl2 _____
Preservative ☐ Number of samples: ☐

Metals:

☐ Ag	☐ Cu	☐ Pb	☐ Other: _____	
☐ Al	☐ Fe	☐ Sb		
☐ As	☐ Hg	☐ Se		
☐ Ba	☐ K	☐ Sn		
☐ Be	☐ Mg	☐ Sr	☐ Total	
85 ☐ Ca	☐ Mn	☐ Tl	☐ Dissolved	
☐ Cd	☐ Mo	☐ V		
☐ Co	☐ Na	☐ Zn		
☐ Cr	☐ Ni			

Organics:

☐ VOC	☐ Pesticides/PCB	☐ Clopyralid	☐ Air VOC
☐ BNA	☐ Dioxin - screen	☐ Dioxin - low resolution	☐ Fixed Gases
☐ TOX	☐ Other: _____	☐ Dioxin - high resolution	☐ GC Sulfur
☐ Herbicides		☐ Tributyltin	☐ Siloxanes

Conventional Chemical:

☐ Alkalinity	☐ MBAS	☐ Solids:
☐ BOD	☐ Nitrogen:	☐ Total Solids
☐ Boron	☐ Ammonia Nitrogen	☐ Total Dissolved Solids
☐ Chloride	☐ Nitrate-N	☐ Total Suspended Solids
☐ COD	☐ Nitrite-N	☐ Settleable Solids
☐ Conductivity	☐ Organic-N	☐ Volatile Suspended Solids
☐ Cyanide (Free)	☐ Kjeldahl Nitrogen	☐ Volatile Total Solids
☐ Cyanide (Total)	☐ Oil & Grease	☐ Sulfates
☐ Flashpoint	☐ pH	☐ Sulfides, Total
☐ Fluoride	☐ Phenols	☐ Sulfides, Dissolved
☐ Grain Size	☐ Phosphate, Total	☐ Thiosulfate
☐ Hardness	☐ Phosphate, Dissolved	☐ TOC
☐ Hexavalent Chromium	☐ Radioactivity	☐ Turbidity
☐ H_2S	☐ Salinity	☐ Other:

Biological:

☐ Total Coliform	☐ Salmonella	☐ Other: _____
☐ Fecal Coliform	☐ Acute Toxicity (Fresh water)	_____
☐ E. coli	☐ Chronic Toxicity (Sea water)	
☐ Enterococcus	☐ Chronic Toxicity (Fresh water)	

Remarks: _____

Environmental Monitoring Division
Microbiology Unit
Chromogenic Substrate Bacteria Densities

Date: _____ Read by: _____ Time: _____

TOTAL COLIFORM

Station	BCB-9		
10 mL		Blank 100 mL	
Large cells			
Small cells			
1 mL		Blank 100 mL	
Large Cells			
Small Cells			

E. COLI

Station	BCB-9		
10 ml		Blank 100 mL	
Large cells			
Small cells			
1 mL		Blank 100 mL	
Large Cells			
Small Cells			

ENTEROCOCCUS

Station	BCB-9		
10 ml		Blank 100 mL	
Large cells			
Small cells			
1 mL		Blank 100 mL	
Large Cells			
Small Cells			

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APPENDIX F

Laboratory Equipment and Supply List

Chromogenic Substrate Method

- **Materials and Equipment**

  - Sterile, transparent, non-fluorescent plastic container - 125 mL volume (use containers provided by Colilert kit if available)
  - Colilert-18 reagent packets
  - Enterolert reagent packets
  - Quantitray/2000 trays
  - Graduated cylinder, sterile - 100 mL (optional)
  - Quantitray/2000 rubber tray insert
  - UV cabinet or lamp - long wave, 366nm
  - Deionized water – sterile
  - Colilert Quantitray/2000 color/fluorescence comparators
  - 70% Ethanol
  - Paper Towels
  - 1 L Erlenmeyer flask
  - 1 mL, 2 mL, 10 mL sterile, disposable pipettes
  - Pipette bulb or automatic pipet-aid
  - Laboratory marking pen
  - Datasheets
  - 35.0 ± 0.5ºCand 41.0 ± 0.5ºC Incubator

Membrane Filtration Method

- **Materials and Equipment**

  - Plate Labeling
    - Indelible marking pen
    - Kimwipes
    - Prepared mEndo, mFC, and mE agar plates
    - Agar plate carrier with dark cover
  - Filtration
    - 1 mL and 10 mL sterile, bacteriological or Mohr disposable pipets
    - Pipet biohazard container
    - Large biohazard container
    - Vacuum pump
    - Filtration manifold

- Microfil vacuum support base
- Microfil filter screen disc (in 95% alcohol jar)
- Sterile, disposable Microfil funnels
- Membrane filters - sterile, white, grid-marked, 7mm diameter filters with 0.45µM pore size
- Labeled mEndo, mFC, and mE agar plates in covered plate carrier
- Alcohol lamp
- 95% and 70% ethanol
- Glass safety jar with lid
- Paper towels
- Sterile, plastic squirt bottle
- Forceps - smooth-tipped stainless steel
- Pipet bulb/automatic pipettors
- Alcohol pads
- Incubator, $35.0 \pm 0.5^{o}C$
- Water bath, $44.5 \pm 0.2^{o}C$
- Incubator, $41.0 \pm 0.5^{o}C$
- Solid heat-sink fecal coliform incubator, $44.5 \pm 0.2^{o}C$
- Matches
- Long-handled forceps
- Sterile, phosphate-buffered rinse water
- Sterile, phosphate-buffered water dilution tubes
- Tupperware w/ wet sponge

o Colony Counting
  - Binocular, stereoscopic microscope with fluorescent lamp
  - Disposable gloves
  - Data worksheets
  - Large biohazard container
  - Incubated mEndo, mFC, and mE agar plates
  - EIA agar plates
  - Counter
  - Timers
  - Forceps

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APPENDIX G

Laboratory Standard Operating Procedures (SOPs)

Chromogenic Substrate Method: Shoreline/Marine Samples
based on HTP SOP #1011

- **Procedure**
  - Disinfect the workbench area with 70% ethanol. Let air-dry.
  - Preparation of sample container:
    - You will need one 125mL sterilized container per sample. Label each sample container with station name and test to be performed (e.g., Container1: S01 (for station name or ID), TC/EC (Total Coliform/E.Coli for type of bacteria to be determined), Container 2: S01, Enterococcus). An additional container is provided for a sample duplicate.
    - Unseal and open the container. . Be careful not to contaminate the inside of the cap when opening.. Pour sterile D.I. water from a flask into each container. Be careful not to contaminate the rim of the D.I. flask or the container. Pour the D.I. water to the 100 mL mark on each container and replace the cap. Cap and store the remaining unused D.I. water in the flask. .
    - If a 10-ml sample aliquot is to be used, remove 10 ml of D.I. water from all sample containers using a sterile 10 ml disposable pipet. If only 1 ml of sample is to be analyzed, skip this step of removing 10 ml of D.I. water.
    - You will need one Coli-18 reagent pak for each sample container labeled TC/EC and one Enterolert reagent pak for each container labeled Entero. Carefully separate one reagent snap pak from the strip, taking care not to accidentally open the adjacent pak. Tap the snap pak to ensure that all of the reagent powder is in the bottom part of the pack.
    - Open the pak by snapping back the top at the scoreline. Do not touch the opening of the pak.
    - Add the reagents to the appropriate sample containers filled with D.I. water. Replace the cap on the container, tighten, and gently mix until the reagent is dissolved. Note that when the Coli-18 reagent is added to the D.I. water in the container, the solution is a clear color and when Enterolert reagent is added to the D.I. water, the solution is a yellow color.
    - Shake vigorously about 25 times. Pipet the necessary amount of each sample into the appropriate sample container. Discard the used pipets into the pipet biohazard container. . Replace the sample container caps and mix gently.
  - Quanti-tray/2000

- Turn on Quanti-tray® sealer at the start of sample preparation to warm up.
- You will need **one** Quanti-tray for **each** labeled sample container.
- Check to see that the green Ready Light (above the amber power light) is illuminated on the sealer. The sealer will not operate until both the amber power light and the green Ready Light are illuminated.
- Using one hand, hold a Quanti-tray upright with the well side (plastic) facing your palm. Squeeze the upper part of the Quanti-tray so that it bends towards the palm of your hand. Using your other hand, gently pull the foil tab at the top of the tray to separate the foil from the top of the tray, creating an open pouch. Avoid touching the inside of the foil or tray and be careful not to tear the foil.
- Pour the reagent/sample mixture directly into the Quanti-tray, avoiding contact with the foil tab at the top of the tray. Tap the small wells at the bottom of the tray to release any air bubbles. Allow any foam present to settle.
- Place the sample-filled tray onto the rubber insert of the sealer with the well side (plastic) of the tray facing down. Align the small and large wells with their corresponding holes in the rubber insert. Make sure the tray is properly seated in the rubber insert. With your hand, gently press on the back of the tray to distribute some of the liquid into the larger wells.
- Slide the rubber insert into the sealer until the motor grabs the rubber insert and begins to draw it into the sealer.
- In approximately 15 seconds, the tray will be sealed and partially ejected from the rear of the sealer. Remove the rubber insert and tray from the rear of the sealer.
- If a misaligned tray is accidentally fed into the sealer, press and hold the "**reverse"** button (located on the top, front center of the sealer). This will reverse the motor and you can then remove the tray. Do not reverse the motor once the rubber insert has been drawn fully into the input slot of the sealer.
- Repeat for each labeled tray. Turn off the sealer and unplug the unit when you are finished sealing all the trays.
- Using a felt-tipped marker, label the front of each tray with the incubation time, analyses, sample date, station name, analyst initial and day of the week it needs to be read after the incubation period.
- Place all Quanti-trays labeled "TC/EC" into the 35°C (Total coliform) incubator for 18-22hours..
- Place all Quanti-trays labeled "Entero" into the 41°C (Enterococcus) incubator for 24-28 hours.

- QA Controls
  - Refer to QA/QC HTP SOP #1011
- Clean-up
  - Dispose of the empty, used sample container in the large, red

biohazard containers.

- Dispose of all pipet wrappers and empty reagent packs in the regular trash receptacle. Return all lab supplies to their proper storage areas.
- Disinfect the workbench area with 70% ethanol. Let air-dry.
- Discard original sample remaining in sample bottle (can discard down sink drain). Rinse with tap water and place empty bottles on trash cart for later cleaning.

- Reading Quanti-Tray Sample Results
  - Disinfect the workbench area with 70% ethanol. Let air-dry.
  - TOTAL COLIFORMS - read 18-22 hours after incubation.
    - Remove the Quanti-trays from the 35°C (Total coliform) incubator.
    - Record the date, time, and analyst name or initials on the sample data sheet for the reading of Total Coliforms.
    - Compare the intensity of the yellow color of the sample wells to the intensity of the yellow color of the Comparator Quanti-tray used as a standard basis. Any well with a yellow color of equal or greater intensity than the Comparator is considered a "positive" well. Wells with a clear color or a yellow intensity less than the Comparator are considered as "negative". **If reaction is unclear or borderline yellow, replace the tray in incubator for further incubation up to a total of 22 hours.**
    - Count the number of positive large wells. Remember that the single, large well at the very top of the Quanti-tray should also be included in the count if it is positive. Record the number of positive large wells on the sample data sheet. Count and record the number of large positive wells for each sample dilution that was set.
    - Count the number of positive small wells. Record the number of positive small wells on the sample data sheet. Count and record the number of small positive wells for each sample dilution that was set.

  - *E. COLI* - read 18-22 hours after incubation.
    - E.Coli reading is taken using the same Total coliform Quanti-trays.
    - Record the date, time, and analyst name or initials on the sample data sheet for the reading of *E. coli*.
    - Place Quanti-tray under a UV cabinet or lamp.
    - Press the red button on the top of the UV lamp to turn the lamp on. Make sure the lamp is pointed away from you.
    - Count the number of large and small fluorescent wells for each sample dilution. Remember that the single, large well at the very top of the Quanti-tray should also be included in the count for the large wells if it is positive. Record the results on the sample data sheet.
    - If in doubt as to the fluorescence of a well, compare it to the negative fluorescence of the Quanti-tray Comparator. This Comparator is "negative" for fluorescence. **If fluorescence on the well(s) is/are still questionable, mark the well(s) with an indelible pen or marker and re-incubate Quanti-tray for an additional 2 - 4 hours**. Read Quanti-tray again following the incubation period.

ENTEROCOCCUS - read 24-28 hours after incubation

- Remove the Quanti-trays from the 41°C (Enterococcus) incubator.
- Record the date, time, and analyst name or initials on the sample data sheet for the reading of Enterococcus.
- Place Quanti-tray under a UV cabinet or lamp
- Press the red button on the top of the UV lamp to turn the lamp on. Make sure the lamp is pointed away from you.
- Shine the UV lamp directly on the sample Quanti-tray within five inches of the tray. Count the number of large and small fluorescent wells for each sample dilution. Remember that the single, large well at the very top of the Quanti-tray should also be included in the count for the large wells if it is positive. Record the results on the sample data sheet.
- If in doubt as to the fluorescence of a well, compare it to the negative fluorescence of the Quanti-tray Comparator. This Comparator is "negative" for fluorescence. **If fluorescence on the well(s) is/are still questionable, mark the well(s) with an indelible pen or marker and re-incubate Quanti-tray for an additional 2 – 4 hours**. Read Quanti-tray again following the incubation period.

- When finished reading all the Quanti-trays, turn off UV lamp and dispose of all trays into the large red biohazard containers.
- Disinfect the work bench area with 70% ethanol. Let air dried..
- Leave the sample data sheets on the clipboard by the Quanti-tray sealer.

- Quanti-Tray Calculations
  - Enter the number of positive large and small wells into the Idexx generator or read from the Idexx MPN table. Multiply the number given in the table by the dilution factor used. If more than one dilution generates a result, take the average.

Example # Positive large wells: 23

Positive small wells: 16

Idexx MPN table: 52.7

Calculation (10 ml aliquot of sample):

52.7 (**number from table**) x 10 (**Result based on a 100 ml sample size**) = 530 MPN/100 ml

Membrane Filtration Method (for Enterococci analysis)

- **Media Preparation**
  - mEndo Agar LES
    - To rehydrate the medium, suspend 51 grams in 1 liter deionized water containing 20 mL 95% ethanol and heat to boiling to dissolve completely. Cool to 45-50^{o}C. (If using the agarmatic, follow the agarmatic directions for making mEndo.) Aseptically dispense 4-5 mL amounts into the lower halves of 60x15 mm sterile, disposable petri dishes and allow it to solidify. Final pH 7.2 ± 0.2. Record pH results in the media prep log book.

    - Set QA media controls.
      - Refer to QA/QC SOP
    - Place agar plates in a labeled media container and refrigerate until needed. The holding time for agar plates is two weeks.
  - mFC Agar
    - To rehydrate the medium, suspend 52 grams in 1 liter deionized water and heat to boiling to dissolve completely. Add 10 mL of a 1% solution of rosolic acid in 0.2 *N* NaOH. Continue heating for 1 minute. Cool to 45 -50^{o}C. (If using the agarmatic, follow the agarmatic directions for making mFC.) Aseptically dispense 4-5 mL amounts into the lower halves of 50-60x15 mm tight-fitting sterile, disposable petri dishes and allow it to solidify. Final pH 7.4 ± 0.2. Record pH results in the media prep logbook.

    - 1% Rosolic Acid Solution - Add 0.1 grams rosolic acid to 10 mL of stock 0.2 *N* NaOH. Mix well.

    - Stock 0.2 *N* NaOH - Add 0.8 grams NaOH to 100 mL deionized water. Mix to dissolve. Store in a labeled polyethylene reagent bottle.

    - Set QA media controls.
      - Refer to QA/QC SOP
    - Place agar plates in a labeled Tupperware container and refrigerate until needed. The holding time for agar plates is two weeks.
  - mE Agar
    - To rehydrate the medium, suspend 7.12 grams in 100 mL of deionized water. Heat to boiling to dissolve completely. Autoclave for 15 minutes at 121^{o}C. Promptly remove from the autoclave and cool to 45-50^{o}C. Add 0.024 grams Nalidixic Acid and 1.5 mL of a 1% solution of triphenyl tetrazolium chloride (TTC). (If using the agarmatic, follow the agarmatic directions for making mE.) Aseptically dispense 4-5 mL amounts into the lower halves of 60x15 mm sterile, disposable petri dishes and allow it to

solidify. Final pH 7.1 ± 0.2. Record pH results in the media prep logbook.

1% TTC Solution - Add 1 gram TTC to 100 mL of deionized water. Mix well. Using a sterile 0.22µm Millex-GS filter, filter-sterilize the solution into a sterile, labeled 500 mL reagent bottle. Store in the refrigerator.

- Set QA media controls.
  - Refer to QA/QC SOP
- Place agar plates in a labeled Tupperware container and refrigerate until needed. The holding time for agar plates is two weeks.

- Esculin Iron Agar (EIA)
  - To rehydrate the medium, suspend 1.65 grams in 100 mL of deionized water. Heat to boiling to dissolve completely. Autoclave for 15 minutes at 121°C. Promptly remove from the autoclave and cool to 45-50°C. (If using the agarmatic, follow the agarmatic directions for making EIA.) Aseptically dispense 4-5 mL amounts into the lower halves of 60x15 mm sterile, disposable petri dishes and allow it to solidify. Final pH 7.1 ± 0.2. Record pH results in the media prep logbook.
  - Set QA media controls.
    - Refer to QA/QC SOP
  - Place agar plates in a labeled Tupperware container and refrigerate until needed. The holding time for agar plates is two weeks.
- Phosphate-Buffered Water
  - 1 *N* NaOH - Carefully add 4 grams NaOH to 100 mL deionized water. Mix to dissolve. Store in a labeled polyethylene reagent bottle.
  - Stock Phosphate Buffer Solution - add 34.0 grams potassium dihydrogen phosphate (KH_2PO_4) to 500 mL deionized water and mix to dissolve. Adjust pH to 7.2 ± 0.5 with 1 *N* NaOH and bring volume to 1 liter, using a 1 liter volumetric flask. Transfer to a reagent bottle and autoclave for 15 minutes at 121°C. Let cool and refrigerate. Discard if turbidity is present.
  - Stock Magnesium Chloride Solution - add 81.1 grams $MgCl_2 \cong 6H_2O$ to 1 liter deionized water and mix to dissolve. Transfer to a reagent bottle and autoclave for 15 minutes at 121°C. Let cool and refrigerate. Discard if turbidity is present.
  - Working Solution of Phosphate-Buffered Dilution/Rinse Water
    - Add 1.25 mL stock phosphate buffer solution and 5 mL stock magnesium chloride solution to 1 liter deionized water. Adjust pH to approximately 7.6-7.7 with 1 *N* NaOH. Mix and dispense approximately 9.5 mL into specially marked dilution test tubes. Autoclave at 121°C for 15 minutes. If phosphate-buffered rinse water is needed, autoclave 1-2 L volumes in large flasks for 45

minutes at 121°C.

- Cool and check that buffered water level is at the marked line (9 mL) on the test tube. Aseptically adjust water level if necessary. Tightened test tube or flask caps and store at room temperature. Holding time for screw-capped media is 3 months. Final pH 7.2 ± 0.1.
- Sterility control - test the sterility of the buffered dilution water by aseptically pouring 2 test tubes of dilution water into a sterile bottle containing 100 mL of Tryptic Soy Broth. Test the sterility of the liter flasks of rinse water by aseptically adding 20 mL buffer to a sterile bottle containing 100 mL TSB. Incubate the bottle for 48 hours at 35.0 ± 0.5°C. Record pH and sterility check results in the media prep logbook.

- Tryptic Soy Broth (TSB)
  - To rehydrate the medium, suspend 30 grams in 1 liter of deionized water and mix to dissolve completely. Dispense 100 mL of broth into 125 mL Nalgene bottles. Autoclave for 15 minutes at 121°C. Promptly remove from the autoclave when done. Let cool and then tighten caps. Final pH 7.3 ± 0.2. Record pH results in the media prep logbook.
  - Set QA media controls.
    - Refer to QA/QC SOP
  - Place TSB bottles in the refrigerator until needed. The holding time for screw-capped media is three months.

- **Plate Labeling Procedure**
  - Clean and wipe the bench-top work area with 70% ethanol and let air-dry.
  - Check the monthly sample calendar for the samples and duplicates scheduled for the day.
  - Check the QA results of the prepared agar plates to be used. These results are recorded in the media prep logbook. Use only media that have passed the sterility, positive control, and negative control checks.
  - Record the media preparation dates for all the agar plates being labeled. The dates are recorded in the media prep logbook under "Prep Date of Media in Use".
  - Inspect all agar plates.
    - Discard any plates that have bubbles that will interfere with bacterial growth when the membrane filter is placed on the agar surface.
    - Check plates for contamination of any kind (bacterial growth, mold, or strange color). Discard any contaminated plates into a biohazard bag.
  - Using an indelible marking pen or pre-printed labels, label each plate with the station name or location at the top of the petri dish, sample volume or dilution in the middle, and sample date at the bottom of the dish.
    - Consult the Sample Dilution Table for the necessary dilutions for

each sample type.
        - mEndo and mFC agar plates are labeled on the bottom (agar side) of the petri dish.
        - mE agar plates are labeled on the top (lid side) and the bottom (agar side) of the petri dish.
    - Stack all the agar plates for the same station together after the plates are labeled. Stack plates by ascending volume order (smallest volume on top).
    - When stacking, be sure to place all plates, agar side up.
    - Place the stack of plates for each sample into a slot in one of the agar plate carriers.
    - Add a small stack of unlabelled mEndo agar plates to the carrier. These plates will be used for QA blanks as needed during filtering.
    - Label the cover of each plate carrier with the sample stations or locations for all plates in the carrier. Include duplicate stations on the label for all boat plate carriers.
    - If plates are labeled one day in advance of use, refrigerate the plate carriers. Labeled plates that are refrigerated need to be taken out of the refrigerator on the day of use.
    - If plates are labeled on the day of use, the plate carriers can be left out at room temperature until needed.

- **Filtration Procedure**
    - Clean and wipe the bench top work area with 70% ethanol and let air-dry.
    - Gather the necessary filtration equipment.
    - Aseptically transfer sterile, phosphate-buffered rinse water into a sterile squirt bottle.
    - Select samples to be filtered. Select the proper agar plates for the samples and check the plate stacking order to make sure sample volumes are in ascending order.
    - Make 1:10 serial dilutions (if needed).
        - Shake the sample vigorously for several seconds (about 25 - 30 times) to break up any bacterial cell aggregates, to separate cells from particulate matter, and to make the sample homogenous.
        - Aseptically pipet 1 mL of the sample into a sterile 9 mL dilution test tube and shake or vortex vigorously. This is a 1:10 (10^{-1}) dilution of the sample.
        - Aseptically pipet 1 mL of the 10^{-1} dilution into a second 9 mL dilution tube and shake or vortex vigorously. This is a 1:100 (10^{-2}) dilution.
        - Aseptically pipet 1 mL from the second (10^{-2}) dilution tube into a third 9 mL dilution tube and shake or vortex vigorously. This is a 1:1000 (10^{-3}) dilution.
        - Continue making 1:10 serial dilutions as needed.
    - Fill the alcohol lamp with 95% ethanol and light it.
    - Prepare filtration equipment, one filtration unit per sample.
        - Wipe the Microfil support base with an alcohol pad. Let dry.
        - Remove filter screen disc from the 95% alcohol jar using the long-

handled forceps. Gently shake the disc over the alcohol jar to remove any excess alcohol. Flame-sterilize the disc. Allow flame to self-extinguish. Place disc onto the Microfil support base.
    - Squirt the disc with a small amount of sterile buffer to wash any residual alcohol off the disc. Apply vacuum to drain the buffer off the disc.
    - Aseptically remove a membrane filter from the filter dispenser, using an alcohol flame-sterilized forceps. Place the filter, grid-side-up on filter support base.
    - Aseptically remove a sterile, disposable Microfil funnel from the funnel dispenser.
    - Put the funnel over the filter on the support base. Place thumbs and index fingers of both hands on the upper, outside ridge of the funnel. Evenly push down on the funnel to securely lock it into place.
- Shake sample vigorously for several seconds (about 25 - 30 times) to break up any bacterial cell aggregates, to separate cells from particulate matter, and to make the sample homogenous. Place bottle at a slant to let any sand or debris in the sample settle to the bottom sides of the bottle.
- Record filtering start time and initials in the LIMS "Micro Log-in" Excel worksheet on the PC computer. Move the cursor to the appropriate cell for the sample being filtered.
    - If the starting time is the current time, press "CTRL+T".
    - Alternately, enter the time using a colon, ex. "10:25 or 14:00".
- Before filtering the sample, determine if a QA sterility blank needs to be done.
    - Refer to QA/QC SOP
- Wet the membrane filter with an adequate amount of sterile rinse water before adding sample aliquots delivered with a pipet. Add the sample aliquot to the filter according to the plate stacking order. Use a new filter for each sample aliquot.
- Use sterile pipets for sample volumes < 20 mL. If the pipet is to be used again, rest the pipet tip against the inner lip of the sample bottle. Do not let the pipet tip rest on the bottom of the sample bottle. Discard used pipets into the pipet biohazard container.
- For sample volumes of 50 mL or 100 mL, aseptically pour the sample to the measured lines on the Microfil funnel. If an excess amount of sample is poured into the funnel, use a sterile pipet to remove the excess. Discard the excess sample along with the pipet into the pipet biohazard container.
- Before applying the vacuum, swirl the sample in Microfil funnel by moving the funnel in a gentle circular motion to evenly distribute bacterial cells on the membrane filter surface.
- Apply vacuum, letting the sample drain through the filter.
- Thoroughly rinse down the walls of the funnel two times with a generous amount of sterile buffer water. This will wash down any bacteria that may adhere to the sides of the funnel.
- With one hand on the outside walls of the funnel, use a backwards and upwards motion to pop the funnel off the support base. Continue to hold

the funnel with your hand. Use your other hand to aseptically remove the filter with a flame-sterilized forceps (one sterile forceps per membrane filter). Aseptically replace the funnel back on the support base.

- Aseptically place the filter on the surface of the appropriate agar plate, using a rolling motion to avoid trapping air between the agar and the filter, which will result in the formation of bubbles. If any air is trapped under the filter, reset the membrane filter onto the agar surface. Place the used forceps into the jar of ethanol.
- Stack finished plates by sample and media type. Remember to always position finished plates agar (bottom) side up. This is to avoid any condensation dripping onto the surface of the filter during incubation, which may interfere with or distort bacterial growth.
- Continue filtering the sample, following the steps detailed above for each sample volume or dilution labeled on the stack of plates.
- If a duplicate sample is being filtered, the same pipets and dilution tubes (if needed) may be used for both the regular sample and the duplicate sample.
- When the sample is finished being filtered, place mEndo and mE agar plates in a covered incubation container (with moist sponges) according to media type. Total coliform mEndo agar plates are incubated for 23 ± 1 hour at 35.0 ± 0.5 ^{o}C. Fecal coliform mFC agar plates are incubated for 24 ± 2 hours at 44.5.0 ± 0.2 ^{o}C. It is important that fecal plates are incubated within 20 minutes of filtration to ensure heat-shock of the non-fecal bacteria. Plates are incubated in either the dry heat-sink incubators or sealed in waterproof bags and placed in the 44.5 ± 0.2^{o}C water bath. Enterococcus mE agar plates are incubated for 48 ± 2 hours at 41.0 ± 0.5^{o}C.
- Record filtration finish time, initials, and incubation time in the LIMS "Micro Log-in" Excel worksheet on the PC computer.
- The incubation containers should be labeled with the indicator bacteria, test date, and incubation time.
- Place used Microfil funnels in the biohazard bag for the funnels. Place sample bottles, empty buffer flasks, and used squirt bottles (if not being used for filtering more samples) in a tub for later washing.
- Wipe down the bench-top work area with 70% ethanol and let air dried.
- To filter another set of samples, wipe the Microfil support base and filter screen disc with a new alcohol pad. Rinse the disc with sterile rinse water. Repeat procedure as detailed in the above sections.
- When taking a long break between filtering samples, wipe the Microfil support base and filter screen disc with a new alcohol pad. Leave the alcohol pad on the screen disc. Place an alcohol-wiped cap over the Microfil unit. Before filtering again, remove the cap and re-wipe the Microfil unit and filter screen disc with the alcohol pad. Rinse the disc with sterile rinse water. Repeat procedure as detailed in the above sections.
- When all samples have been filtered, remove the filter screen disc from the Microfil support base and put in the 95% alcohol jar. Wipe the Microfil support base with a new alcohol pad. Leave the alcohol pad in

the empty disc space. Place an alcohol-wiped cap over the Microfil unit.

- **Colony Counting Procedure**
  - Check the LIMS "Micro Log-in" Excel worksheet for the incubation times of the plates that need to be read that day. Determine when the plates can be read according to their required incubation times.
  - Gather the necessary data worksheets for all samples to be read. Each test and sample type has separate data worksheets.
  - Record the time the plates are read and analyst initials in the LIMS "Micro Log-in" Excel worksheet and also on the data worksheets.
    - If the read time is the current time, press "CTRL+T".
    - Alternately, enter the time using a colon, ex. "10:25 or 14:00".
  - If desired, wear disposable gloves when handling and reading the plates.
  - Remove plates from the incubator when it is time to read them and arrange them in ascending volume order for each station.
  - Use the stereoscopic microscope with a fluorescent lamp to aid in identifying and counting colonies.
  - Starting with the control blank plate if one was done, examine the filter for bacterial contamination or any notable changes on the filter or agar media.
  - Examine and count all the plates set for a single sample, starting with the smallest sample volume filtered or the most dilute sample.
  - Colonies that have grown into each other should be counted individually. Separate nuclei or a fine line of contact may usually be seen.
  - Colonies in each and every filter grid square within the filtering area are to be counted.
  - To make counting easy and simple, start counting at the top of the filter. Count from left to right, following the grid lines, and continue to the bottom of the filter.
  - Countable ranges - Due to the possible adverse effect of colony crowding on sheen or color development on the filter membrane, and to be assured of a statistically valid colony count, minimum and maximum bacterial levels have been set for each of the indicator bacteria.
    - Total bacteria: <200 total colonies (background and indicator bacteria).
    - Total Coliform: 20 - 80 coliform colonies
    - Fecal Coliform: 20 - 60 fecal coliform colonies
    - Enterococcus: 20 - 60 enterococcus colonies
  - Colony Morphology
    - Total Coliforms
      - The typical colony has a pink to dark-red color with a shiny, greenish-gold, metallic surface sheen. The sheen may cover the entire colony, or it may appear only in the central area or on the periphery.
      - This sheen is produced as a by-product of lactose fermentation (acid aldehyde complex) in combination with the Schiff's reagent (fuschin sulfite) in the mEndo media.
    - Fecal Coliforms
      - Any colony exhibiting any light or dark blue color, whether

covering the entire colony or only in or on part of the colony.
- This blue color is a result of the acid produced by the fermentation of lactose combining with the aniline blue dye in the mFC media.
- Colonies exhibiting a cream or grey color are not fecal coliforms.

▪ Enterococcus
- After 48 ± 2 hours incubation, mE filters with growth on them are transferred to room temperature EIA plates.
- Using forceps, remove the filter (handling the filter by its edge, outside of the filtration area) from the mE plate and roll it onto the agar surface of the EIA plate.
- Replace the top of the EIA plate with the labeled top lid of the original mE plate.
- Incubate the EIA plates for 20 minutes at 41.0 ± 0.5^{o}C.
- Enterococci are pink to carmine-red colonies with black or reddish-brown precipitate or halos on the underside of the filter when placed on EIA agar.
- The colony color is due to the reduction of the vital indicator TTC (2,3,5-Triphenyl tetrazolium chloride) to non-reversible formazin. The dark precipitate or halo is the result of the hydrolysis of esculin.

o Record all colony counts and any other notable information on the data worksheet. Comments should include information about unusual conditions on the filter, such as the presence of solids, artifacts or high background counts. The condition of the growth on the filter should also be noted, such as confluent areas or confluent growth over the filter.
▪ CG = confluent bacterial growth with indistinct or non-discrete colonies.
▪ TNTC = Too Numerous To Count
▪ >200 = greater than 200 background and indicator colonies on a filter.

o If there are any questions regarding counting colonies or any unusual or suspicious plates, save all plates for that sample and show them to a microbiologist.

o Dispose of all plates and gloves in a biohazard container. Autoclave at the end of the day.

▪ **Calculations**

o Due to the possible adverse effect of colony crowding or color development on the filter membrane, and to be assured of a statistically valid colony count, minimum and maximum bacterial levels have been set for each of the indicator bacteria.
▪ Total bacteria: <200 total colonies (background and indicator bacteria).
▪ Total Coliform: 20 - 80 coliform colonies

- Fecal Coliform: 20 - 60 fecal coliform colonies
- Enterococcus: 20 - 60 enterococcus colonies

- Indicator bacteria are expressed as bacterial density (CFU) per 100 mL of sample.
- The raw bacterial counts from the data worksheets are entered into LIMS "Sample Data Entry" Excel worksheets on the PC computer by a technician. The computer calculates the final bacterial densities for each sample and prints a copy of the data worksheet. See the LIMS Data Entry SOP for more details.
- The supervisor verifies the daily calculated bacterial densities. Daily bacterial density reports are printed out by the computer and E-mailed to the primary leads of the jurisdictional groups, who in turn will communicate this data to its jurisdictional members. The data reports are kept in a labeled notebook and the original data worksheets are kept in the data file cabinet. See the LIMS Data Validation SOP for more details.
- If the final bacterial densities need to be calculated by hand, the following guidelines should be used. All calculated values should have only 1 or 2 significant figures, depending on the colony counts.

  - Countable Range (Std.Meth., EPA):

    Countable range number of colonies x 100 = (value) CFU/100 **mL filter volume**

Disregard non-countable range counts and volumes.

Volume	Count	
blank	0	
0.5	0	
5.0	6	$\frac{35}{20} \times 100 =$ **180 CFU/100 mL**
20	35	
50	95	

  - Two volumes in the countable range (EPA):

    Calculate each count independently as in 6.4.1. above and then average the results.

Volume	Count		
blank	0	$\frac{20}{20} \times 100 = 100$	$\frac{60}{50} \times 100 = 120$
0.5	0		
5.0	6		
20	20	$\frac{100 + 120}{2} =$ **110 CFU/100 mL**	
50	60		

  - Counts less than the countable range (Std. Meth.):

Add all colonies x 100 = (value) CFU/100 mL
Total all volumes

Volume	Count	
blank	0	
0.5	0	
5.0	1	$\frac{19 + 4 + 1 + 0}{50 + 20 + 5 + 0.5}$ x 100 = **32 CFU/100 mL**
20	4	
50	19	

- No counts on any filter volume (EPA):

$\frac{1 \times 100}{\text{Largest vol filtered}}$ = < (value) CFU/100 mL

Volume	Count	
blank	0	
0.5	0	$\frac{1}{50}$ x 100 = **<2 CFU/100 mL**
5.0	0	
20	0	
50	0	

- Counts greater than the countable range - too numerous to count (TNTC) or confluent growth (CG) (EPA):

$\frac{\text{Highest upper limit count x 100}}{\text{Smallest vol filtered}}$ = >(value) CFU/ 100 mL

Volume	Count	For Total Coliforms:
blank	0	$\frac{80}{0.5}$ x 100 = **>16,000 CFU/100 mL**
0.5*	TNTC or CG	
5.0	TNTC or CG	For Fecal Coliforms or Enterococci:
20	TNTC or CG	$\frac{60}{0.5}$ x 100 = **>12,000 CFU/100 mL**
50	TNTC or CG	

*NOTE: If the count at the lowest dilution is TNTC, try to estimate the count on the plate. Estimate the count in a quadrant if necessary. Use this number to calculate the count per 100 mL.

- Confluent Growth Counts (Std. Meth., EPA):

Disregard all dilution volumes that are confluent growth.
Analyze remaining counts and volumes.

Volume	Count	Volume	Count
blank	0	blank	0
0.5	0	0.5	3
5.0	CG	5.0	20

20	CG	20	CG
50	CG	50	CG

1 x 100 = **<200 CFU/100 mL** 20 x 100 = **400 CFU/100 mL**
0.5 5.0

- Total bacterial count (background bacteria plus indicator bacteria) greater than 200 colonies (Std. Meth.):

  Analyze counts and volumes. Report as a greater than value.

Volume	Count	Volume	Count
blank	0	blank	0
0.5	0 (>200)	0.5	0
5.0	0 (>200)	5.0	3
20	CG	20	18 (>200)
50	CG	50	60 (>200)

1 x 100 = **>20 CFU/100 mL** 60 x 100 = **>120 CFU/100 mL**
5 50

- Total colonies less than 200, but indicator bacteria greater than upper limit (Std. Meth.):

  If plate has well isolated, discrete colonies that can be easily counted, use the higher count.

Volume	Count	Volume	Count
blank	0	blank	0
0.5	85	0.5	2
5.0	TNTC	5.0	95
20	TNTC	20	TNTC
50	TNTC	50	CG

85 x 100 = **17,000 CFU/100 mL** 95 x 100 = **1,900 CFU/100 mL**
0.5 5

APPENDIX H

Data Acquisition, Reduction, Validation, and Reporting Standard Operating Procedure (SOP)

When performing analyses, results are generally tabulated onto laboratory worksheets (see Appendix E, Field and Laboratory Worksheets) but sometimes are generated electronically via instrumentation. Data on laboratory worksheets are entered into the Laboratory Information Management System using an Excel interface. These data are then validated through a quality assurance process that checks for correctness of data entry and validity of results. The analyst who generates the data has the initial and primary responsibility for the completeness and correctness of the data. The data are then checked by the unit supervisor (or designee). The following procedures describe the data acquisition and entry process then the quality assurance and quality control procedures.

Data Acquisition

Both raw and calculated data are acquired in the laboratory by manual, electronic, or direct computer acquisition. Acquired data are properly and securely stored for the duration specified by regulatory agencies or the customer. Guidelines for documentation and recording of information are as follows:

- Manual (Hand-written) Data Entry
    - Data are entered directly into the notebook or worksheet with non-erasable ink.
    - Data entries are signed and dated by the analyst making the entry. If the entry is more than one page, each page is signed and dated.
    - Mistakes are canceled by drawing a line through the entry, entering the correct value, and signing and dating the correction. The use of correction fluid is not acceptable.
    - Blank pages or substantial portions of pages with no entries are marked with a large "X" to indicate that they were intentionally left blank.
- Direct Computer Acquisition
    - In EMD's Microbiology Unit, the program/software used to generate results is prepared internally. A designated staff member of the Information & Control System Division (ICSD) at Hyperion has the responsibility of preparing the program and maintaining the supporting documents.
    - The laboratory relies on vendor-supplied information for the validity and integrity of instruments equipped with significant computer functions as an integral part of the system.

Data Reduction

Data reduction, where applicable, is described in specific SOP's. It involves reporting values with the appropriate significant figures in the concentration units established by the regulatory agency or the data user.

Procedure for Entering Microbiology Data into LIMS

- Log-On to LIMS Computer System
  - To log onto the LIMS system, double-click on the "Data Entry" icon on the PC computer screen.
  - A Microsoft Excel dialog box will appear. Select the "Enable Macros" button.
  - Wait until the "Microbiology Laboratory Worksheet StartDialog" dialog box appears.

- Data Entry for CS
  - Enter the sample date in the dialog box. Please note that current date is filled in by default.
  - Select the sample type. There is a list of sample locations to choose from. (E.g. 5-Mile, Ballona Creek, Cabrillo Beach, LAH Plume, SMB Plume Day1, Shoreline, Inshore, and so on.)
  - Dilutions for the CS method are not modified for rain events. For this method always make sure the "No" button is selected.
  - Select the "OK" button.
  - A computer form similar to the raw data worksheet will appear. Select the Excel worksheet tab for the type of test data to be entered. (ex. Total, *E. coli*, or Total & *E. coli*)
  - Enter analyst initials, date, and time into the computer in the designated cells.
  - Check to make sure the sample volumes or dilutions in the computer match the volumes or dilutions on the raw data worksheet. In the case of Ballona Creek, make changes to the volumes on the computer form, if necessary.
  - Enter the number of large and small positive wells.
  - Check to make sure all data has been entered correctly. If a calculated value does not appear for a sample, notify a microbiologist or the supervisor.
  - At the top of the computer worksheet, select the "Send Data to LIMS/Wisard" button.
  - Select the "Print" button at the top of the computer worksheet. A printed hardcopy of the raw data worksheet will print out on the printer in the micro lab.
  - Select the "New Worksheet" button at the top of the computer screen if entering data for another sample location. Select the "Save/Exit" button if all the data entry has been done.
  - If there are any problems or error messages regarding sending the data to

LIMS, please contact LIMS staff at 55749 or 55120.

- Data Entry for MF
  - Enter the sample date in the dialog box. Please note that current date is filled in by default.
  - Select the sample type. There is a list of sample locations to choose from. (E.g. 5-Mile, Ballona Creek, Cabrillo Beach, LAH Plume, SMB Plume Day1, Shoreline, Inshore, and so on.)
  - If rain dilutions were used on the data worksheet, select "Yes" in the small "Rain" box. If normal dilutions were used, make sure the "No" button is selected.
  - Select the "OK" button.
  - A computer form similar to the raw data worksheet will appear. Select the Excel worksheet tab for the type of test data to be entered. (ex. Total, Fecal, Entero, or Total & Fecal)
  - Enter analyst initials, date, and time into the computer in the designated cells.
  - Check to make sure the sample volumes or dilutions in the computer match the volumes or dilutions on the raw data worksheet. In the case of Ballona Creek, make changes to the volumes on the computer form, if necessary.
  - Enter the bacterial colony counts.
  - Check to make sure all data has been entered correctly. If a calculated value does not appear for a sample, notify a microbiologist or the supervisor.
  - At the top of the computer worksheet, select the "Send Data to LIMS/Wisard" button.
  - Select the "Print" button at the top of the computer worksheet. A printed hardcopy of the raw data worksheet will print out on the printer in the micro lab.
  - Select the "New Worksheet" button at the top of the computer screen if entering data for another sample location. Select the "Save/Exit" button if all the data entry has been done.
  - If there are any problems or error messages regarding sending the data to LIMS, please contact LIMS staff at 55749 or 55120.

Review and Validation

Review

Data review is the process of comparing results to all available information, such as sample preparation and QC sample data, to evaluate the validity of the results. It supports the contention that the data are:

- Reasonable (experience with similar situations, common sense), and
- Capable of supporting a defensible decision.

The analyst and the unit supervisor (or designee) are responsible for reviewing the data relative to the following:

- Method blanks and QC sample
- Raw data
- Calculations
- Transcription

Validation

Data validation is the systematic procedure of reviewing data against a set of criteria to provide assurance of its validity before reporting the data. It is accomplished through routine examination of data collection, flow procedures, and QC sample results. It uses QC criteria to reject or accept specific data

- Validation includes the following:

    - Dated and signed entries by analysts on the worksheets and logbooks used for all samples.
    - Use of QC criteria to reject or accept specific data.
    - Checking of LIMS data entry and reporting

Validation Guidelines include the following:

- Documentation of methods used and QC applied.
- Maintenance performed on instruments.
- Documentation of sample preservation, transport, and storage.
- Review of QC sample data.

Data validation is performed, signed, and dated by the analyst, the unit supervisor (or designee), and where applicable, the laboratory manager.

Reporting

Data prepared for release to the Legal Reporting Unit are checked and approved by the unit supervisor (or designee) by the 5th of the following month for the previous month's data. The final report is prepared by the Legal Reporting Unit of EMD. The report is again scanned for missing data and outliers. Regulatory limitation calculations will be applied to the data set and exceedances clearly listed. If stations are out-of-compliance, accelerated monitoring will be indicated. Any regulatory required summary reports of source identification findings or sanitary surveys will be included. The report is signed by the Division Manager before distribution and may include the following:

- Sample ID used by the laboratory and the client (if available).
- Sample matrix type, description, and method number.
- The chemical/physical/biological parameters analyzed with the reported values and units of measurement.
- Data for all parameters reported with consistent number of significant figures.

- Results of QC samples, if appropriate.
- Footnotes referenced to specific data, if required, to explain reported values.
- If there are regulatory limits applicable to specific analyses, then limits are clearly notated and exceedances listed.
- Discussion on non-compliance data
- Report transmittal letter or memorandum identifying the person sending the report and the person(s) receiving the data.

APPENDIX I

Quality Assurance/Quality Control (QA/QC)

The quality assurance objectives for measurement of data are unique to the particular program for which the data are collected and utilized. They describe the overall uncertainty that the data user is willing to accept in order to make decisions for environmental or other concerns. This uncertainty describes the data quality that is needed, which are usually expressed in terms of precision, bias, representativeness, comparability, and completeness.

The participating laboratories will use approved and recognized test methods, and comply with uncertainty requirements of the method. Quality control samples are measured and uncertainties are assessed and results must be within the range prescribed by the methods. Internal acceptance criteria are established by analyzing laboratory control samples on a daily basis. The participating laboratories will strive to meet the QA/QC goals described in this section and, therefore, be able to attest to the integrity of the sampling and analytical process.

The following QA/QC procedures will be conducted for shoreline sample collection, laboratory analyses, and data management to ensure the production of reliable and defensible data for Ballona Creek, estuary and tributaries.

Sample Collection

Only trained laboratory staff will be assigned to collect samples using proper sampling procedures, appropriate sampling equipment, required containers, and proper preservation techniques.

General guidelines for sample collection by laboratory staff are as follows:

- Assure sterility check on sample bottles and avoid contamination.
- Label sample containers with sample date, sample time, sampling point, sample type (grab/composite), preservatives added (if needed), the name of the sampler, and analyses needed.
- Use aseptic technique when collecting samples to prevent contamination (e.g. the inner surfaces or lip edges of the bottle or cap are not to be touched).
- Avoid collecting sample in multiple sweeps and no refilling of the sample bottle.
- Once the sample is collected, immerse at least one-third of the sample bottle in ice.
- Do not exceed maximum allowable transport time (time of sample collection to sample analysis) of 6 hours.
- Once received, log the samples into the laboratory system as soon as possible, assigned a unique login number, and properly stored.

- Sample preparation steps done prior to analysis, such as sample preservation are described in individual test SOP's.

Sample Handling

Chain-of-Custody

The purpose of the chain-of-custody is to establish detailed written and legal documentation of all transactions in which samples are transferred from the custody of one individual to another. The custody procedure is also used whenever samples are submitted to a laboratory within the division or to a contract laboratory. The chain-of-custody begins at the sample collection site and includes couriers or messengers who handle the sample in transit. It follows the sample in the laboratory until its ultimate disposal. It is a form of proof used to establish the authenticity and integrity of the sample, since the results will be used to show compliance with the TMDL requirements, i.e., numeric targets and waste load allocations.

A Chain-of-Custody (COC) must accompany each sample submitted to a participating laboratory. If a COC has not been filled out prior to delivery of the sample, a form will be provided to the delivery person prior to acceptance of said sample. The COC will be reviewed to make sure that all of the needed information has been supplied. As an example, the Chain-of-Custody Form being used at EMD is attached (Appendix E).

Samples that are collected by EMD's Microbiology Unit staff for bacteriological testing are delivered directly to the microbiology laboratory. A COC sheet is not required since technically there is no sample exchange, i.e., the sample collection staff and the analytical staff are ones and the same.

Sample Holding & Preservation

Samples must meet EPA holding time requirements for each testing parameter. The sample refrigeration and holding time of six hours until analyses are performed are crucial for microbiological testing. Microbiological samples must be handled and stored under contamination free environments.

After the sample is received, the participating laboratory will enter the sample information into the Laboratory Information Management System (LIMS) or comparable database and a unique laboratory registration number will be generated for that sample.

Sample Disposal

After the analyses are completed, the sample will be retained as legal evidence or legally disposed of as determined by the microbiological analysis of the sample.

Analyzed samples and standards used in analyses are disposed of in accordance with the laboratories written procedures, e.g., EMD's Chemical Hygiene Plan.

Analytical Procedures

Analyses

Analyses performed at EMD laboratories are generally driven by regulatory concerns and plant operations' requirements. There are many different analytical methods applicable to environmental analyses. EMD's methods are generally based on those specified by EPA, Federal and State regulatory agencies, or professional organizations. As a guide, references for the microbiological procedures are listed below. "Standard Methods for the Examination of Water and Wastewater", 18th – 20thedition, 1992, 1998 respectively, APHA, AWWA, WPCF, Washington, DC. "Microbiological Methods for Monitoring the Environment, Water, and Wastes", EPA-600/8-78-017.

Standard Operating Procedures (SOPs)

Routine analyses are defined in Standard Operating Procedures (SOPs), which are detailed descriptions of how to use and what to expect from a method. They contain method-specific QC criteria (i.e., instrument calibration, reagent blank, method blank, calibration standards, etc.), and QC requirements such as duplicate analysis, spike recoveries, holding time, etc. EMD follows a standardized SOP format, its content and application is presented in Appendix H of this document.

Microbiological Analyses

The following methods and target organisms are used in analysis of shoreline samples for Ballona Creek, estuary and tributaries:

- Membrane Filtration
  - Total coliform
  - Fecal coliform
  - *Enterococcus*
- Chromogenic Substrate
  - Total coliform
  - *E.coli*
  - *Enterococcus*

For the Ballona Creek Bacterial TMDL Monitoring Program, all the methods used will be EPA, Regional Board, or Standard Methods for Examination of Water and Wastewater (APHA, 18th – 20th 1992, 1998, respectively) approved methods.

The following QA/QC checklist is applicable for the chromogenic substrate and membrane filtration methods.

Chromogenic Substrate

- QC Checks on Idexx Reagent
    - Colilert-18 and Enterolert –sterility check performed with each use; autofluorescence, positive and negative controls; performed on each new lot of reagent
    - Monthly QC verification of at least 10 positive wells/target organism

- Quanti-trays:
    - Leak test performed on each new lot of trays

- DI Water
    - Sterility check performed with each autoclaved batch
    - Heterotrophic plate count performed monthly
    - Amm-N, Org-N, and TOC performed monthly
    - Heavy metals, total and single, performed annually
    - Total chlorine performed with each new batch
    - Water suitability test performed with change of E-Pure system filters or water source

- Equipment and Laboratory Environment:
    - Incubator temperatures checked twice daily (morning and late afternoon)
    - Refrigerator temperatures checked twice daily (morning and late afternoon)
    - Thermometers calibrated semiannually
    - Autoclaves calibrated semiannually; preventative maintenance performed quarterly
    - Air and Rodac testing for laboratory air and surface environments performed monthly.
    - Balances calibrated semiannually; weight check with each use
    - PH meters- calibrated semiannually; standardized with each use
    - Quanti-tray sealers checked and cleaned weekly

- Personnel QA checks
    - Reagent blanks
    - Sample duplicates (done on 10% of the samples per month)
    - Standard sample analysis and comparison count performed monthly

Membrane Filtration

- QC Checks on Media (mEndo, mFC, mE, EIA; phosphate buffered water):
    - mEndo, mFC, mE, EIA: pH, sterility check, and positive and negative controls with each new batch
    - Phosphate buffered water: pH and sterility check with each new batch
    - Monthly QC verification of at least 10 positive colonies/target organism

- Equipment and Laboratory Environment:
    - Incubator temperatures checked twice daily (morning and late afternoon)

- Refrigerator temperatures checked twice daily (morning and late afternoon)
- Thermometers calibrated semi-annually
- Autoclaves calibrated semi-annually; preventative maintenance performed quarterly
- Air and Rodac testing for laboratory air and surface environments performed monthly.
- Balances calibrated semi-annually; weight check with each use
- PH meters- calibrated semi-annually; standardized with each use
- Residue on glass- performed annually for glassware and petri dishes
- Water suitability test performed with change of E-Pure system filters or water source

- Personnel QA checks (performed by all technical lab staff)
  - Reagents blanks
  - Sample duplicates (done on 10% of the samples per month)
  - Standard sample analysis and comparison count performed monthly for MF analysis:

System and Performance Audits

An audit is a periodic check to ensure that the laboratory operates according to the policies and procedures described in the Quality Assurance Manual, complies with good laboratory practices, and meets the requirements of regulatory agencies. It may be either a system or performance audit.

System Audit

A system audit is a review of laboratory operations conducted to verify that the laboratory has the necessary facilities, equipment, staff, and procedures in place to generate acceptable data. It is an on-site inspection of the laboratory's system of operations. It may be an internal or external audit. Internal inspections may be performed by quality assurance personnel. External audits are generally laboratory certification-related activities.

1. Internal

   Periodically, the QA Officer (or designee) audits the laboratories and reports the results to the Division Manager (or laboratory director), laboratory managers, and unit supervisors.

2. External

   State-certified laboratories are site visited every two years by auditors from the Environmental Laboratory Accreditation Program (ELAP) of the California Department of Health Services (CA DOHS). Accreditation is by

scientific discipline or field of testing. Non-compliances with good laboratory practices are identified and reported as deficiencies and are subject to corrective action before accreditation is renewed.

Performance Audit

A performance audit is a review to evaluate the laboratory's analytical activities as well as the data produced by analysts. It verifies the ability of the laboratory to correctly identify and quantify compounds in unknown samples submitted by the auditing entity. The purpose of these audits is to determine the laboratory's capability to generate scientifically sound data.

1. Internal

Periodically, the QA staff submits unknown samples to most of the laboratories. These samples are usually from the inventory of previous Performance Evaluation (PE) samples from EPA. Analysis of these samples is also a corrective action requirement for Discharge Monitoring Report (DMR) and/or Water Pollution (WP) samples evaluated with "unacceptable results". The QA staff may also conduct intra- and inter-comparison studies.

2. External

All laboratory units, including the Microbiology laboratory, at EMD participate in mandatory QA Performance Evaluation (PE) Study Programs.

a. Mandatory PE Programs

* Water Pollution QA Study Program (WP) serves a dual purpose. It satisfies EPA's wastewater testing laboratory requirements and meets one of ELAP's laboratory certification criteria. Test samples are analyzed for parameters listed under each field of testing on our certifications and are specified in the WP Program following certified procedures. A laboratory can participate in a WP Study twice a year.

* For the Microbiology Performance Evaluation (PE) Study, Drinking Water/Wastewater Enumeration is required for ELAP certification. Like all the other PE programs, the samples are acquired from NIST-approved vendors and analyses are done for certified analytes.

b. Voluntary PE Program

The Microbiology Unit also takes part in the inter-laboratory calibration studies with EPA. These programs are performance based.

Assessment of Precision and Accuracy

Data quality may be assessed in terms of precision, accuracy, representativeness, completeness, and comparability. The latter three are usually determined outside of the laboratory operations and with limited involvement of laboratory staff. These measures are not included in this section. The internal quality control measures (i.e., precision and accuracy) that are performed in the laboratory to evaluate data quality are described in this section.

Precision

Precision is the agreement among a set of replicate measurements without knowledge of the true value. It is the degree to which a measurement is reproducible. Precision, expressed as Relative Percent Difference (RPD), is determined for each laboratory unit by analyzing replicates of the same sample, a number of duplicate pairs, or matrix-spiked duplicate samples.

Accuracy

Accuracy is a measurement of how close the result is to the true value. Each laboratory unit establishes its accuracy of measurement by analyzing QC check samples (spiked samples, standard reference materials from a reliable source, etc). The results of the QC samples are correlated to documented, certified values. Results of spiked samples are calculated as Percent Recovery. Actual Percent Recovery is compared to established reference data. The degree of closeness of the QC check sample contributes to the general assurance that the accuracy of the data is within acceptable limits.

Corrective Action

Laboratory events and data that fall outside established quality acceptance criteria may require investigation or corrective action. The corrective action implemented depends on the type of analysis, the extent of the error, and whether the error can be determined and corrected. The purpose of the corrective action is to resolve the problem and to restore the system to proper operation. Investigative steps and corrective actions implemented are documented.

Corrective Action Procedures

1. The initial corrective action procedures may be handled at the bench level. The unit supervisor is immediately notified of the deviation. The analyst reviews the sample preparation for possible errors and checks the instrument calibration, calibration and spike solutions, instrument sensitivity, etc.

2. If the error cannot be resolved by the analyst, the unit supervisor has the responsibility of resolving the problem with assistance, if needed, from the laboratory manager and/or the QA Officer.

3. The corrective action adopted may be determined by the analyst, the unit supervisor, the laboratory manager, the QA Officer, or through a consensus. If needed, the final decision for corrective action rests with the laboratory manager after consultation with the QA Officer.

4. The unit supervisor shall maintain an accurate and up-to-date record of corrective actions taken in the unit. A corrective action report form (included herein as an attachment) is available for use.

5. The laboratory manager shall periodically review corrective action records and plan for system improvement by involving analysts, unit supervisors, and QA personnel.

General Guidelines for Initiating a Corrective Action

1. Identify/define the problem.
2. Assign responsibility for investigating the problem.
3. Investigate and determine the causes.
4. Develop corrective action to eliminate the problem.
5. Measure the effectiveness of the corrective action.
6. Analyst, unit supervisor, laboratory manager, and the QA Officer meet to review and evaluate the process, if necessary.
7. Document the process by filling out the Corrective Action Report Form.

APPENDIX J

Data Format

Data format. List of fields, type of data, whether it is required, and description of data format to be used for submission for archival.

Field Name	*Type*	*Required*	*Description*
Agency	Text	Y	A unique code used by the submitting agency (luAgency)
Account	Text	Y	Place-holder code to contain “TMDL”.
Program	Text	Y	Place-holder code to contain “BC TMDL”.
StationID	Text	Y	The station name from the list of stations provided in lookup list (luStations).
AgencySampleID	Text	N	The laboratory internal sample identifier
SampleDate	Date/Time	Y	The date the sample was analyzed (must be the same date as when the sample was taken) expressed as dd-mmm-yyyy
SampleTime	Number	Y	The time the sample was collected expressed as hh:mm
SamplerID	Text	Y	Name of person collecting sample
AnalysisDate	Date/Time	Y	The date the sample was analyzed (must be the same date as when the sample was taken) expressed as dd-mmm-yyyy
AnalysisTime	Number	Y	The time the testing was started expressed as hh:mm
AnalystID	Text	Y	Name of person analyzing sample
ParameterCode	Text	Y	What type of bacteria are being tested
Qualifier	Text	N	Qualifier for the result
Result	Number	Y	The numerical results of the test
ResultUnits	Text	Y	The units for the results
TextValue	Text	Y	Explanation for sample not analyzed, default None, luAnalyticalFailure
Dilution	Number	Y	The dilution factor associated with the result.
LabRep	Text	Y	The count of the lab replicate.
AnalysisMethod	Text	Y	The Method used to do the analysis
Comments	Text	N	Additional comments

luAgencyCode	
Agency Code	**Agency Description**
CLA EMD	City of Los Angeles, Environmental Monitoring Division
DHS	Department of Health Services
LACSD	Los Angeles County Sanitation Districts

luStations	
Station Name	**DESCRIPTION**

luAnalyticalFailure	
None	No analytical failure, default value
AE	Analyst error
NA	Station not accessible, no sample taken
NS	No sample
NT	Sample not tested

APPENDIX K

Final Resolution and Attachment to Final Resolution

State of California
California Regional Water Quality Control Board, Los Angeles Region

RESOLUTION NO. 2006-011
June 8, 2006

Amendment to the Water Quality Control Plan for the Los Angeles Region to Incorporate a Total Maximum Daily Load for Bacteria in Ballona Creek, Ballona Estuary and Sepulveda Channel.

WHEREAS, the California Regional Water Quality Control Board, Los Angeles Region, finds that:

1. The Federal Clean Water Act (CWA) requires the California Regional Water Quality Control Board (Regional Board) to develop water quality objectives which are sufficient to protect beneficial uses for each water body found within its region.

2. A consent decree between the U.S. Environmental Protection Agency (USEPA), Heal the Bay, Inc. and BayKeeper, Inc. was approved on March 22, 1999. This court order directs the USEPA to complete Total Maximum Daily Loads (TMDLs) for all impaired waters within 13 years. A schedule was established in the consent decree for the completion of the first 29 TMDLs within 7 years. The remaining TMDLs will be scheduled by Regional Board staff within the 13-year period.

3. The elements of a TMDL are described in 40 CFR 130.2 and 130.7 and section 303(d) of the CWA, as well as in USEPA guidance documents (Report No. EPA/440/4-91/001). A TMDL is defined as the sum of the individual waste load allocations for point sources and load allocations for nonpoint sources and natural background (40 CFR 130.2). Regulations further stipulate that TMDLs must be set at levels necessary to attain and maintain the applicable narrative and numeric water quality standards with seasonal variations and a margin of safety that takes into account any lack of knowledge concerning the relationship between effluent limitations and water quality (40 CFR 130.7(c)(1)). The regulations in 40 CFR 130.7 also state that TMDLs shall take into account critical conditions for stream flow, loading and water quality parameters.

4. The numeric targets in this TMDL are not water quality objectives and do not create new bases for enforcement against dischargers apart from the water quality objectives they translate. The targets merely establish the bases through which load allocations (LAs) and waste load allocations (WLAs) are calculated. WLAs are only enforced for a discharger's own discharges, and then only in the context of it National Pollutant Discharge Elimination System (NPDES) permit, which must be consistent with the assumptions and requirements of the WLA. The Regional Board will develop permit requirements through a subsequent permit action that will allow all interested persons, including but not limited to municipal

storm water dischargers, to provide comments on how the WLA will be translated into permit requirements.

5. Upon establishment of TMDLs by the State or USEPA, the State is required to incorporate the TMDLs along with appropriate implementation measures into the State Water Quality Management Plan (40 CFR 130.6(c)(1), 130.7). This Water Quality Control Plan for the Los Angeles Region (Basin Plan), and applicable statewide plans, serve as the State Water Quality Management Plans governing the watersheds under the jurisdiction of the Regional Board.

6. As envisioned by Water Code section 13242, the TMDL contains a "description of surveillance to be undertaken to determine compliance with objectives." The Compliance Monitoring and Special Studies elements of the TMDL recognize that monitoring will be necessary to assess the on-going condition of the Ballona Creek, Estuary, and their tributaries and to assess the on-going effectiveness of efforts by dischargers to reduce bacteria loading to these waterbodies. Special studies may also be appropriate to provide further information about new data, new or alternative sources, and revised scientific assumptions. The TMDL does not establish the requirements for these monitoring programs or reports, although it does recognize the type of information that will be necessary to secure. The Regional Board's Executive Officer will issue orders to appropriate entities to develop and to submit monitoring programs and technical reports. The Executive Officer will determine the scope of these programs and reports, taking into account any legal requirements, and issue the orders to the appropriate entities.

7. Ballona Creek flows as an open channel for just under 10 miles from Los Angeles (South of Hancock Park) through Culver City, reaching the Pacific Ocean at Playa del Rey. It is entirely lined in concrete and is fed by a complex underground network of storm drains, which reaches north to Beverly Hills and West Hollywood. Tributaries of the creek include Centinela Creek, Sepulveda Canyon Channel, Benedict Canyon Channel, and numerous other storm drains. The creek meets Ballona Estuary, at Centinela Avenue, where concrete is replaced by grouted riprap side slopes and an earthen bottom. Ballona estuary flows into the Santa Monica Bay, and its water quality affects the adjacent shoreline of Dockweiler Beach.

8. The Regional Board's goal in establishing the Ballona Creek, Ballona Estuary, and Sepulveda Channel TMDL is to reduce the risk of illness associated with recreating in waters contaminated with human sewage and other sources of bacteria. Local and national epidemiological studies compel the conclusion that there is a causal relationship between adverse health effects, such as gastroenteritis, and recreational water quality, as measured by bacteria indicator densities.

9. The Regional Board recognizes that there are two broad approaches to implementing the TMDL. One approach is an integrated water resources approach. An integrated water resources approach has been previously defined by the Regional Board in the Santa Monica Bay Beaches Bacteria Wet Weather TMDL (Regional Board Resolution No. 2002-022 and attachments). For clarification, the Regional Board considers natural treatment systems (e.g. grassy swales, wetlands, vegetated buffers) to be consistent with an integrated water resources approach.

10. Regional Board staff have prepared a detailed technical document that analyzes and describes the specific necessity and rationale for the development of this TMDL. The technical

document entitled "Total Maximum Daily Loads for Bacterial Indicator Densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel" is an integral part of this Regional Board action and was reviewed, considered, and accepted by the Regional Board before acting. Further, the technical document provides the detailed factual basis and analysis supporting the problem statement, numeric targets (interpretation of the numeric water quality objective, used to calculate the load allocations), source analysis, linkage analysis, waste load allocations (for point sources), load allocation (for nonpoint sources), margin of safety, and seasonal variations and critical conditions of this TMDL.

11. On June 8, 2006, prior to the Board's action on this resolution, public hearings were conducted on the "Total Maximum Daily Loads for Bacterial Indicator Densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel". Notice of the hearing for the "Total Maximum Daily Loads for Bacteria Densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel" was published in accordance with the requirements of Water Code section 13244. This notice was published in the Los Angeles Times on April 3, 2006.

12. The public has had reasonable opportunity to participate in review of the amendment to the Basin Plan. A draft of the TMDL for bacteria densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel was released for public comment on April 3, 2006; a Notice of Hearing and Notice of Filing were published and circulated 45 days preceding Board action; Regional Board staff responded to oral and written comments received from the public; and the Regional Board held a public hearing on June 8, 2006 to consider adoption of the TMDL. In addition, input from participants in the stakeholder group "Cleaner Rivers through Effective Stakeholder TMDLs" (CREST) was solicited in developing potential implementation options to achieve compliance with the waste load allocations, and in estimating associated costs of selected strategies. CREST is a stakeholder effort initiated by the City of Los Angeles geared towards collaborative TMDL development in the Los Angeles River and Ballona Creek watersheds.

13. In amending the Basin Plan, the Regional Board considered the factors set forth in sections 13240 and 13242 of the Water Code.

14. The amendment is consistent with the State Antidegradation Policy (State Board Resolution No. 68-16), in that the changes to water quality objectives (i) consider maximum benefits to the people of the state, (ii) will not unreasonably affect present and anticipated beneficial use of waters, and (iii) will not result in water quality less than that prescribed in policies. Likewise, the amendment is consistent with the federal Antidegradation Policy (40 CFR 131.12).

15. Pursuant to Public Resources Code section 21080.5, the Resources Agency has approved the Regional Water Boards' basin planning process as a "certified regulatory program" that adequately satisfies the California Environmental Quality Act (CEQA) (Public Resources Code, Section 21000 et seq) requirements for preparing environmental documents. (14 Cal. Code Regs. § 15251(g); 23 Cal. Code Regs. § 3782.) As such, the Regional Water Board's basin planning documents together with an Environmental Checklist, are the "substitute documents" that contain the required environmental documentation under CEQA. (23 Cal Code Regs. § 3777.) The detailed technical report entitled "Total Maximum Daily Load for Bacteria Densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel," responses prepared by staff to address comments raised during the development of the TMDL, this resolution, and the Environmental Checklist serve as the substitute documents for this project. The project itself is the establishment of a TMDL for bacteria in Ballona Creek, Ballona

Estuary, and Sepulveda Channel. While the Regional Board has no discretion to not establish a TMDL (the TMDL is required by federal law) or for determining the water quality standard to be applied (the Basin Plan establishes the numeric water quality objectives that must be implemented), the Board does exercise discretion in assigning waste load allocations and load allocations, determining the program of implementation, and setting various milestones in achieving the numeric water quality standards established in the Basin Plan.

16. A CEQA Scoping hearing was conducted on June 12, 2003 at the Los Angeles Regional Water Quality Control Board, 320 W. 4th Street, Los Angeles, CA 90013. A notice of the CEQA Scoping hearing was sent to interested parties including cities and/or counties with jurisdiction in or bordering the Ballona Creek watershed.

17. The lengthy implementation period allowed by the TMDL, will allow many compliance approaches to be pursued. In preparing the accompanying CEQA substitute documents, the Regional Board has considered the requirements of Public Resources Code section 21159 and California Code of Regulations, title 14, section 15187, and intends the substitute documents to serve as a tier 1 environmental review. Nearly all of the compliance obligations will be undertaken by public agencies that will have their own obligations under CEQA. Project level impacts will need to be considered in any subsequent environmental analysis performed by other public agencies, pursuant to Public Resources Code section 21159.2. If not properly mitigated at the project level, there could be adverse environmental impacts. The substitute documents for this TMDL, and in particular the Environmental Checklist and staff's responses to comments, identify broad mitigation approaches that should be considered at the project level. Consistent with CEQA, the substitute documents do not engage in speculation or conjecture and only consider the reasonably foreseeable environmental impacts of the methods of compliance, the reasonably foreseeable feasible mitigation measures, and the reasonably foreseeable alternative means of compliance, which would avoid or eliminate the identified impacts.

18. The proposed amendment could have a significant adverse effect on the environment. However, there are feasible alternatives, feasible mitigation measures, or both that would substantially lessen any significant adverse impact. The public agencies responsible for those parts of the project can and should incorporate such alternatives and mitigation into any subsequent projects or project approvals. Possible alternatives and mitigation are described in the CEQA substitute documents, specifically the TMDL technical report and the Environmental Checklist. To the extent the alternatives, mitigation measures, or both are not deemed feasible by those agencies, the necessity of implementing the federally required bacteria TMDL and reducing the elevated bacteria densities from Ballona Creek, Ballona Estuary, and Sepulveda Channel (an action required to achieve the express, national policy of the Clean Water Act) outweigh the unavoidable adverse environmental effects.

19. The regulatory action meets the "Necessity" standard of the Administrative Procedures Act, Government Code, Section 11353, Subdivision (b). As specified above, federal regulations require that TMDLs be incorporated into the water quality management plan. The Regional Board's Basin Plan is the Regional Board's component of the water quality management plan, and the Basin Plan is how the Regional Board takes quasi-legislative, planning actions. Moreover, the TMDL is a program of implementation for existing water quality objectives, and is, therefore, appropriately a component of the Basin Plan under Water Code section 13242. The necessity of developing a TMDL is established in the TMDL staff report, the section 303(d) list, and the data contained in the administrative record documenting the bacteria impairments of the Ballona Creek, Ballona Estuary, and Sepulveda Channel.

20. The Basin Plan amendment incorporating a TMDL for Bacteria Densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel must be submitted for review and approval by the State Water Resources Control Board (State Board), the State Office of Administrative Law (OAL), and the USEPA. The Basin Plan amendment will become effective upon approval by OAL and USEPA. A Notice of Decision will be filed.

21. If during its approval process Regional Board staff, the SWRCB or OAL determines that minor, non-substantive corrections to the language of the amendment are needed for clarity or consistency, the Executive Officer may make such changes, and shall inform the Board of any such changes.

THEREFORE, be it resolved that pursuant to sections 13240 and 13242 of the Water Code, the Regional Board hereby amends the Basin Plan as follows:

1. Pursuant to sections 13240 and 13242 of the California Water Code, the Regional Board, after considering the entire record, including oral testimony at the hearing, hereby adopts the amendments to Chapters 3 and 7 of the Water Quality Control Plan for the Los Angeles Region, as set forth in Attachment A hereto, to incorporate the elements of the bacteria TMDL for Ballona Creek, Ballona Estuary, and Sepulveda Channel.

2. The Executive Officer is directed to forward copies of the Basin Plan amendment to the State Board in accordance with the requirements of section 13245 of the California Water Code.

3. The Regional Board requests that the State Board approve the Basin Plan amendment in accordance with the requirements of sections 13245 and 13246 of the California Water Code and forward it to OAL and the USEPA.

4. If during its approval process the State Board or OAL determines that minor, non-substantive corrections to the language of the amendment are needed for clarity or consistency, the Executive Officer may make such changes, and shall inform the Board of any such changes.

5. The Executive Officer is authorized to sign a Certificate of Fee Exemption.

I, Jonathan S. Bishop, Executive Officer, do hereby certify that the foregoing is a full, true, and correct copy of a resolution adopted by the California Regional Water Quality Control Board, Los Angeles Region, on June 8, 2006.

Chief Deputy

Jonathan S. Bishop
Executive Officer

As adopted on June 8, 2006

Attachment A to Resolution No. 06-011

Amendment to the Water Quality Control Plan – Los Angeles Region to incorporate the TMDL for Bacterial Indicator Densities in Ballona Creek, Ballona Estuary, and Sepulveda Channel.

Adopted by the California Regional Water Quality Control Board, Los Angeles Region on June 8, 2006.

Amendments:

Table of Contents
Add:

Chapter 7. Total Maximum Daily Loads (TMDLs) Summaries
7-21 Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL

List of Figures, Tables and Inserts
Add:

Chapter 7. Total Maximum Daily Loads (TMDLs)
Tables
7-21 Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL
7-21.1. Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL: Elements
7-21.2a. Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL: Final Allowable Exceedance Days by Reach
7.21.2b. Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL: WLAs and LAs for tributaries to the Impaired Reaches.
7-21.3. Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL: Significant Dates

Chapter 7. Total Maximum Daily Loads (TMDLs) Summaries, Section 7-21 (Ballona Creek, Ballona Estuary, and Sepulveda Channel Bacteria TMDL)

This TMDL was adopted by the Regional Water Quality Control Board on June 8, 2006.

This TMDL was approved by:

The State Water Resources Control Board on [Insert Date].
The Office of Administrative Law on [Insert Date].
The U.S. Environmental Protection Agency on [Insert Date].

The following table includes all the elements of this TMDL.

Table 7-21.1. Ballona Creek, Estuary, and Tributaries s Bacteria TMDL: Elements

Element	Key Findings and Regulatory Provisions
Problem Statement	Elevated bacterial indicator densities are causing impairment of the water contact recreation (REC-1) beneficial use designated for Ballona Estuary and Sepulveda Channel, limited water contact recreation (LREC) designated for Ballona Creek Reach 2, and non-contact recreation (REC-2) beneficial uses of Ballona Creek Reach 1. Recreating in waters with elevated bacterial indicator densities has long been associated with adverse human health effects. Specifically, local and national epidemiological studies compel the conclusion that there is a causal relationship between adverse health effects and recreational water quality, as measured by bacterial indicator densities.
Numeric Target *(Interpretation of the numeric water quality objective, used to calculate the waste load allocations)*	The TMDL has a multi-part numeric target based on the bacteriological water quality objectives for marine and fresh water to protect the contact and non-contact recreation uses. These targets are the most appropriate indicators of public health risk in recreational waters. These bacteriological objectives are set forth in Chapter 3 of the Basin Plan.[1] The objectives are based on four bacterial indicators and include both geometric mean limits and single sample limits. The Basin Plan objectives that serve as the numeric targets for this TMDL are: In Marine Waters Designated for Water Contact Recreation (REC-1) 1. Geometric Mean Limits a. Total coliform density shall not exceed 1,000/100 ml. b. Fecal coliform density shall not exceed 200/100 ml. c. Enterococcus density shall not exceed 35/100 ml. 2. Single Sample Limits a. Total coliform density shall not exceed 10,000/100 ml. b. Fecal coliform density shall not exceed 400/100 ml. c. Enterococcus density shall not exceed 104/100 ml. d. Total coliform density shall not exceed 1,000/100 ml, if the ratio of fecal-to-total coliform exceeds 0.1. In Fresh Waters Designated for Water Contact Recreation (REC-1) 1. Geometric Mean Limits a. *E. coli* density shall not exceed 126/100 ml. b. Fecal coliform density shall not exceed 200/100 ml. 2. Single Sample Limits a. *E. coli* density shall not exceed 235/100 ml. b. Fecal coliform density shall not exceed 400/100 ml.

[1] The bacteriological objectives were revised by a Basin Plan amendment adopted by the Regional Board on October 25, 2001, and subsequently approved by the State Water Resources Control Board, the Office of Administrative Law and finally by U.S. EPA on September 25, 2002.

Element	Key Findings and Regulatory Provisions
	In Fresh Waters Designated for Limited Water Contact Recreation (LREC-1)[2] 1. Geometric Mean Limits a. *E. coli* density shall not exceed 126/100 ml. b. Fecal coliform density shall not exceed 200/100 ml. 2. Single Sample Limits a. *E. coli* density shall not exceed 576/100 ml. In Fresh Waters Designated for Non-Contact Water Recreation (REC-2) 1. Geometric Mean Limits a. Fecal coliform density shall not exceed 2000/100 ml. 2. Single Sample Limits a. Fecal coliform density shall not exceed 4000/100 ml. The targets apply throughout the year. Determination of attainment of the targets will be at in-stream monitoring sites to be specified in the compliance monitoring report. Implementation of the above REC-1 and LREC-1 bacteria objectives and the associated TMDL numeric targets is achieved using a 'reference system/anti-degradation approach' rather than the alternative 'natural sources exclusion approach subject to antidegradation policies' or strict application of the single sample objectives. As required by the CWA and Porter-Cologne Water Quality Control Act, Basin Plans include beneficial uses of waters, water quality objectives to protect those uses, an anti-degradation policy, collectively referred to as water quality standards, and other plans and policies necessary to implement water quality standards. This TMDL and its associated waste load allocations, which shall be incorporated into relevant permits, and load allocations are the vehicles for implementation of the Region's standards. The 'reference system/anti-degradation approach' means that on the basis of historical exceedance levels at existing monitoring locations, including a local reference beach within Santa Monica Bay, a certain number of daily exceedances of the single sample bacteria objectives are permitted. The allowable number of exceedance days is set such that (1) bacteriological water quality at any site is at least as good as at a designated reference site within the watershed and (2) there is no degradation of existing bacteriological water quality. This approach recognizes that there are natural sources of bacteria that may cause or contribute to exceedances of the single sample objectives and that it is not the intent of the Regional Board to require treatment or diversion of natural coastal creeks or to require treatment of natural sources of bacteria from undeveloped areas.

[2] The bacteriological objectives for the LREC-1 use designation were provided in a Basin Plan Amendment adopted by State Board on January 20, 2005, and subsequently approved by the Office of Administrative Law and finally by U.S. EPA on February 17, 2006

Element	Key Findings and Regulatory Provisions
	The geometric mean targets may not be exceeded at any time. The rolling 30-day geometric means will be calculated on each day. If weekly sampling is conducted, the weekly sample result will be assigned to the remaining days of the week in order to calculate the daily rolling 30-day geometric mean. For the single sample targets, each existing monitoring site is assigned an allowable number of exceedance days for three time periods (1) summer dry-weather (April 1 to October 31), (2) winter dry-weather (November 1 to March 31), and (3) wet-weather (defined as days with 0.1 inch of rain or greater and the three days following the rain event.) Implementation of the REC-2 target will be as specified in the Basin Plan. The REC-2 bacteria objectives allow for a 10% exceedance frequency of the single sample limit in samples collected during a 30-day period. This allowance, which is based on an acceptable level of health risk, will be applied in lieu of the allowable exceedance days discussed earlier. As with the other REC-1 and LREC-1 objectives, the geometric mean target for REC-2, which is based on a rolling 30-day period, will be strictly adhered to and may not be exceeded at any time.
Source Analysis	The major contributors of flows and associated bacteria loading to Ballona Creek and Estuary, are dry- and wet-weather urban runoff discharges from the storm water conveyance system. Run-off to Ballona Creek is regulated as a point source under the Los Angeles County MS4 Permit, the Caltrans Storm Water Permit, and the General Construction and Industrial Storm Water Permits. In addition to these regulated point sources, the Ballona Estuary receives input from the Del Rey Lagoon and Ballona Wetlands through connecting tide gates. Preliminary data suggest that the Ballona Wetlands are a sink for bacteria from Ballona Creek and it is therefore not considered a source in this TMDL. Inputs to Ballona Estuary from Del Rey Lagoon, are considered non-point sources of bacterial contamination. This waterbody may be considered for a natural source exclusion if its contributing bacteria loads are determined to be as a result of wildlife in the area, as opposed to anthropogenic inputs. The TMDL will require a source identification study for the lagoon in order to apply the natural source exclusion. Other nonpoint sources in Ballona Creek and Estuary include natural sources from birds, waterfowl and other wildlife. Data do not currently exist to quantify the extent of the impact of wildlife on bacteria water quality in the Estuary.
Loading Capacity	The loading capacity is defined in terms of bacterial indicator densities, which is the most appropriate for addressing public health risk, and is equivalent to the numeric targets, listed above.
Waste Load Allocations *(for point sources)*	The Los Angeles County MS4 and Caltrans storm water permittees and co-permittees are assigned waste load allocations (WLAs) expressed as the number of daily or weekly sample days that may exceed the single sample targets equal to the TMDLs established for the impaired reaches (see Table 7.21.2a), and Waste Load Allocations assigned to waters tributary to impaired reaches (Table 7.21.2b). Waste load allocations are expressed as allowable exceedance days because the bacterial density and frequency of single sample

Element	Key Findings and Regulatory Provisions
	exceedances are the most relevant to public health protection. For each monitoring site, allowable exceedance days are set on an annual basis as well as for three time periods. These three periods are: 1. summer dry-weather (April 1 to October 31) 2. winter dry-weather (November 1 to March 31) 3. wet-weather days (defined as days of 0.1 inch of rain or more plus three days following the rain event). The County of Los Angeles, Caltrans, and the Cities of Los Angeles, Culver City, Beverly Hills, Inglewood, West Hollywood, and Santa Monica are the responsible jurisdictions and responsible agencies[3] for the Ballona Creek Watershed. The responsible jurisdictions and responsible agencies within the watershed are jointly responsible for complying with the waste load allocation in each reach. For the single sample objectives of the impaired REC-1 and LREC-1 reaches, the proposed WLA for summer dry-weather are zero (0) days of allowable exceedances, and those for winter dry-weather and wet-weather are three (3) days and seventeen (17) days of exceedance, respectively. In the instances where more than one single sample objective applies, exceedance of any one of the limits constitutes an exceedance day. The proposed waste load allocation for the rolling 30-day geometric mean for the responsible agencies and jurisdictions is zero (0) days of allowable exceedances. For the single sample objectives of the impaired REC-2 reach, the proposed WLA for all periods is a 10% exceedance frequency of the REC-2 single sample water quality objectives. The proposed waste load allocation for the rolling 30-day geometric mean for the responsible agencies and jurisdictions is zero (0) days of allowable exceedances. In addition to assigning TMDLs for the impaired reaches, Waste Load Allocations and Load Allocations are assigned to the tributaries to these impaired reaches. These WLAs and LAs are to be met at the confluence of each tributary and its downstream reach (see Table 7.21.2b).
Load Allocations *(for nonpoint sources)*	Load allocations are expressed as the number of daily or weekly sample days that may exceed the single sample targets identified under "Numeric Target" at a monitoring site, along with a rolling 30-day geometric mean. Load allocations are expressed as allowable exceedance days because the bacterial density and frequency of single sample exceedances are the most relevant to public health protection. Del Rey Lagoon is considered a nonpoint source and is therefore subject to load allocations. The proposed LA for summer dry-weather are zero (0) days of allowable exceedances, and those for winter dry-weather and wet-weather are three (3) days and seventeen (17) days of exceedance, respectively. In the instances where more than one single sample objective applies, exceedance of any one of the limits constitutes an exceedance day. The proposed load allocation for the rolling 30-day geometric mean for the responsible agencies and

[3] For the purposes of this TMDL, "responsible jurisdictions and responsible agencies" are defined as (1) local agencies that are permittees or co-permittees on a municipal storm water permit, (2) local or state agencies that have jurisdiction over Ballona Creek and Estuary, and (3) the California Department of Transportation pursuant to its storm water permit.

Element	Key Findings and Regulatory Provisions
	jurisdictions is zero (0) days of allowable exceedances (see Table 7.21.2a). The City of Los Angeles is the responsible jurisdiction for the Del Rey lagoon, and is responsible for complying with the assigned load allocations presented in Table 7.21.2b at the tide gate(s) between the Lagoon and the Estuary. If other unidentified nonpoint sources are directly impacting bacteriological water quality and causing an exceedance of the numeric targets, within the Estuary, the permittee(s) under the Municipal Storm Water NPDES Permits are not responsible through these permits. However, the jurisdiction or agency adjacent to the monitoring location may have further obligations to identify such sources.
Implementation	The regulatory mechanisms used to implement the TMDL will include the Los Angeles County Municipal Storm Water NPDES Permit (MS4), the Caltrans Storm Water Permit, general NPDES permits, general industrial storm water permits, general construction storm water permits, and the authority contained in Sections 13263 and 13267 of the Water Code. Each NPDES permit assigned a WLA shall be reopened or amended at re-issuance, in accordance with applicable laws, to incorporate the applicable WLAs as a permit requirement. Each responsible jurisdictions and agency will be required to meet the storm water waste load allocations shared by the LA County MS4 and Caltrans permittees at the designated TMDL effectiveness monitoring points. An iterative implementation approach using a combination of non-structural and structural BMPs may be used to achieve compliance with the waste load allocations. The administrative record and the fact sheets for the MS4 and Caltrans storm water permits must provide reasonable assurance that the BMPs selected will be sufficient to implement the waste load allocation. Load allocations for nonpoint sources will be incorporated into Waste Discharge Requirements and MOUs with the responsible jurisdictional agencies. This TMDL will be implemented in two phases over a ten-year period (see Table 7-21.3). Within six years of the effective date of the TMDL, compliance with the allowable number of summer dry-weather (April 1 to October 31), winter dry-weather exceedance days (November 1 to March 31) and the rolling 30-day geometric mean targets for both periods must be achieved. Within ten years of the effective date of the TMDL, compliance with the allowable number of wet-weather exceedance days and rolling 30-day geometric mean targets must be achieved. In order to clearly justify an extended implementation schedule beyond 10 years and up to 14 years from the effective date of the TMDL, the responsible agencies are required to submit additional quantifiable analyses as described below to demonstrate (1) the proposed plans will meet the final WLAs and (2) the proposed implementation actions will achieve multiple water quality benefits and other public goals. The types of approaches proposed coupled with quantifiable estimates of the integrated water resources benefits of the proposed structural and non-structural BMPs included in the Implementation Plan would provide the obligatory demonstration that an integrated water resources approach is being

Element	Key Findings and Regulatory Provisions
	pursued. This demonstration shall include numeric estimates of the benefits, including but not limited to reductions in other pollutants, groundwater recharged, acres of multi-use projects and water (e.g. urban runoff) beneficially reused. The responsible jurisdictions and the responsible agencies must submit a report to the Executive Officer (see Table 7-21.3) describing how they intend to comply with the dry-weather and wet-weather WLAs. As the primary jurisdiction, the City of Los Angeles is responsible for submitting the implementation plan report described above. In addition, as the responsible agency for Del Rey Lagoon, the City of Los Angeles must submit a report detailing how it intends to comply with the load allocations assigned to this waterbody. Alternatively, the City of Los Angeles may submit data clearly demonstrating that Del Rey Lagoon is not a source, for the Regional Board's consideration.. The Regional Board intends to reconsider this TMDL, within 4 years of its effective date to incorporate modifications to the WLAs based on results of the scheduled reconsideration of the Santa Monica Bay (SMB) beaches TMDLs. The SMB beaches TMDLs are scheduled to be reconsidered in four years to re-evaluate the allowable winter dry-weather and wet-weather exceedance days based on additional data on bacterial indicator densities in the wave wash; to re-evaluate the reference system selected to set allowable exceedance levels; to re-evaluate the reference year used in the calculation of allowable exceedance days, and to re-evaluate the need for revision of the geometric mean implementation provision. The Regional Board also intends to re-asses the WLAs for Benedict Canyon Channel, Sepulveda Channel, and Centinela Creek based on results of the required compliance monitoring, and/or any voluntary beneficial use investigations.
Margin of Safety	By directly applying the numeric water quality standards and implementation procedures as Waste Load Allocations, there is little uncertainty about whether meeting the TMDLs will result in meeting the water quality standards.
Seasonal Variations and Critical Conditions	Seasonal variations are addressed by developing separate waste load allocations for three time periods (summer dry-weather, winter-dry weather, and wet-weather) based on public health concerns and observed natural background levels of exceedance of bacterial indicators. The critical condition for bacteria loading to the Ballona Creek, Ballona Estuary, and Sepulveda Channel is during wet weather when monitoring data indicate greater exceedance probabilities of the single sample bacteria objectives than during dry-weather. The Santa Monica Bay Beaches Bacteria TMDL identified the critical condition within wet weather more specifically, in order to set the allowable number of exceedances of the single sample limit days. The 90^{th} percentile storm year in terms of wet days was used as the reference year. The 90^{th} percentile year was selected for several reasons. First, selecting the 90^{th} percentile year avoids an untenable situation where the reference system is frequently out of compliance. Second, selecting the 90^{th} percentile year allows responsible jurisdictions and responsible agencies to plan for a 'worst-case scenario', as a critical condition is intended to do

Element	Key Findings and Regulatory Provisions
Monitoring	The TMDL effectiveness monitoring program will assess attainment of the allowable exceedances for Ballona Creek, Ballona Estuary, and Sepulveda Channel, and the WLAs for the tributaries. Responsible jurisdictions and responsible agencies shall conduct daily or systematic weekly sampling at a minimum of two locations within Ballona Estuary and Reach 2 of Ballona Creek, at least one location each in Reach 1 of Ballona Creek and Sepulveda Channel, and at the confluence with Centinela Creek and Benedict Canyon Channel, to determine compliance. Similar monitoring at the connecting tide gates of Del Rey Lagoon is also required. Where monitoring locations are located at or close to the boundary of two reaches, data from sampling points will also be used to assess the immediate downstream reach. This will ensure that the downstream reaches, which have more stringent water quality objectives, are adequately protected. If the number of exceedance days is greater than the allowable number of exceedance days in the REC-1 and LREC-1 waters, and/or the frequency of exceedance is greater than 10% in the REC-2 waters, the responsible jurisdictions and/or responsible agencies shall be considered not to be attaining the TMDLs and/or assigned allocations (non-attaining). Responsible jurisdictions or agencies shall not be deemed non-attaining if the investigation described in the paragraph below demonstrates that bacterial sources originating within the jurisdiction of the responsible agency have not caused or contributed to the exceedance. If an in-stream location is non-attaining as determined in the previous paragraph, the Regional Board shall require responsible agencies to initiate an investigation, which at a minimum shall include daily sampling at the existing monitoring location until all single sample events meet bacteria water quality objectives.
Special Studies	Should the jurisdictional agency for Del Rey Lagoon opt for the natural source exclusion, the TMDL requires that a separate bacteria source identification study be conducted to determine its eligibility.. The study should identify all probable sources of bacteria loads, their estimated contributions to the Lagoon, and a determination of the frequency of exceedances of the single sample bacteria objectives caused by the identified natural sources.

Table 7.21.2a: Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL: Final Allowable Exceedance Days by Reach

Time Period	Ballona Estuary, Ballona Creek Reach 2, and Sepulveda Channel *	Ballona Creek Reach 1**
Summer Dry-Weather (April 1 to October 31)	Zero (0) exceedance days based on the applicable Single Sample Bacteria Water Quality Objectives Zero (0) exceedance days based on the Rolling 30-Day Geometric Mean Bacteria Water Quality Objectives	No more than 10% of the Single Sample Bacteria Water Quality Objectives Zero (0) exceedance days based on the Rolling 30-Day Geometric Mean Bacteria Water Quality Objectives
Winter Dry-Weather (November 1-March 31)	Three (3) exceedance days based on the applicable Single Sample Bacteria Water Quality Objectives Zero (0) exceedance days based on the Rolling 30-Day Geometric Mean Bacteria Water Quality Objectives	No more than 10% of the Single Sample Bacteria Water Quality Objectives Zero (0) exceedance days based on the Rolling 30-Day Geometric Mean Bacteria Water Quality Objectives
Wet-Weather (days with ≥0.1 inch of rain + 3 days following the rain event)	17*** exceedance days based on the applicable Single Sample Bacteria Water Quality Objectives Zero (0) exceedance days based on the Rolling 30-Day Geometric Mean Bacteria Water Quality Objectives	No more than 10% of the Single Sample Bacteria Water Quality Objectives Zero (0) exceedance days based on the Rolling 30-Day Geometric Mean Bacteria Water Quality Objectives

* Exceedance days for Ballona Estuary based on REC-1 marine water numeric targets; for Ballona Creek Reach 2 based on LREC-1 freshwater numeric targets; and for Sepulveda Channel, based on fresh water REC-1 numeric targets

**Exceedance frequency for Ballona Creek Reach 1 based on freshwater REC-2 numeric targets

*** In Reach 2, the greater of the allowable exceedance days under the reference system approach or high flow suspension shall apply.

Table 7.21.2b: Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL: WLAs and LAs for tributaries to the Impaired Reaches.

Tributary	Point of Application	Water Quality Objectives	Waste Load Allocation (No. exceedance days)
Ballona Creek Reach 1	At confluence with Reach 2	LREC-1 Freshwater	For single sample objectives: *(0) summer dry weather,* *(3) winter dry weather* *(17*) winter wet weather* For geometric mean objectives: *(0) for all periods*
Benedict Canyon Channel	At confluence with Reach 2	LREC-1 Freshwater	For single sample objectives: *(0) summer dry weather,* *(3) winter dry weather* *(17*) winter wet weather* For geometric mean objectives: *(0) for all periods*
Ballona Creek Reach 2	At confluence with Ballona Estuary	REC-1 Marine water	For single sample objectives: *(0) summer dry weather,* *(3) winter dry weather* *(17) winter wet weather* For geometric mean objectives: *(0) for all periods*
Centinela Creek	At confluence with Ballona Estuary	REC-1 Marine water	For single sample objectives: *(0) summer dry weather,* *(3) winter dry weather* *(17) winter wet weather* For geometric mean objectives: *(0) for all periods*
Del Rey Lagoon	At confluence with Ballona Estuary	REC-1 Marine water	For single sample objectives: *(0) summer dry weather,* *(3)winter dry weather* *(17) winter wet weather* For geometric mean objectives: *(0) for all periods*

*At the confluence with Reach 2, the greater of the allowable exceedance days under the reference system approach or high flow suspension shall apply.

Sepulveda Channel was not assigned a waste load allocation at its confluence with Reach 2 since the TMDL requires the more stringent REC-1 objectives to be met in this waterbody, which should lead to the attainment of the less stringent LREC-1 objectives of the downstream reach.

Table 7.21.3 Ballona Creek, Ballona Estuary and Sepulveda Channel Bacteria TMDL: Significant Dates

Date	Action
Responsible Jurisdictions for the Waste Load Allocations	
12 months after the effective date of the TMDL	Responsible jurisdictions and responsible agencies must submit, for Regional Board approval, a comprehensive bacteria water quality monitoring plan for the Ballona Creek Watershed. The plan must be approved by the Executive Officer before the monitoring data can be considered during the implementation of the TMDL. The plan must provide for analyses of all applicable bacteria indicators for which the Basin Plan and subsequent amendments have established objectives The plan must also include a minimum of two sampling locations (mid-stream and downstream) in Ballona Estuary, Ballona Creek (Reach 1 and 2), and their tributaries. The draft monitoring report shall be made available for public comment and the Executive Officer shall accept public comments for at least 30 days. Once the coordinated monitoring plan is approved by the Executive Officer, monitoring shall commence within 6 months.
$2^1/_2$ years after the effective date of the TMDL	Responsible jurisdictions and agencies must provide a draft Implementation Plan to the Regional Board outlining how each intends to cooperatively achieve compliance with the dry-weather and wet-weather TMDL Waste Load Allocations. The report shall include implementation methods, an implementation schedule, and proposed milestones. The description of the implementation methods and milestones shall include a technically defensible quantitative linkage to the interim and final waste load allocations (WLAs). The linkage should include target reductions in stormwater runoff and/or fecal indicator bacteria. The plan shall include quantitative estimates of the water quality benefits provided by the proposed structural and non-structural BMPs. Estimates should address reductions in exceedance days, bacteria concentration and loading, and flow in the drain and at each beach compliance monitoring location. As part of the draft plan, responsible agencies must submit results of all special studies and/or Environmental Impact Assessments, designed to determine feasibility of any strategy that requires diversion and/or reduction of Creek flows. If a responsible jurisdiction or agency is requesting a longer schedule for wet-weather compliance based on an integrated approach, the plan must include a clear demonstration that the plan meets the criteria of an IWRA, and a clear demonstration of the need for the proposed schedule. Compliance with the wet-weather allocations shall be as soon as possible but under no circumstances shall it exceed the time frame adopted in the

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Date	Action
	TMDL for non-integrated approaches or for an integrated approach. The draft Plan shall be made available for public comment and the Executive Officer shall accept public comments for at least 30 days.
3 months after receipt of Regional Board comments on the draft plan	Responsible jurisdictions and agencies submit a Final Implementation Plan to the Regional Board.
Responsible agencies for Load Allocations	
1 year after the effective date of the TMDL	Responsible agencies must submit, for Regional Board approval, separate comprehensive bacteria water quality monitoring plans for inputs from Del Rey Lagoon and the Ballona Wetlands to the Ballona Estuary. Each plan must be approved by the Executive Officer before the monitoring data can be considered during the implementation of the TMDL. The plan must provide for analyses of all applicable bacteria indicators for which the Basin Plan and subsequent amendments have established objectives The plan must also include a minimum of one sampling location at the connecting tide gate(s). The draft monitoring reports shall be made available for public comment and the Executive Officer shall accept public comments for at least 30 days. Once a coordinated monitoring plan is approved by the Executive Officer, monitoring shall commence within 6 months.
3 years after the effective date of the TMDL.	If the responsible agency for the Del Rey Lagoon intends to pursue a natural source exclusion, it shall submit the results of separate natural source study for the Lagoon to the Executive Officer of the Regional Board. The study shall include a comprehensive assessment of all sources of bacteria loads to the Lagoon and estimates of their individual contributions. In addition, a determination of the number of exceedance days caused by these sources should be made These studies shall be made available for public comment and the Executive Officer shall accept public comments for at least 30 days.
Responsible Agencies for WLAs and LAs* *(*Only if not eligible for natural source exclusion(s)*	
4 years after the effective date of the TMDL:	The Regional Board shall reconsider this TMDL to: (1) Re-assess the allowable winter dry-weather and wet-weather exceedance days based on a re-evaluation of the selected reference watershed and consideration of other reference watersheds that may better represent reaches of Ballona

Date	Action
	Creek and Estuary, (2) Consider whether the allowable winter dry-weather and wet-weather exceedance days should be adjusted annually dependent on the rainfall conditions and an evaluation of natural variability in exceedance levels in the reference system(s), (3) Re-evaluate the reference year used in the calculation of allowable exceedance days, and (4) Re evaluate whether there is a need for further clarification or revision of the geometric mean implementation provision. (5) Consider natural source exclusions for bacteria loading from Del Rey Lagoon and the Ballona Wetlands based on results of the source identification study. (6) Re-assess WLAs for Benedict Canyon Channel, Sepulveda Channel, and Centinela Creek based on results of the required compliance monitoring, and/or any voluntary beneficial use investigations.
6 years after the effective date of the TMDL:	Achieve compliance with the allowable exceedance days for summer and winter dry-weather as set forth in Table 6-1 and rolling 30-day geometric mean targets.
10 years after effective date of the TMDL or, if an Integrated Water Resources Approach is implemented, up to July 15, 2021.*	Achieve compliance with the allowable exceedance days as set forth in Table 6-1 and rolling 30-day geometric mean targets during wet-weather.

*July 15, 2021 is the final compliance date of the Santa Monica Bay Beaches Bacteria Wet-Weather TMDL.

APPENDIX L

Laboratory Safety

The collection and analysis of environmental samples involves contact with samples that may contain agents that pose a chemical and microbiological hazard. The primary means of exposure to these chemical and microbiological hazards involve body contact during sample collection and hand-mouth or nose contact while handling the samples, eyes can get accidentally splashed and contaminated with water sample when the bottle with sample accidentally fell and cap opened. Personal protective measures are mandatory while working in the field and laboratory. Following are some key steps to be followed by all laboratory analysts:

a. Assure that all persons wear appropriate eye protection when toxic materials (chemicals or biochemicals) are handled. Contact lenses should not be worn when working with chemicals.
b. Material Safety Data Sheet (MSDS) for each chemical must be available at all time and should be accessible to all lab staff.
c. Before working in the laboratory, know where the eye wash, shower or other first aid kits are located.
d. Handle chemicals or biochemicals under the exhaust hood.
e. Always wear gloves, goggles, laboratory coat, proper laboratory shoes, face shield (when necessary) when working in the laboratory. You can be handling the sample or just working with the person handling the samples but you can still be in danger of contamination.
f. Consider all samples to have a possible potential hazard when handling them. After all, these samples are being monitored for any toxic substance. All safety gears must be worn at all times in the field or in the laboratory.
g. Wear appropriate gloves when the potential for contact with toxic materials exists; inspect gloves before each use, wash them before removal, and replace them periodically.
h. Persons doing sampling must wear boots. The boots must be cleaned before entering the building. Boots, laboratory coats and other safety gears must not be cannot be worn in the lunchroom, under any circumstances. Steel-toed chemical resistant boots should be worn for the harshest environments, where there is also risk of injury to the foot and toes. Hard hat must also be worn on field sampling when necessary.
   (Note: I won't be focusing more on safety on field sampling because the title says Laboratory Safety. Please let me know if you want me to.)
i. Use any other protective and emergency apparel and equipment as appropriate.
j. Remove laboratory coats immediately on significant contamination.
k. Report any accident to your supervisor.
l. Regular safety meeting should be provided to the staff.

In addition, persons who work in biological laboratories are often at risk of exposing themselves to a number of infectious agents, especially those known to be indigenous to wastewater. Most persons trained in biological and especially microbiological fields usually are aware of the risks involved, and even if precautions are taken, most of the work-related infections are due to certain practices conducted in the laboratory resulting in the generation of aerosols or through cutaneous pathways. The following guidelines are designed to prevent any exposure of personnel to infectious agents.

1. General chemical hygiene practices apply as well to the biological laboratories.
2. All work areas must be disinfected before and after all laboratory operations.
3. Hazardous areas and receptacles of contaminated items are to be marked with a biohazard sign.
4. No eating or drinking in the laboratory. No food or drink is to be stored in laboratory refrigerators, incubators or on bench tops.
5. Store personal effects outside the microbiology laboratory area to prevent contamination. Manager and supervisors are responsible for enforcing this rule.
6. It is policy to wear a lab coat while working in the microbiology lab. Lab coats and street clothes should be stored separately. Lab coats are prohibited in the lunchroom.
7. Latex or plastic gloves are to be provided and used by employees.
8. Always wash your hands thoroughly after handling sewage, sludge, or receiving water samples of any source before handling food or leaving the lab. "All" samples should be treated as potentially hazardous. Germicidal soap is to be available to all employees, and should be kept in stock.
9. Laboratory workers should not touch their hands to their face, especially the eyes, nose, and mouth when working with wastewater and sludge samples.
10. For workers who handle wastewater and its byproducts, it is recommended that they have been vaccinated for polio and tetanus. Persons in poor health and at risk of infection should inform their supervisor, and arrange for an improvement in their personal protection.
11. Handle all microorganisms as if they are pathogenic. The principle of sterile technique should be understood and applied during the handling of cultures and their related equipments.
12. Never pipette by mouth. Use bulbs or other mechanical means to draw up the liquid. Discard all used pipettes into a jar containing disinfectant solution for decontamination before washing them.
13. Avoid generation of aerosols during operations such as inoculation, pipetting, mixing, or centrifuging.
14. Equipment:
    a. Microscopes, colony counters, etc. are to be kept in the work area and be dust free; they are to be cleaned after use.
    b. Water baths should be kept free of growth deposits.
    c. Autoclaves, hot air sterilizing ovens, and water distilling equipment and centrifuges should be cleaned regularly to ensure safe operating.

    d. Employees are to be trained in autoclave operation and operating instructions posted near each instrument.
    e. Performance checks of autoclaves and hot air sterilizers should be conducted with the use of spore strips, spore ampoules, indicators, etc.
15. Safety cabinets of the appropriate type and class are to be supplied, maintained, and used.
16. Personnel are to be trained in the proper procedures for handling lyophilized (freeze-dried) cultures when used.
17. Employees should use the provided bottle carriers when moving reagents, acids, and solvents through the building.
18. Laboratory personnel must follow labeling protocols in the laboratory to prevent mix-ups of reagents, and when possible use the pre-labeled or permanently labeled bottles. Secondary containers are to be labeled as well.
19. In the event of a spill, all possible contaminated surfaces and tools are to be disinfected and the absorbent material placed in a biohazard bag for disposal.
20. All contaminated plates and Quanti-trays are to be autoclaved in biohazard bags at the end of the analysis and then disposed of in the labeled bags as regular trash.
21. Sterilize biological waste materials and contaminated equipment (cultures, glassware, etc.) before washing, storage, or disposal by autoclaving or decontaminating.
22. Eliminate flies and other insects to prevent contamination vectors of sterile equipment, media, samples, cultures, and infection of personnel (i.e., provide screens on windows and doors to outside if there is no air conditioning).

APPENDIX M

Participating Organizations and Contacts

Responsible Agency:

	Contact	Phone	E-mail
City of Los Angeles	Reza Iranpour	(213) 485-0577	Reza.Iranpour@lacity.org
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