



2007 QUALITY ASSURANCE MANUAL



MARINE MICROBIOLOGY LABORATORY AND VECTOR MANAGEMENT



**METROPOLITAN WASTEWATER DEPARTMENT
ENVIRONMENTAL MONITORING AND
TECHNICAL SERVICES DIVISION**

QUALITY ASSURANCE MANUAL



City of San Diego Marine Microbiology and Vector Management

July 2007

MISSION STATEMENT

“Striving for the utmost in professionalism and efficiency as we provide high quality laboratory services for the City of San Diego’s discharge permit programs”

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Table of Contents

MISSION STATEMENT	i
CREDITS AND ACKNOWLEDGEMENTS.....	v
INTRODUCTION.....	1
GENERAL INTRODUCTION	1
<i>Marine Microbiology.....</i>	<i>1</i>
<i>Vector Management.....</i>	<i>3</i>
SITE AND FACILITY	4
ISO 14001	5
LAB SAFETY.....	6
FIELD SAMPLING.....	7
FIELD SAMPLING PROCEDURES	7
ANALYTICAL PROCEDURES.....	11
MICROBIOLOGICAL PROCEDURES	11
<i>Heterotrophic Plate Count.....</i>	<i>11</i>
<i>Chromogenic Substrate.....</i>	<i>11</i>
<i>Multiple Tube Fermentation</i>	<i>11</i>
<i>Membrane Filtration.....</i>	<i>11</i>
<i>Coliphage.....</i>	<i>12</i>
MICROBIOLOGICAL QUALITY ASSURANCE PROCEDURES	12
<i>Media Preparation.....</i>	<i>12</i>
<i>Microbiology.....</i>	<i>13</i>
DISCHARGE MONITORING REPORT-QUALITY ASSURANCE	15
DATA	17
DATA HANDLING	17
CORRECTIVE ACTIONS	17
REFERENCES.....	19
APPENDICES	
APPENDIX A: ISO14001 DOCUMENTATION	
APPENDIX B: LABORATORY ORGANIZATION	
APPENDIX C: CERTIFICATIONS	
APPENDIX D: MISCELLANEOUS QA TEST RESULTS	

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CITY OF SAN DIEGO QUALITY ASSURANCE MANUAL MARINE MICROBIOLOGY AND VECTOR MANAGEMENT 2007

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INTRODUCTION



City of San Diego
Metropolitan Wastewater Department
Environmental Monitoring & Technical Services Division
Marine Microbiology & Vector Management Section

INTRODUCTION

GENERAL INTRODUCTION

The Quality Assurance/Quality Control Program for the City of San Diego's Marine Microbiology and Vector Management Laboratory includes various practices that have been instituted to ensure the accuracy and reliability of the laboratory's field sampling, laboratory analysis, records keeping, manual data entry and reporting. The procedures are reviewed and updated to reflect ongoing changes in sampling requirements, sample collection, methods, technology, and applicability of new analytical methods. Documents describing these and other procedures are maintained in accordance with Metropolitan Wastewater Department, Environmental Monitoring and Technical Services Division (MWWD-EMTS) ISO 14001 certification. An inventory of these documents is presented in [Appendix A](#).

The Marine Microbiology and Vector Management (MMVM) section is part of the Environmental Monitoring and Technical Services (EMTS) Division of the Metropolitan Wastewater Department (MWWD), City of San Diego. This manual provides a brief description of Marine Microbiology and Vector Management structure and personnel, and includes information concerning field sampling protocols and equipment, analytical techniques, training, as well as QA procedures utilized in conducting permit mandated, contractual, and voluntary work.

Marine Microbiology

The Marine Microbiology laboratory is certified by the State of California Department of Health Services, Environmental Laboratory Accreditation Program (ELAP). The laboratory undergoes onsite audits every two years to evaluate overall lab performance, field-of-testing (FOT) methods proficiency and the state of the quality assurance/quality control (QA/QC) program. We also participate in yearly Performance Evaluation (PE) and Discharge Monitoring Report-Quality Assurance (DMR-QA).

The Marine Microbiology Laboratory staff is responsible for sample collection, quantification and identification of bacteria as well as the quantification of coliphage. Microbiological tests are conducted on environmental samples collected from ocean, bay, estuary, watershed, and storm drains. In addition, tests are run on other types of matrices such as wastewater, reclaimed water, treated water, bio-solids, and membrane bioreactor products. The laboratory is involved with planning, designing, prioritizing, and implementing microbiological studies and programs. The laboratory interfaces with public health regulators and other similar agencies regarding microbiological data to identify potential solutions to common monitoring and technical problems. New microbiological procedures and rapid diagnostic tests are also evaluated by the laboratory.

The Marine Microbiology Laboratory is responsible for complying with the bacteriological monitoring and reporting requirements of the Point Loma Ocean Outfall (PLOO) and the South Bay Ocean Outfall (SBOO) National Pollution Discharge Elimination System (NPDES) permits. Offshore and shoreline samples are collected for PLOO and SBOO with additional samples from

Mexican waters to satisfy the SBOO permit. All samples are analyzed to determine fecal indicator bacteria densities. The purpose of bacterial sampling is to track the wastewater plume, evaluate kelp bed water quality against permit standards, and to address beach water quality issues at shoreline stations. Test results are reported to the Regional Water Quality Control Board 9 (RWQCB9) as required by permit and to the San Diego County Department of Environmental Health (SDCDEH) to fulfill the sampling and reporting requirements mandated by the Beach Safety Bill (AB411) recreational body contact standards. Bacterial exceedances of the California Ocean Plan (COP) or AB411 standards require re-sampling until compliance criteria are met. Recent changes to the PLOO permit require that, in addition to the core monitoring mentioned above, the laboratory participates in regional studies that are coordinated by the Southern California Coastal Water Research Project (SCCWRP). Additionally, Marine Microbiology personnel are on-call seven days a week, 24 hours a day, working closely with SDCDEH to monitor waters in order for SDCDEH to post and un-post beaches which may be impacted by sewage seepages or spills.

The laboratory currently provides microbiological services and technical consulting for the following MWWDD Divisions, City departments and external entities:

- Stormwater NPDES permit
 - Coastal Storm-Drain Outfall and Receiving Water Monitoring Program
 - Dry Weather Field Testing Program
 - Investigation of Illegal Connections/Illicit Discharges (IC/ID)
 - Additional studies or projects
- Collections Division
 - Storm drains
 - Investigation of potential sewage system infrastructure seepage or spills
- Wastewater Treatment and Disposal Division NPDES permit
 - Assistance in meeting NPDES compliance with bacteriological monitoring and reporting requirements
 - Treatment plant process control
 - Analysis of treatment plant and pump station stormwater run-off
 - Special studies and projects through contract work
- Environmental Services Department (ESD) NPDES permit
 - Analysis of Miramar Landfill compost and soil
 - Analysis of Miramar landfill stormwater run-off
- International Border and Water Commission NPDES permit
 - Sampling and analysis of receiving water
 - Analysis of offshore waters
 - Technical assistance
- The University of California, San Diego, Scripps Institute of Oceanography (UCSD-SIO) NPDES permit
 - Ocean shoreline testing
 - Outfall discharge testing
 - Dry and wet weather special studies
 - UV/Ozone treatment efficacy testing
- Permits and Compliance

- Annual storm water runoff bacterial monitoring for wastewater treatment facilities and pump stations.

Vector Management

The Vector Management group's main responsibility is to keep MWWD's facilities free of insects that are capable of transmitting diseases to humans. In its efforts to do so, staff is highly trained in controlling insects with innovative and environmentally friendly techniques.

Vector Management provides monitoring, surveillance, control, and prevention of insects and other invertebrates that are either vectors of diseases, or a nuisance to humans. The group provides routine control of insect infestations which negatively impact the treatment process at four major treatment plants. Environmental conservation measures, education, and water management techniques, aided by appropriate chemical and biological control technology are used to prevent the spread of mosquitoes including those responsible for the transmission of West Nile Virus. Vector Management staff are certified by the State of California Department of Environmental Health for the application of biological and chemical control agents.

All four of MWWD's treatment plants (Point Loma Wastewater Treatment Plant, North City and South Bay Water Reclamation Plants, and Metro Biosolids Center), four major and 85 minor pump stations, buildings and office facilities are continually monitored to census invertebrate populations in order to determine the effectiveness of the vector control program.

The Vector Management group actively participates, in conjunction with the San Diego County's Mosquito and Vector Control Division (MVCD), in coordinating vector surveillance and control at large water bodies such as the San Diego River, Peñasquitos Preserve, coastal lagoons and storm drain outlets.

The Vector Management group coordinates the collection, identification, and initial testing (with the San Diego County's Mosquito and Vector Control Division) of birds suspect of dying as a result of West Nile virus infection. Confirmation tests using Real Time-Polymerase Chain Reaction (RT-PCR) Taqman Assay Technology are performed at the Center for Vector borne Diseases (CVEC), located at the University of California Davis. The group provides vector control assistance and West Nile Virus surveillance to other City Departments such as the Water and the Park and Recreation Departments.

In addition to mosquitoes and flies, the Vector Management group also assists and advises other Divisions and Departments in the following areas:

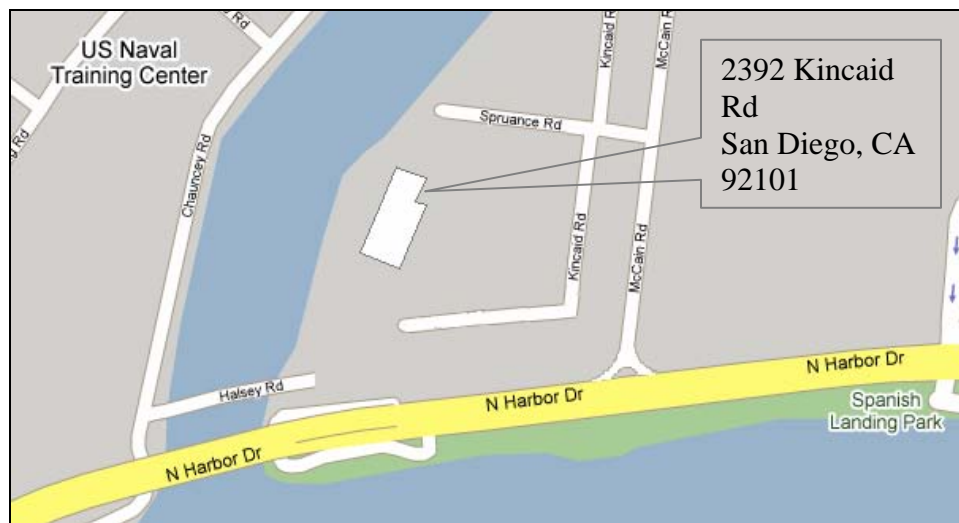
- Rat and Mice infestations
- Ant problems, esp. imported Red Fire Ants
- Bees: Africanized and European Honey Bees
- Termites
- Fleas and Ticks

Environmental surveys are conducted by Vector Management after spills originating from the treatment plants or pump stations. These surveys are to determine if an adverse environmental impact has taken place as a result of the spill. Where necessary, Vector Management monitors Benthic Macroinvertebrate communities to evaluate the biological health of streams and creeks.

During the Dry Weather Field Testing and the Coastal Storm Drain Outfall and Receiving Water seasons, the Storm Water PPP staff monitors and collects samples from over 300 storm drains for water quality analyses. During these sampling events, if SWPPP staff observes mosquito larvae present in the storm drains, Vector Management is notified via e-mail. Vector Management staff then create a service call and follow up to mitigate the problem.

SITE AND FACILITY

The Environmental Monitoring and Technical Services (EMTS) facility which houses the Marine Microbiology laboratory became operational in February of 2004. It is located within the City of San Diego at 2392 Kincaid Rd which is situated at the site east of the boat channel and west of the San Diego International Airport.



In addition to housing the MMVM laboratory, the EMTS facility serves as the base of operation for other groups and functions of the Division. These include Administration, Business Support, Permits & Compliance, Data Management & Reporting, IT/GIS Systems, Ocean Operations, Toxicology and Taxonomy.

The facility is protected 7/24 by an electronic security system requiring an authorized swipe card and security code for entry. Access to the facility, during the normal hours of operation, is through the double door main entrance. A fire alarm system is tied into the security system such that, in the event a fire detection sensor is triggered, an automatic signal is relayed to the local fire department enabling a quick response. A secured refrigerator is located at the loading area for receiving and preserving after hour samples.

The MMVM laboratory occupies an area of approximately 9,000 square feet on the second floor of the two story facility. The component laboratory areas include those for the preparation of microbiological media, reagents and implements, glass and plastic ware decontamination, washing and drying, bacteriology, virology assay and clean room activities, vector management functions, storage and office suites.

A computerized HVAC system controls and maintains the facility environment, creates positive pressure in critical laboratory areas and provides total recurrent air recycling. An emergency back up generator provides electrical services to key laboratory outlets for uninterrupted analytical processing in the event the conventional electricity supply is not available.

Built-in laboratory components include sterilizers, each with their own integral steam generators, an air dryer, fume hoods, and industrial washer/dryers. Other laboratory equipment include the following devices: point of use Milli-Q water pure system, laminar flow hood, Vitek 2 Compact, analytical balance, a biological safety cabinet pH meters, water baths, air incubators, refrigerators, freezers, sub-zero freezers, centrifuges, and microscopes. For those units that are considered critical to uninterruptible operations, redundant pieces of equipment are in place.

Water, vacuum, air and natural gas services are available at dedicated bench areas and a DI water treatment system provides polished water to preparatory and analytical laboratory areas. A water softening system provides softened water to sterilizers and glassware washers.

Qualified vendors are under annual contract to provide routine maintenance and emergency services for the laboratory water softener system, de-ionized water system, autoclaves, washers, dryers, hoods, and other specialized laboratory equipment.

The near-coast location of the laboratory is well situated for the timely and efficient transport of offshore samples from docked seagoing vessels and established coastal shoreline sample sites. From the facility, it is a 5 minute drive to the docked, oceangoing, sampling vessels and a 40 minute drive to the farthest shoreline sample site. In the future, a boat dock will be constructed on the boat channel shoreline adjacent to the NTC facility so that samples can be offloaded directly to the MMVM laboratory.

ISO 14001

The International Standardization of Organization 14001 (ISO 14001), a Swiss based organization for standardization, is a widely recognized voluntary international standard that represents the state of the art in global environmental management practices. In June 2002 the Environmental Monitoring and Technical Services Division (EMTS) became the first publicly owned laboratory in the Nation to become [ISO certified](#).

On a yearly basis, a review of the environmental aspects associated with sampling activities, laboratory services, and products or byproducts of the section's operations is performed. Existing or potential air emissions, releases to water, waste management, soil contamination, use of raw materials and natural resources, and local environmental and community issues in both normal and emergency operating conditions are considered. Operational controls and section-specific environmental objectives and targets are then established.

As required by the EMTS division environmental policy, all employees are required to undergo regular environmental awareness training. It is also obligatory that contracted entities follow and visitors are informed of this environmental policy. An active recycling program takes care of laboratory generated recyclable items such as paper, cardboard, plastic and glass. This reflects the section's commitment to its environmental policy which stresses the prevention of pollution, the

maintenance of regulatory compliance, and continual improvement of its environmental management practices.

Under supervision of the Environmental Management System Coordinator (EMSC) and Section Head, a system for managing all documents pertinent to the section's operations is in place. This ensures that all documents are reviewed, approved, issued, distributed and revised according to the MMVM Document Control Group and program guidelines.

ISO 14001 complements the Environmental Laboratory Accreditation's Program (ELAP), a quality assurance monitoring program, by ensuring environmentally sound practices in its daily operations.

LABORATORY SAFETY

Safety is taken very seriously and is a prime consideration in the operation of the laboratory. A significant amount of time and effort is dedicated to employee training and awareness. Training is administered according to the employee's job classification and two prioritized categories: 1) "mandated by law" and 2) "department required". Training requirements are reviewed annually by supervisors so that employees are scheduled for classes based on the review of each individual's past record.

Recurring essential training courses include First Aid/CPR/AED, Injury and Illness Prevention, Fire Prevention and Protection, Emergency Preparedness and Hazmat Handler Review. Routine safety tailgates are held monthly with presentations assigned on a rotational basis among all employees in the section.

The laboratory is equipped throughout with state standard fire extinguishers, fire blankets, spill kits, first aid kits, eye washes and emergency showers. An automated defibrillator is located in a central part of the building. There are fume hoods and a biological cabinet for use when necessary. Personal protective equipment is made available and required, where applicable, for laboratory and field work. Access to City storerooms and an open PO make immediate acquisition of safety equipment simple and timely. The Business plan, Chemical Hygiene plan, and Material Safety Data Sheets (MSDS) are on file and available for viewing.

The section provides a representative to serve on the Division's laboratory Safety Committee. The committee meets every two months to discuss and act upon pending safety related matters and to plan for future internal safety audits of the various laboratory facilities. Safety Committee audits are conducted once per year as are combined Safety Committee-Safety Officer audits. Audit deficiencies are submitted to the section head for corrective action to be taken within a prescribed time limit depending on the deficiency.

For facility Hazmat certification, the laboratory undergoes periodic San Diego County Hazmat inspections. Additionally, the scope of the International Standardization of Organization 14001 (ISO 14001) audit places a special emphasis on safety and training records and the proper disposal of hazardous materials and waste.

FIELD SAMPLING PROCEDURES



FIELD SAMPLING

FIELD SAMPLING PROCEDURES

The Marine Microbiology laboratory is tasked with sampling duties that are required by a variety of discharge permits, supplemental environmental projects, special projects, studies, and sewage spill events. Types of sampling sources include ocean, bay, estuary, watershed, storm drains, treatment plant effluent, and potable water. The laboratory's technical staff has the primary responsibility for all of the aspects of sample collection and delivery.

Samplers are trained in proper sampling technique including how to use sampling gear and personal protective equipment, the proper handling of samples using aseptic technique, the appropriate procedures for labeling, the requirements of sample preservation, and observation of holding times. Standard Operating Procedures (SOPs) reflect the current method of field sampling. Samplers are required to complete thorough documentation of sample site visual observations including chain-of-custody paperwork for each of the samples obtained. Samplers are trained to document and report any unusual events or peculiarities which may have an impact on analytical results. These may include environmental aspects at the sample site, sample transit conditions, equipment fitness, weather, as well as other physical and biological conditions at the site.

Samplers use GPS instrumentation and maps to correctly locate the established sample sites and to assign coordinates when establishing new sites. Upon establishing new sample sites, whether for purposes of special studies, projects or spill sampling, the GPS coordinates are determined, documented, and archived for future use.

To appropriately respond to emergencies such as sewage spills and to carry out urgent sampling duties, samplers rotate through 24 hour 7 days a week on-call duty. In such cases, the sampler either collects samples from pre-arranged sample locations and/or conducts a survey to determine the most suitable sampling site(s). Tidal cycles must also be considered in determining the appropriate time to sample. At the time of sample collection, the sampler must evaluate and determine if the sample can be safely collected.

Samplers are evaluated on an annual basis for general sampling competency. Communication skills are emphasized between the technical staff to carry out the necessary planning for proper sample collection. The sample vehicles are equipped with 4x4 drives and lift gates. The sample vehicles are routinely serviced and maintained to ensure safe and reliable transportation.

MAPS

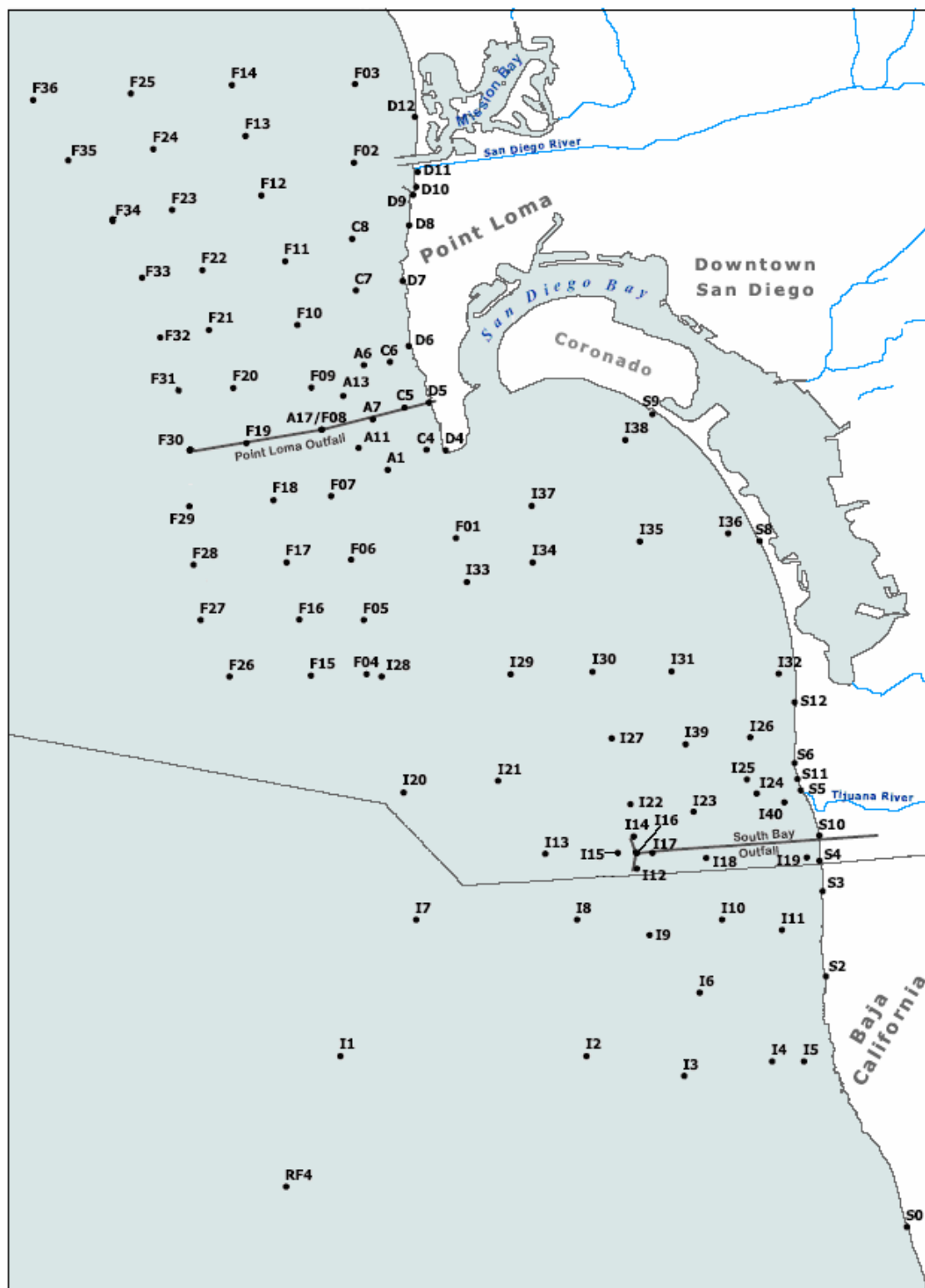


Figure 1. All stations

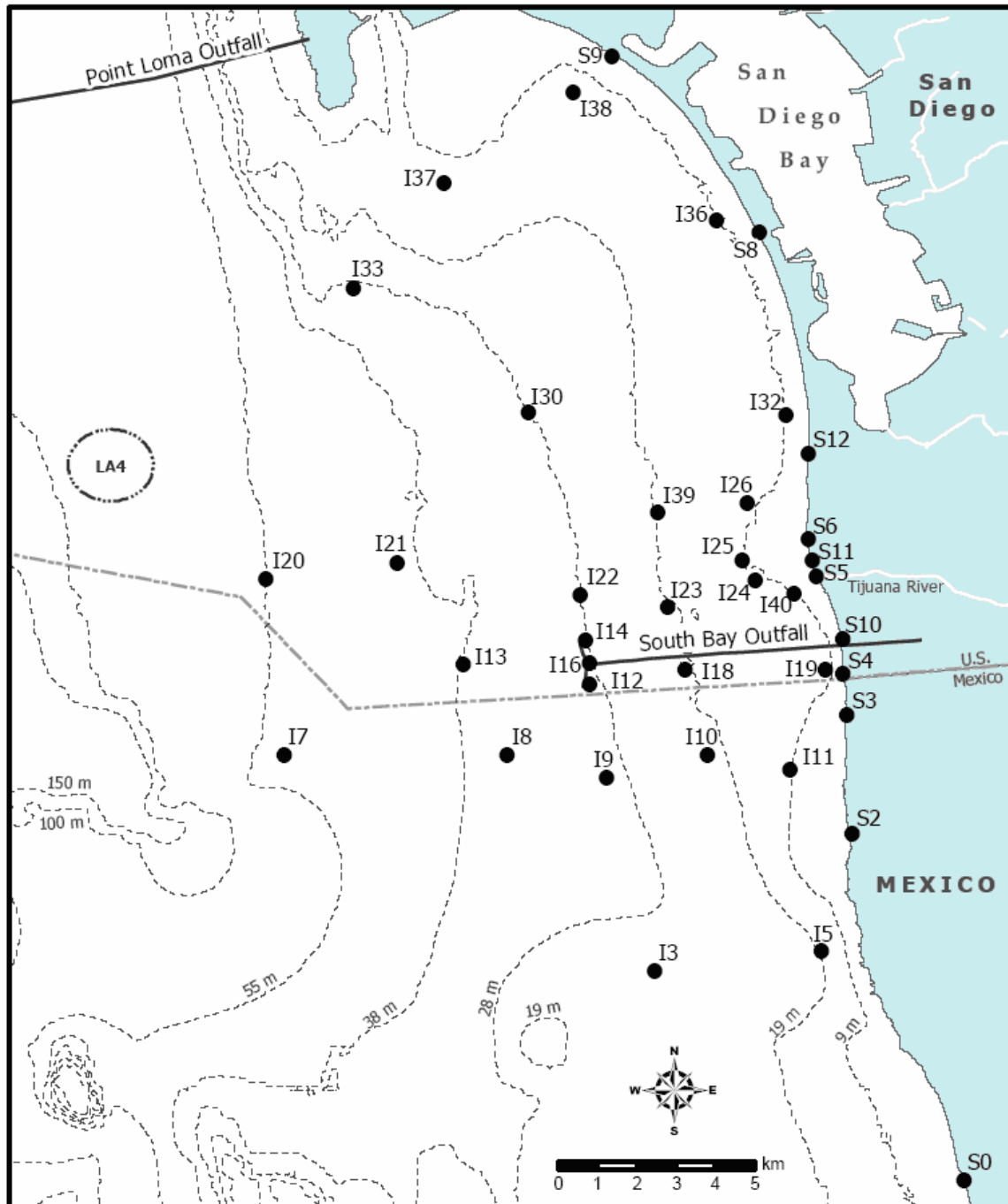


Figure 2. Routine PLOO stations

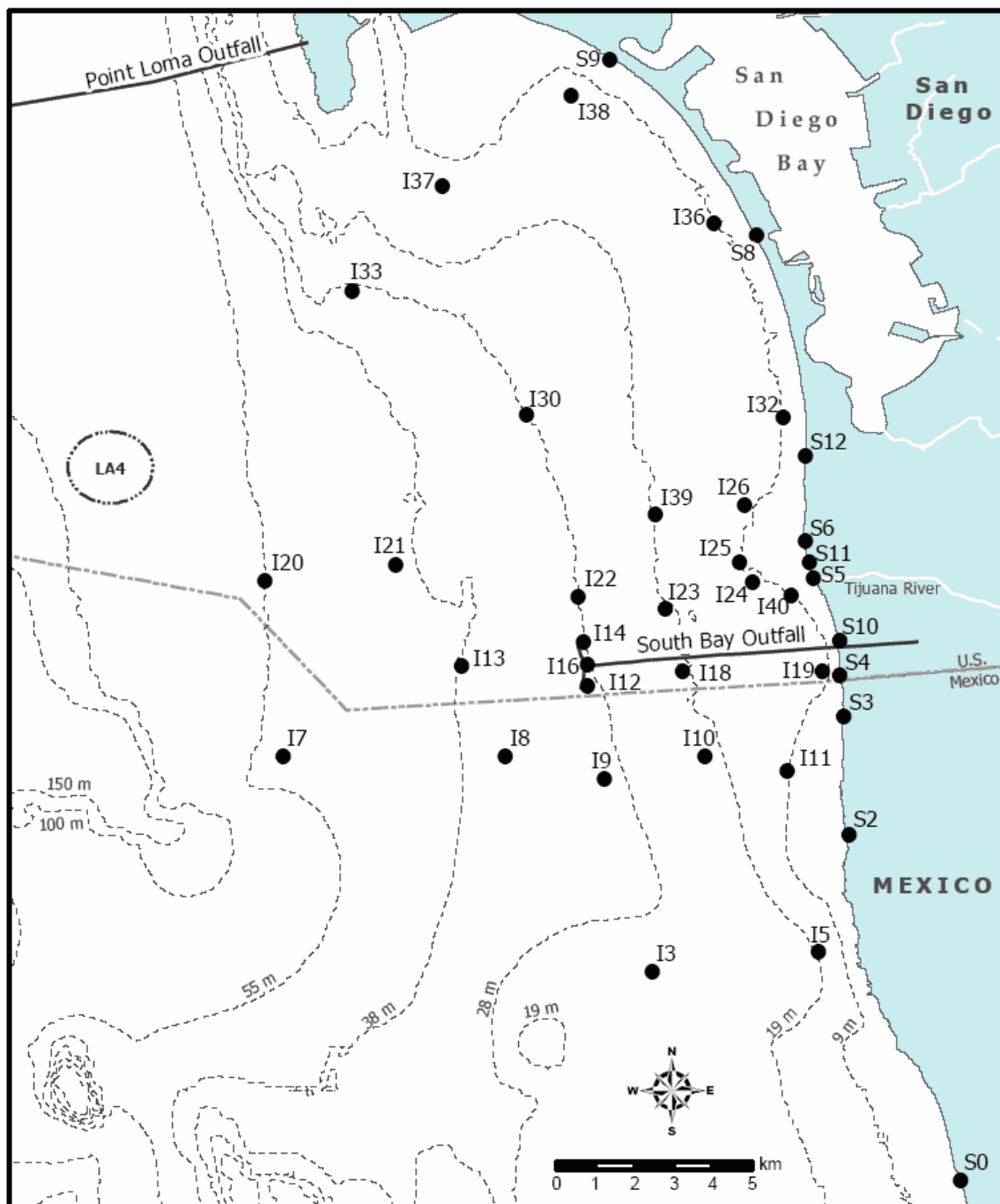
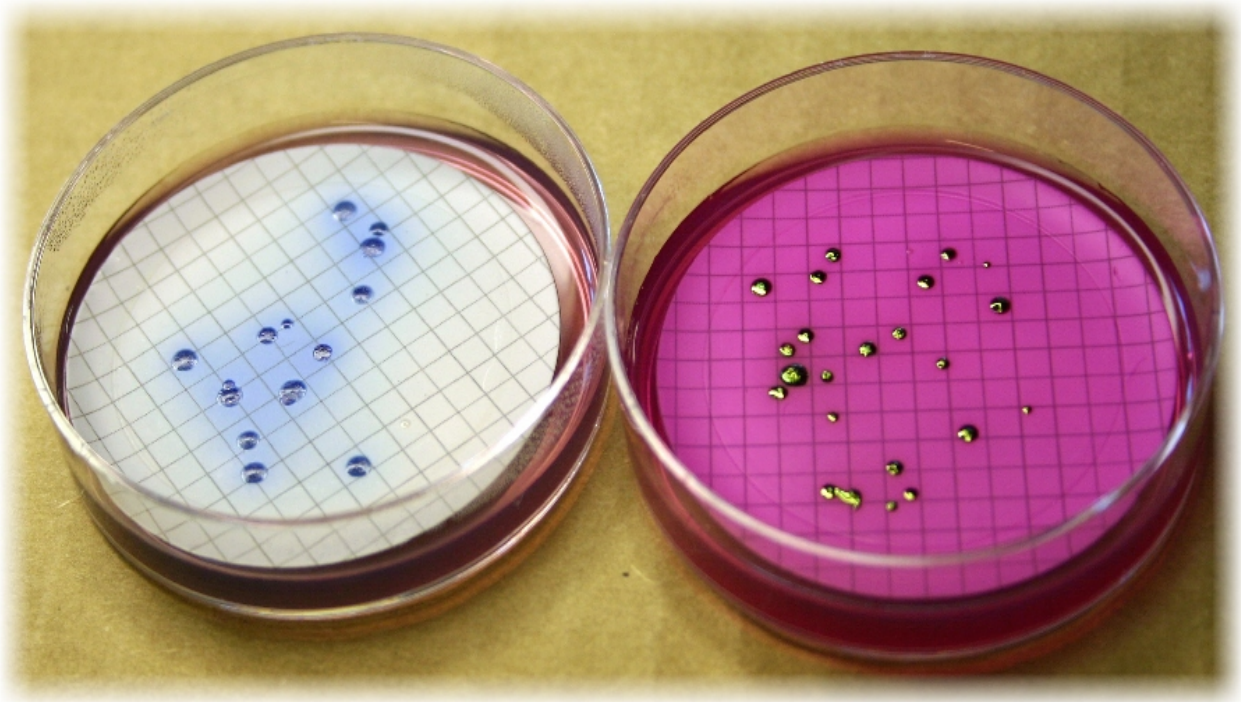


Figure 3. Routine SBOO stations

LABORATORY AND ANALYTICAL PROCEDURES



ANALYTICAL PROCEDURES

MICROBIOLOGICAL PROCEDURES

The Marine Microbiology group follows guidelines issued by the EPA's Water Quality Office and the California State Department of Health Services', Water Laboratory Approval Group with respect to sampling and analytical procedures. The 19th edition of Standard Methods for the Examination of Water and Wastewater, (Eaton et al., 1995) and the EPA's Microbiological Methods for Monitoring the Environment, Water and Waste (Bordner et al., 1978) and EPA 1600 (2006) are referred to for standard methodologies as well as in-house Standard Operating Procedures (SOPs). ([Appendix A](#)).

Heterotrophic Plate Count

The heterotrophic plate count (HPC) is used to estimate the number of viable heterotrophic bacteria in water and measuring changes during water treatment and distribution. The Pour Plate Method is used (SM 9215 B). HPC analyses are run monthly to check the quality of the laboratory's filtered water supply, as needed on Treatment Plant process control samples and periodically on contract samples when specified. Results are reported as colony forming units per milliliter of sample (CFU/mL).

Chromogenic Substrate

Chromogenic substrate analyses, Colilert (SM 9223B, IDEXX) and Enterolert (IDEXX) are used to statistically estimate the mean density of total coliforms, *E. coli* and enterococcus present in liquid, solid, or semisolid samples. Colilert and Enterolert analyses are run weekly on the Tijuana River samples from Dairymart Road and Hollister Street, and on occasional non-receiving water samples as a result of sewage spill or storm water investigations. The Colilert and Enterolert formulations are used in conjunction with the IDEXX Quantitray. A Most Probable Number (MPN) Index value is determined from the IDEXX MPN Index table and reported as MPN per 100 milliliters of sample (MPN/100mL).

Multiple Tube Fermentation

The Multiple Tube Fermentation (MTF) analysis is used to statistically estimate the mean density of total coliforms (SM 9221 B) and fecal coliforms (SM 9221 E) present in liquid, solid, or semisolid samples. MTF analyses are run daily on water reclamation plants, storm drains, monthly on treatment plant sewage effluent, and as-needed for compost, treatment plant storm run-off, dry weather field testing, sewage spills, spill investigations and occasional special studies. Laurel Tryptose Broth (LTB) is used as the presumptive medium and Brilliant Green Bile (BGB) Broth is used as the confirmatory medium for the total coliform test. EC broth is the confirmatory medium used for the fecal coliform test. Tubes are set up in a 5-5-5 decimal dilution configuration. An MPN calculation is performed on the results and reported as MPN per 100 milliliters of sample (MPN/100mL) for liquids and, after factoring % Total Solids, MPN per gram dry weight (MPN/gDW) for solids and semisolids.

Membrane Filtration

The membrane filtration (MF) technique is used for direct enumeration of total coliforms (SM 9222 A, B), fecal coliforms (9222D) and enterococcus (EPA 1600) in seawater samples and, to some extent, storm water samples. MF results are highly reproducible, can be used to test larger sample volumes and yield numerical results more rapidly than the MTF method. This technique has limitations when testing waters with high turbidity or non-coliform (background) bacteria. Bay, ocean shoreline and ocean inshore and offshore station samples are filtered and incubated in the laboratory. Sterile 0.45 µm membrane filters on glass or plastic funnels are used for filtering seawater samples. Total coliforms are enumerated on M-Endo LES agar, fecal coliforms on M-FC agar and enterococcus on MEI agar. Results are reported as colony forming units per 100 milliliters of sample (CFU/100mL).

Coliphage

The coliphage membrane filtration technique is used for the enumeration of male specific phage (MS2) in water samples. Exclusively contract work, projects include testing the efficacy of wastewater treatment systems such as Membrane Bio-Reactors (MBR) and Biological Aerated Filters (BAF). In addition, various projects tested membrane effectiveness based on phage removal for the purposes of membrane certification by the National Sanitation Foundation (NSF) and California State Department of Health Services (CA-DHS). The testing process involves seeding filtration units with coliphage, determining influent and effluent concentrations and assessing the reduction rate of coliphage after treatment.

Sterile 0.45µm filters are utilized for filtering water samples. Filters are inverted, placed on tryptone agar plates which contain a layer of host bacteria and incubated. Results are reported as plaque forming units per 100 milliliters of sample (PFU/100mL) (Sobsey, et al., 1990, J. AW WA 82:52-59).

MICROBIOLOGICAL QUALITY ASSURANCE PROCEDURES

QA procedures suggested by the EPA's Water Quality Office, Water Hygiene Division and the California State Department of Health Services' Water Laboratory Approval Group are followed and documented in all areas involved in microbiological testing.

Media Preparation

A Millipore Milli-Q unit provides analytical grade deionized water for preparation of reagents and media. The Prep room's deionized and Milli-Q ultra pure water are analyzed daily for conductivity, pH, and total chlorine residual. The Bacteriology laboratory's deionized water is analyzed monthly for conductivity, pH, and total chlorine residual. TOC and Ammonia are analyzed monthly and Heavy Metals are analyzed annually. In addition, a heterotrophic plate count for bacteria is performed monthly on the Prep room's deionized and Milli-Q water, and on the Bacteriology Laboratory's deionized water. A "Test for Bacteriological Quality of Reagent Water" is run annually on the deionized and Milli-Q water ([Appendix D](#)).

Media are prepared from dehydrated stock according to manufacturer and EPA specifications (see Bordner *et al.*, 1978). Upon receipt, all media and reagents are logged in and given

accession numbers. Accession number, date received, and date opened are written on each container. Each batch of prepared media is tested for pH immediately after preparation. Positive, negative, and media sterility control plates are run with each preparation.

Three percent of the buffer dilution water is tested by adding 50 mL of the product water to approximately 50 mL of double strength Nutrient Broth medium which is then incubated at the appropriate temperature for 48 hours.

A media preparation log is kept showing type of media, amount prepared, final pH and preparer's initials.

Records are kept of operation, calibration, and maintenance of all laboratory equipment. Maintenance contracts provide regular service for the autoclave, microscopes, balances, and bacteriological safety cabinets. Balances are checked prior to each day's media preparation against certified weight standards. Autoclave performance is documented with strip charts and verified with spore strip testing once per week. Temperature sensitive tape is used on all items to be autoclaved. Items are labeled with all required information. Autoclave logs are kept on the type of material, length of sterilization, actual cycle maximum temperature and pressure, and the operator's initials. Temperature records are kept daily for all refrigerators and drying/sterilizing ovens and twice daily for all incubators and water baths.

Disposable Petri dishes, pipettes, and culture tubes are used whenever possible to reduce the possibility of contamination. Reusable glass and plastic laboratory ware are washed in a Steris Reliance 400 Laboratory Glassware Washer. Sample bottles are sterilized by autoclaving at 121°C for 30 minutes. Sterility is verified by adding Tryptic Soy Broth. After incubation, a bottle from each tray is analyzed for bacterial growth and the result is entered into the appropriate log. If growth occurs, the entire batch is re-autoclaved. Glass and plastic ware used in media preparation and sample collection are tested for soap residue by using bromothymol blue pH indicator solution and the results are entered into the appropriate log. A "Test for Inhibitory Residues" is run when changes are made in detergent brand or washing procedures. (Eaton *et al.*, 1995). ([Appendix D](#)).

Microbiology

Lyophilized cultures are ordered from Microbiologics and working cultures are inoculated every two months. A propagation log is kept indicating date of inoculation and stock culture information. Because of difficulties encountered when using ATCC cultures for *E. coli*, every two years (or as needed), a wild type strain of *E. coli* is identified using the Vitek 2 Compact. This strain is propagated and preserved with glycerol. It is subdivided into cryovials and stored in -80°C to serve as stock culture. Positive, negative and buffer and media sterility control plates are run with each analysis.

Bacteriological duplicate and split samples are performed as quality assurance checks to measure variability between samples and analysts precision, respectively (Table 1). A duplicate sample is obtained by taking two distinct samples at a given station in the field and then analyzing them in exactly the same way. A split sample is obtained by taking aliquots of a single field sample and then having two different analysts perform the dilutions, filtration and plating. Duplicate samples are performed on approximately 5% of the water quality samples. Split samples are performed once each month. The sign test (see Gilbert, 1987) is

used to statistically compare the results of the paired duplicate and split samples. ([Appendix D](#)). Funnel sterility and rinse efficiency checks are performed with each batch.

The laboratory conducts monthly comparisons of bacterial colony counts to quantify the counting precision of each analyst. Each analyst must be able to duplicate his/her own prior colony counts within 5% and counts by any two analysts must fall within 10% of each other. Routine plate bacterial density calculations are checked and initialed by a member of the Microbiology Group who was not involved with the original counts or calculations.

Monthly verifications of positive or negative results are performed using EPA approved methods which include an automated identification system (Vitek 2 Compact). The Vitek 2 Compact is also used to identify any questionable bacterial colonies. All laboratory Quality Assurance and Quality Control processes are documented and filed.

The laboratory has a library of SOPs which describe each of procedures routinely performed including quality assurance practices. The SOPs are reviewed annually and reflect current laboratory practices. ([Appendix A](#)).

The laboratory maintains an ELAP certification that involves yearly performance evaluations. The laboratory also participates in intercalibration studies with different agencies on a regular basis.

DISCHARGE MONITORING REPORT-QUALITY ASSURANCE

(DMR-QA) Study 26

In 2006, the Marine Microbiology laboratory was required to participate in the DMR-QA Study 26. This is an Environmental Protection Agency (EPA) mandated study administered by the Office of Enforcement and Compliance Assurance. It is the first such study in which the laboratory has taken part. Participation will be required in future DMR-QA studies.

DMR-QA is mandatory for major and selected minor permit holders under the Clean Water Act's National Pollution Discharge Elimination System (NPDES). The study addresses the analytical processing and reporting abilities of the laboratory according to the specific bacteriological requirements of the NPDES permit(s). The microbiological testing for Study 26 included total and fecal coliforms analyzed by the membrane filter and multiple fermentation tube methods.

The study samples must come from an accredited proficiency test (PT) provider that is appropriately accredited by the National Institute of Standards and Technology National Voluntary Laboratory Accreditation Program (NIST NVLAP). Environmental Resource Associates (ERA) is the provider the Marine Microbiology laboratory contracts to supply proficiency test samples required for ELAP certification and now for the Study 26. ERA supplied PT samples and instructions, and evaluated the reported results. ERA was also responsible for receiving the complete DMR-QA data package for each permit, preparing an evaluation report for each data package, and sending these evaluation reports to each permit holder and the state or EPA regional DMR-QA Coordinator.

Results are reported as either "Acceptable" or "Not Acceptable". The total coliform and fecal coliform results reported by the Marine Microbiology laboratory for Study 26 were acceptable. Should it occur, the laboratory will identify and report the causes of a "Not Acceptable" result and also identify system changes to correct the discrepancies in order to avoid their recurrence.

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DATA

PL Loma Ocean Outfall

Sample Date: 10-JUN-07

Station	Depth (m)	QA/QC Procedure	Analyst	TOTAL CFU/100 mL	FECAL CFU/100 mL	ENTERO CFU/100 mL
A6	18	DUPLICATE	JLT	4e	<2	<2
A17	60	DUPLICATE	JLT	220e	30e	<2

Sample Date: 11-JUN-07

Station	Depth (m)	QA/QC Procedure	Analyst	TOTAL CFU/100 mL	FECAL CFU/100 mL	ENTERO CFU/100 mL
D6	0	DUPLICATE	JLT	<20	<2	<2

Sample Date: 17-JUN-07

Station	Depth (m)	QA/QC Procedure	Analyst	TOTAL CFU/100 mL	FECAL CFU/100 mL	ENTERO CFU/100 mL
D6	0	DUPLICATE	JT	<20	<2	<2

Sample Date: 18-JUN-07

Station	Depth (m)	QA/QC Procedure	Analyst	TOTAL CFU/100 mL	FECAL CFU/100 mL	ENTERO CFU/100 mL
A6	18	DUPLICATE	JLT	50	<2	<2
A17	60	DUPLICATE	JLT	50	<2	<2

DATA

DATA HANDLING

Experienced biologists read and record analytical data within each method's specified times. The recorded analytical results are reviewed by a peer and a supervisor before entry into the Oracle database. Biologists are responsible for analytical data and field data entry. Once the data is entered, it is reviewed by the Data Management and Reporting group personnel as well as the database administrator. The data is then sent back to Marine Microbiology for final review.

Data is evaluated, and the appropriate responses to action limits are carried out. A highly experienced workforce ensures vigilance, historical perspective, as well as intuitive assessment.

Reports are issued by the Biologist III, according to the contract specifications. Monthly, semi-annual, annual, as well as compliance reports are generated upon the client's request. In compliance with the Beach Safety Bill (AB 411) regulations, exceedances of recreational waters are reported to the San Diego County Department of Environmental Health Services. The client is also informed of these exceedances to conduct any required follow up actions. Special data packages and Electronic Data Delivery (EDD) are provided as specified by contract.

In accordance with the laboratory's ISO 14001 certification, hardcopy data records, QA data, and field sheets are kept on hand for at least 7 years. They are then archived and kept indefinitely or as specified by contract. The database is backed up by the database administrator on a daily basis and backup files are kept indefinitely.

CORRECTIVE ACTIONS

Corrective actions are taken to correct errors and to eliminate their cause in order to prevent future recurrences. They are initiated upon detecting equipment failure, audit non-conformance, or failure to follow established procedures for sampling, analyses or reporting. Actions are initiated by the section's supervisor, the QA/QC officer, and the ISO 14001 Program Coordinator. Corrective actions are communicated to the section in the form of an email, tailgate, or formal memo. They are also documented using proper forms and reported through the appropriate chain of command ([Table 1](#)).

Table 1: Corrective Action Table

Error Type	Corrective Action
Sampling	<ul style="list-style-type: none"> - Review sampling record & SOP with sampler - Verify sampling location using GPS - Review sampling technique - Retrain & document the retraining - Follow up & test
Mathematical/Calculation	<ul style="list-style-type: none"> - Check formulas & verify calculation with analyst - Correct and re-check values - Verify and correct all records including hard copy & electronic - Provide training if needed - Communicate to section to prevent recurrence
Method/ Procedural	<ul style="list-style-type: none"> - Review SOP with analyst - Discuss analyst's techniques - Discuss equipment failures, if applicable - Recollect or reanalyze samples if needed - Retrain & document the retraining
Instrument Calibration	<ul style="list-style-type: none"> - Check standards i.e. expiration date, clarity...etc. - Review SOP - Recalibrate - Reanalyze samples if possible
Instruments/ Equipment	<ul style="list-style-type: none"> - Review maintenance record - Perform simple trouble shooting techniques - Notify contracted service technician.
Reporting Overlimits & Exceedances	<ul style="list-style-type: none"> - Notify appropriate personnel as soon as error is detected - If re-sampling is required, arrange for it immediately - Report oversight to appropriate personnel - Review Laboratory Action Limit SOP - Communicate to section to prevent reoccurrence - If necessary, complete documentation in Communication Log
QA/QC	<ul style="list-style-type: none"> - Keep a schedule of performance evaluation tests & inspection dates - Ensure that daily, monthly, quarterly and annual QA analyses are completed on time - Frequently review record to ensure that the QA requirements are completed and on time.
Power Outage	<ul style="list-style-type: none"> - Ensure that periodic checks are conducted on the performance of the back-up generator - Ensure that critical pieces of equipment are connected to power supply and that the appropriate amount of amperage is available for the equipment - Notify appropriate entities - Survey effect on the various equipment and analysis - Reanalyze samples if possible

REFERENCES

REFERENCES

- Bordner, R.H., J.A. Winter and P.V. Scarpino (eds.) (1978). Microbiological Methods for Monitoring the Environment: Water and Waste, EPA Research and Development, EPA-600/8-78-017, 337 pp.
- Eaton, A.D., Clesceri, L.S., A.E. Greenberg and (eds.). (1995). Standard Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association, American Water Works Association, and Water Environment Federation.
- EPA. (2006) Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl- β -D-Glucoside Agar (mEI)
- Gilbert, R.O. (1987). Statistical Methods for Environmental Pollution Monitoring, Van Nostrand Reinholdn Company, 320 pp.
- Sobsey, M.D., K.J. Schwab and T.R. Handzel. (1990). A simple membrane filter methods to concentrate and enumerate male-specific RNA coliphages. Journal AWWA, Vol. 82 (9):52-59.
- City of San Diego. (2008). Quality Assurance/Quality Control Report – Calendar Year 2006. City of San Diego, Metropolitan Wastewater Department, Environmental Monitoring and Technical Services Division, San Diego, CA.

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APPENDICES

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Appendix A

ISO14001 Documentation

An inventory of Marine Microbiology and Vector Management Laboratory Standard Operating Procedure (SOP) Documents

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Marine Microbiology and Vector Management

Index of Standard Operating Procedures

Document Number	Document Name	Review Date/Initials
MMVM-SOP-001.2-03082005	Maintenance and Monitoring of Lab Incubators, Waterbaths, and Refrigerators	01/16/08 AR
MMVM-SOP-002.3-03082005	Calibration of Lab Thermometers	01/16/08 AR
MMVM-SOP-003.1-03082005	Laboratory Maintenance	01/16/08 AR
MMVM-SOP-004.1-04102007	Vitek 2 Compact Verification	01/22/08 AR
MMVM-SOP-005.6-02082007	Membrane Filtration for Total and Fecal Coliforms	01/16/08 SER
MMVM-SOP-006.6-04012008	Membrane Filtration: Enterococcus	04/01/08 SER
MMVM-SOP-007.3-03082005	Heterotrophic Plate Count	01/22/08 AR
MMVM-SOP-008.3-06062007	Multiple Tube Fermentation: Total Coliforms	01/24/08 GBA
MMVM-SOP-009.4-06062007	Multiple Tube Fermentation: Fecal Coliforms	01/24/08 GBA
MMVM-SOP-0010.1-05212004	Multiple Tube Fermentation: Enterococcus	01/15/08 RD
MMVM-SOP-0011.1-02082007	Completed Test	01/15/08 RD
MMVM-SOP-0012.5-03082005	Colilert-18 (Idexx): Total Coliforms & E. Coli	02/20/07 JLT
MMVM-SOP-0013.2-05212004	Enterolert (Idexx): Enterococcus	02/20/07 JLT
MMVM-SOP-0014.1-04222004	Sampling Procedure for NCWRP and SBWRP	02/20/07 JLT
MMVM-SOP-0015.2-03022005	Coliphage Membrane Filtration Technique	01/16/08 SER
MMVM-SOP-0016.0-03042002	Coliphage Top Overlay Agar Method	01/16/08 SER
MMVM-SOP-0017.0-03042002	Enteric Virus: Plaque Assay	01/16/08 SER
MMVM-SOP-0018.0-03042002	Enteric Virus: Cytopathic Effect (CPE)	01/16/08 SER
MMVM-SOP-0019.0-03142002	Virus Adsorption-Elution-Precipitation Procedure	01/16/08 SER
MMVM-SOP-0020.0-03042002	Tissue Culture Procedures	02/08/07 RD
MMVM-SOP-0021.1-06172003	Inhibitory Test	01/24/08 GBA
MMVM-SOP-0022.3-04122007	Lab Tech Daily Duties	01/25/08 ZPR

Marine Microbiology and Vector Management

Index of Standard Operating Procedures

Document Number	Document Name	Review Date/Initials
MMVM-SOP-0023.2-03242005	Assistant Lab Tech Daily Duties	01/25/08 ZPR
MMVM-SOP-0024.0-03042002	Measuring Stream Flow with a Simple Float	01/25/08 ZPR
MMVM-SOP-0025.1-03242005	Shoreline Sampling	01/25/08 ZPR
MMVM-SOP-0026.3-04122007	Autoclave	01/25/08 ZPR
MMVM-SOP-0027.2-01252008	Balance	01/25/08 ZPR
MMVM-SOP-0028.0-03062002	Chlorine Colorimeter	01/25/08 ZPR
MMVM-SOP-0029.0-03062002	Conductivity Meter	01/25/08 ZPR
MMVM-SOP-0030.0-03062002	Dissolved Oxygen Meter/Corning	01/25/08 ZPR
MMVM-SOP-0031.0-03062002	Dissolved Oxygen Meter/Orion	01/25/08 ZPR
MMVM-SOP-0032.0-03062002	Fluorometer	01/25/08 ZPR
MMVM-SOP-0033.0-03062002	Glass and Plastic Labware: Cleaning and Sterilizing	01/25/08 ZPR
MMVM-SOP-0034.2-04042005	Glassware Washer	01/25/08 ZPR
MMVM-SOP-0035.1 -06172003	Milli-Q Laboratory Water Purification System	4/12/07 ZPR
MMVM-SOP-0036.2-06262003	pH Meter	01/25/08 ZPR
MMVM-SOP-0037.1-03242005	Wheaton Unispense Media Dispenser	01/25/08 ZPR
MMVM-SOP-0038.0-03062002	Media Dispensing Syringe	01/25/08 ZPR
MMVM-SOP-0039.1-06172003	mENDO Agar LES	01/25/08 ZPR
MMVM-SOP-0040.2-03242005	mFC Agar	01/25/08 ZPR
MMVM-SOP-0041.0-03062002	Plate Count Agar	01/25/08 ZPR
MMVM-SOP-0042.0-03062002	BEA Agar	01/25/08 ZPR
MMVM-SOP-0043.0-03062002	Trypticase Soy Agar	01/25/08 ZPR
MMVM-SOP-0044.1-06262003	mEI Agar	01/25/08 ZPR
MMVM-SOP-0045.0-03062002	Brain Heart Infusion Broth & Agar	01/25/08 ZPR
MMVM-SOP-0046.1-03242005	Lauryl Tryptose Broth	01/25/08 ZPR

Marine Microbiology and Vector Management

Index of Standard Operating Procedures

Document Number	Document Name	Review Date/Initials
MMVM-SOP-0047.1-03242005	Brilliant Green Bile Broth	01/25/08 ZPR
MMVM-SOP-0048.0-03062002	EC Broth	01/25/08 ZPR
MMVM-SOP-0049.1-06262003	Phosphate Saline Buffered Water (for mEI)	01/25/08 ZPR
MMVM-SOP-0050.0-03082002	Phosphate Buffered Dilution Water	01/25/08 ZPR
MMVM-SOP-0051.0-03082002	Nutrient Agar	01/25/08 ZPR
MMVM-SOP-0052.0-03082002	Nutrient Broth	01/25/08 ZPR
MMVM-SOP-0053.1-06162003	Sterilization of Wooden Applicator Sticks	01/25/08 ZPR
MMVM-SOP-0054.0-03082002	Azide Dextrose Broth	01/25/08 ZPR
MMVM-SOP-0055.1-04222004	Non-Shoreline Sampling	01/25/08 ZPR
MMVM-SOP-0056.0-03082002	Ascaris Ova Procedure	01/24/08 GBA
MMVM-SOP-0057.3-04102008	Recycling SOP	04/10/08 JLT
MMVM-SOP-0058.0-03112002	Chain of Custody	01/24/08 GBA
MMVM-SOP-0059.1-06162003	Avon Boat	01/25/08 ZPR
MMVM-SOP-0060.0-03112002	Multiple Tube Fermentation for Salmonella	01/15/08 RD
MMVM-SOP-0061.0-03112002	Preparation of Bacterial Slants for QC	01/25/08 ZPR
MMVM-SOP-0062.1-04292002	Competency Training and Evaluation	01/24/08 GBA
MMVM-SOP-0063.0-03142002	Midge Prevention and Control Program	01/15/08 AM
MMVM-SOP-0064.0-03142002	Vector Control	01/15/08 AM
MMVM-SOP-0065.0-04172002	ISO Document Control	01/24/08 GBA
MMVM-SOP-0068.0-04252002	Sewage Spill	01/24/08 GBA
MMVM-SOP-0069.0-04252002	Incident Communication Log	01/22/08 AR
MMVM-SOP-0070.1-04222004	Biological Waste Disposal	01/16/08 RS
MMVM-SOP-0071.1-04222004	SOP Management and Use	01/24/08 GBA
MMVM-SOP-0073.1-04222004	Spill Kits	01/22/08 AR

Marine Microbiology and Vector Management

Index of Standard Operating Procedures

Document Number	Document Name	Review Date/Initials
MMVM-SOP-0074.1-04042005	Alcohol Hazardous Waste Disposal	01/15/08 AM
MMVM-SOP-0075.1-06262003	Ocean Vessel Supply and Sample Transport	01/16/08 RS
MMVM-SOP-0076.0-02032003	Miscellaneous Reagents	01/16/08 RS
MMVM-SOP-0077.0-05072003	Hot Air Oven	01/16/08 RS
MMVM-SOP-0078.0-02242004	Acid Washing	01/16/08 RS
MMVM-SOP-0079.0-02242004	Surf Zone Sampling	01/16/08 RS
MMVM-SOP-0080.0-02242005	Calcium Saline Buffer	01/16/08 RS
MMVM-SOP-0081.0-02242005	Milli-Q Water Blanks (90 & 100 mL)	01/16/08 RS
MMVM-SOP-0082.0-05062005	Pesticide Use Reporting	01/15/08 AM
MMVM-SOP-0083.0-05062005	SPWRP Vector Control (Shut-Down)	01/15/08 AM
MMVM-SOP-0084.0-02072007	Gram Staining	01/24/08 GBA
MMVM-SOP-0085.1-04042008	Obsolete	01/24/08 GBA
MMVM-SOP-0086.0-02072007	Trypticase Soy Broth	01/24/08 GBA
MMVM-SOP-0087.0-04122007	Nutrient Broth II	01/24/08 GBA
MMVM-SOP-0088.0-10182007	Wild E. coli Culture Propagation	01/24/08 GBA
MMVM-SOP-0089.0-01022008	Bacterial Overlimit Responses	03/25/08 SER

Appendix B

Laboratory Organization

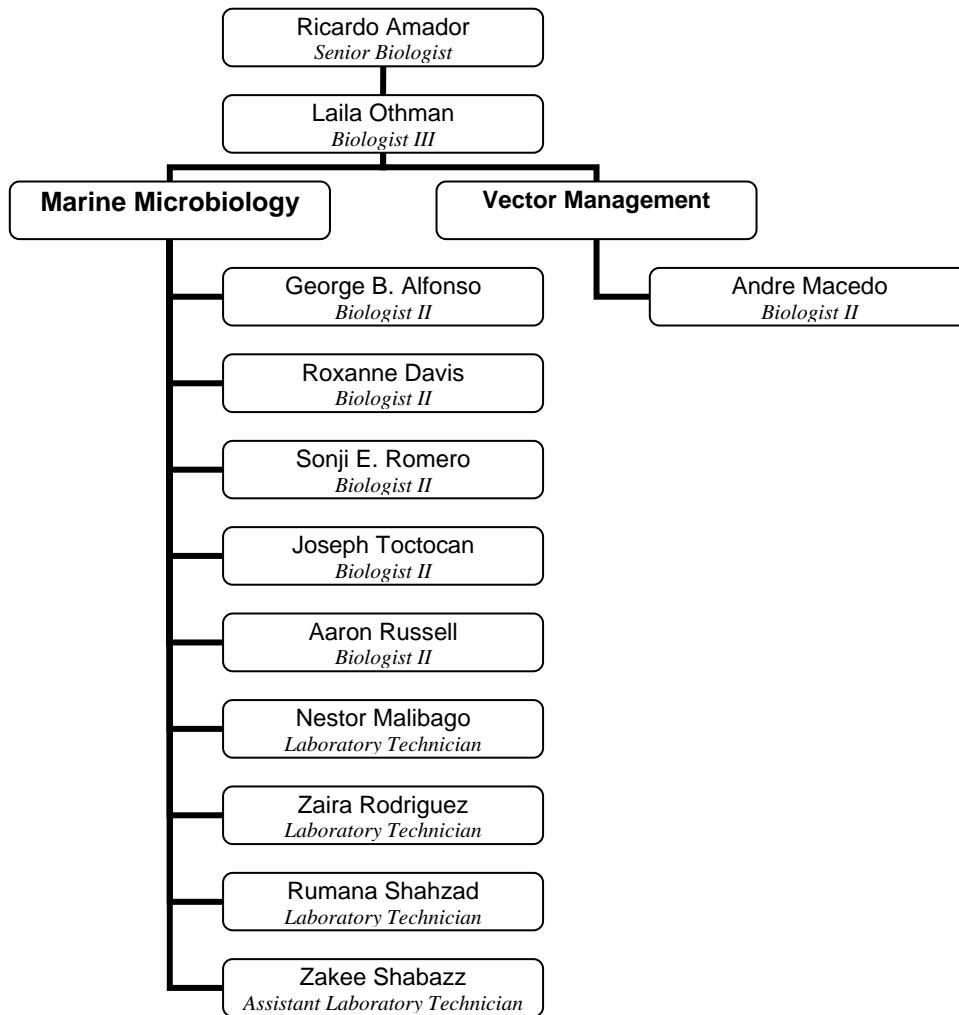
Section flowchart, laboratory positions, and staff biographies

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**City of San Diego
Metropolitan Wastewater Department
Environmental Monitoring & Technical Services Division**

Alan Langworthy, *Deputy Director, MWWD/EMTS*

Marine Microbiology and Vector Management



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LABORATORY STRUCTURE OF MARINE MICROBIOLOGY AND VECTOR MANAGEMENT

Laboratory staff consists of one Senior Biologist, one Biologist III, six Biologists II, three Laboratory Technicians and one Assistant Laboratory Technician. Following the job descriptions below, a summary of each employee's qualifications is included.

Senior Biologist (1): Laboratory supervisor responsible for overseeing the MMVM Laboratory and Programs and for implementing client contracts and service level agreements.

Biologist III (1): Supervisor responsible for all functions of the group. Duties include supervision, coordination with other groups, quality assurance, reporting of data to appropriate agencies and all phases of field and laboratory operations.

Biologist II (5): Fully trained professionals responsible for all phases of microbiological analyses. Duties include the analysis of ocean offshore and receiving waters, watersheds, effluent waters treatment plants for total coliform, fecal coliform / *E. coli*, and enterococci bacteria, microbiological analysis of biosolids, laboratory water quality analysis and design, data entry, specified interagency communications and performance of special projects as requested.

Biologist II/Entomologist (1): Fully trained professional, in the scientific identification of freshwater macro invertebrates, responsible for the performance of biological assessment of streams and creeks. Duties include sampling, monitoring, collection and taxonomic identification of arthropod and other insect vectors; is State Certified to handle and apply pesticides for mosquito and other invertebrate control; accurately reviews, researches, calculates, evaluates, and reports entomological data; abides by SOPs, QA/QC rules, and County Department of Health Services (CDHS) regulations.

Biologist I(0): Entry-level professionals who are being trained in various aspects of the City's field and microbiology laboratory operations.

Laboratory Technician (3): Technical support staff responsible for sample collection and microbiology preparation activities. Duties include quality assurance of all field and prep-room equipment, processes, instrumentation and other supplies; maintains stock of prep-room and microbiology supplies; conducts media preparation and quality control; operates and maintains City vehicles and watercraft; assists with sample site assessment and selection; conducts routine bacteriological analyses under guidance of professional staff.

Assistant Laboratory Technician (1): Technical support staff responsible for equipment and supply transport, preparation of media and glassware for field and laboratory operations, and field sampling.

In addition to routine work performed during the workweek, scheduling includes weekend overtime work in support of the treatment plant processes and offshore and/or shoreline permit work. Also, a team of one biologist and one laboratory tech are on call 24 hours per day / 7 days per week for the purpose of call-out in the event of an emergency.

Marine Microbiology and Vector Management Laboratory (MWWD/EMTS)

Ric Amador Senior Biologist

Ric Amador is a Senior Biologist and manages the Marine Microbiology and Vector Management Groups. He received his B.S. degree in Zoology at San Diego State University in 1975. He served in the U.S. Army, was an Environmental Health Technician Course graduate and worked as a Health and Environment Technician from 1975 to 1978. During this time he completed Environmental Health coursework offered by Baylor University. While in the Army Ric conducted a variety of health related surveys, inspections and investigations and performed bacteriological tests on water and wastewater samples. Ric began his career with the City in the Water Department Water Quality Laboratory as a Microbiologist in 1978. His responsibilities included bacteriological, chemical and plankton sampling and analyses appropriately applied to water samples from raw drinking water sources, water treatment plants, potable water distribution systems, reclamation plants, sewage sources and wells. In 1989 Ric promoted to supervisor of the Water Quality Microbiology Laboratory. Ric developed procedures for handling water quality complaints, a protocol for new main training, sampling and testing as well as helping to design the microbiology database. He led the implementation of the Total Coliform Rule sample siting plan and coordinated the laboratory's effort in the Mission Bay Rainfall Study. In 1997 Ric transferred to the Metropolitan Wastewater Department to supervise the Marine Microbiology and Vector Management group. He directed the group's work for the Bight'98, Bight'03, EPA Impact and Bonair Storm Drain studies. He is the project manager for the Mission Bay Water Quality Supplemental Environmental Project. Ric promoted to Senior Biologist in 2001.

Laila Othman Biologist III

Laila Othman is the supervising biologist for the Marine Microbiology and Vector Management Section. She received a B.A. in Chemistry at San Diego State University in 1995, an A.S. in Liberal Arts from Southwestern Community College in 1992, and an A.S. in Pharmacy at Ramallah Women's Training Center (Junior College). Laila began her career with the city in 1989 as an Assistant Laboratory Technician responsible for media preparation, and field sampling. In 1996, she was promoted to Biologist I/II and performed bacterial and viral analyses of environmental water samples. Her primary responsibilities included membrane filtration, multiple tube fermentation, chromogenic, and phage analyses, in addition to participating in special and regional studies. In 2002, Laila was promoted to Biologist III, responsible for supervising a large and varied section. Laila also plays an important role in the City's Diversity Program and produces the Division's Diversity newsletter.

George B. Alfonso
Biologist II/Microbiology

George Alfonso is a Microbiologist in the Marine Microbiology and Vector Management Section. George received his B. S. in Medical Technology degree in 1975 at St. Louis University, in the Philippines. He immediately began employment with the Bureau of Research and Laboratories of the Department of Health in the Philippines, where he worked in the rabies vaccine production for both human and veterinary use. In 1978, George went to work for the Microbiology section of Nestle Philippines, until leaving for Saudi Arabia in 1982. He worked for sometime in the flour milling industry as a shift technician in the quality control laboratory, and then in the Water Quality Laboratory of the Water Treatment Plant for King Khalid International Airport in Riyadh, Saudi Arabia. In 1991, George brought his family to the United States and worked as a laboratory technician at Pacific Treatment and Analytical Services. He came to the City in 1991 to work as Biologist I in the Water Quality Laboratory of the Water Department. Besides his regular laboratory work, he got involved in the development on the new database system (LIMS) for the Water Quality Laboratory of Water Department. He joined the Marine Microbiology and Vector Management Group in 1999.

Roxanne Davis
Biologist II

Roxanne Davis is a biologist in the Marine Microbiology and Vector Management Section. She received her B.S. in Microbiology at San Diego State University in 1986 and her A.S. in Animal Health Technology at San Diego Mesa Community College in 1981. Roxanne began her career with the City as an Assistant Laboratory Technician in the Industrial Waste Laboratory in 1991, where she was responsible for field sampling and testing. In 1994, she was promoted to Biologist I with the Marine Microbiology Section. Her primary responsibilities include bacterial and viral analyses on ocean, shoreline, storm drain, and tributary water samples using membrane filtration, multiple tube fermentation, IDEXX, and phage analyses. Roxanne also creates and updates the laboratory's SOPs and orders all supplies for the laboratory. Roxanne has also worked for The Salk Institute in 1985, preparing tissue culture media and for Scripps Clinic and Research Foundation from 1987-1989, conducting experiments for cancer research that involved tissue culture, monoclonal antibody production, and DNA/RNA preparations. In 1991, she worked as a research technician at the San Diego State University Foundation and performed injections, blood collection, and surgeries while studying fetal alcohol syndrome in laboratory rats.

Sonji E. Romero
Biologist II

Sonji Romero is a biologist for the Marine Microbiology and Vector Management Section. Her duties include bacteriological/viral analyses of various environmental receiving waters, storm water, wastewater, sludge, and solids. These analyses include Membrane Filtration, Multiple Tube Fermentation, and chromogenic substrate analyses. In 1992, she received her B.S. Degree in Microbiology from SDSU. Prior to becoming a biologist with the Marine Microbiology section, Sonji worked as a Laboratory Technician for Wastewater Chemistry where her duties included washing

laboratory glassware, performing TKN analyses and pesticides extraction, and sample collection. Sonji has also worked as a chemist with Analytical Technologies, Inc., as a VOC Gas Chromatography Chemist, where she performed Purge and Trap (EPA 601/602, 8010/8020/8021, 501/502) analyses on various industrial and environmental matrices, GC maintenance, data input and review. She is also an active member of the San Diego Stream Team, sampling for BMI and chemistries in Peñasquitos Creek and the San Diego River.

Joseph Toctocan
Biologist II

Joseph Toctocan is a biologist in the Marine Microbiology and Vector Management Section. He received his B. S. degree in Biology from St. Louis University, Baguio City, Philippines in 1980. He started his career with the City of San Diego, in October 1993 as a laboratory technician for the drinking water department. He transferred to his present position in September 2001 where he performs bacteriology tests (HPC, MPN, Colilert and MF) for the ocean and storm drain monitoring programs, reclaimed water processing plants, and other special studies. In the Philippines, he worked as a medical laboratory manager before immigrating to the U.S.A. in 1993. Joseph is also certified as a Medical Assistant, EKG operator and Phlebotomist in the state of California, and has worked as a Medical Assistant at the Amigo Medical Clinic in Chula Vista and a Medical Assistant instructor at the Pima Medical Institute in San Diego, CA. Joseph has also provided medical volunteer service for the San Ysidro Health Center and the Paradise Valley Hospital in National City, CA.

Aaron Russell
Biologist II

Aaron Russell is a biologist in the Marine Microbiology and Vector Management Section. He received his B.S. Degree in Biology from San Diego State University in 2005. His duties include bacteriological analysis of various environmental receiving waters, storm water, reclaimed water, sewage, and sediment. The analyses frequently used include membrane filtration, multiple tube fermentation, IDEXX, and phage analysis. In 1997, Aaron began working as an Assistant Laboratory Technician in the group performing laboratory prep work and field sampling. In 2002, Aaron filled a position in the City's Storm Water Pollution Prevention Program where he worked to comply with the City's Storm Water Discharge Permit 2002. His areas of focus included the development and implementation of the Dry Weather Stormdrain Monitoring Program, Coastal Storm drain Monitoring Program, and the Illicit Connection Illegal Discharge Program. In 2003, Aaron promoted to the Environmental Services Department where he expanded his ecology background by working with the City's solid waste regulations, Multiple Species Conservation Program, local fire ecology, invasive plant eradication, and public environmental education

André S. Macedo

Biologist II

André Macedo is a Biologist in the Marine Microbiology and Vector Management Section. André's primary area of responsibility includes the prevention and control of insects of medical importance and that are capable of transmitting diseases to humans, such as mosquitoes and flies. He is also trained as a First Responder in situations involving fire ants and Africanized honeybee attacks. André graduated in 1990 from SDSU with a B.S. in Biology, and since 1993 has been certified by the California DHS's Vector Borne Disease Control Section as a Vector Control Specialist. He started working for the City in 1989 as a Student Intern at the AQUA II Project. He became a Biologist in 1995 after serving as an Administrative intern and Laboratory Technician in the Organic Chemistry and Entomology work groups. Between 1997 and 2000, André worked as a Special Adviser to the Federal Ministry of Health of Brazil, and for the Health Departments of the Brazilian states of Pernambuco and Bahia. Currently André is responsible for all aspects of vector monitoring and control at all MWWD's wastewater, water reclamation, and sludge processing facilities.

Nestor Abenojar Malibago

Laboratory Technician

Nestor Abenojar Malibago is a Laboratory Technician in the Marine Microbiology and Vector Management Section. He was in his fourth year in B.S. Mechanical Engineering at Saint Louis University, Philippines when he joined the U.S. Navy and was classified as a Boiler Technician until December 1995. He received his Water/Wastewater Technology certificate at Mesa College, San Diego in June 1997. In May of 1996, Nestor began his career in the City as Utility Worker I in the Metropolitan Wastewater Department, Collections Division. In June 1998, he was hired in Industrial Waste Laboratory as a Laboratory Technician. Nestor transferred to Marine Microbiology and Vector Management Section in October 2002. His responsibilities include media and reagent preparation, quality assurance and control, shore and bay sampling, supply inventory and maintenance, preparation and transport of reusable supplies, and media and buffer water preparation. He is also on call to respond to accidental sewage spills investigations, participates in sample site assessment and G.P.S. verification, and assists Marine Microbiologist in routine analysis as required.

Zaira Rodriguez

Laboratory Technician

Zaira is a Laboratory Technician in the Marine Microbiology and Vector Management Section. She received her B. S. as Pharmacobiological Chemist (1995) and Industrial Chemist (1987) at UABC (Campus Tijuana, Mexico). Zaira started her career working at Hospital IMSS in Tijuana as Chemist and Microbiologist in the areas of Microbiology, Parasitology, Serology, Immunology, Endocrinology, Clinical Chemistry, Blood Bank and Hematology. She performed two research projects "Sensitivity to Antibiotics of Bacteria Isolated in the Hospital" and "Retrospective Study of Antibodies against CMV in Patients in the Hospital" (1988-1999). In 1999 she started working at the San Diego County Public Health Laboratory as a Laboratory Assistant for three years. Her main duties at the County Laboratory were performing IDEXX, MPN, Q.A., Q.C. and analysis on water samples, also in the area of Mycobacteria Isolation, Serology and Parasitology. She participated in

two Binational Projects in Tijuana and San Diego with the San Diego County Health and Human Services. Zaira started her career with the City of San Diego in January 2003 in the Marine Microbiology and Vector Management Section as a Laboratory Technician. She is a sampler involved in Water Monitoring Programs, performs Q. A., Q. C. of materials and supplies necessary for the microbiological analysis of water, and provide analytical help to the Biologist as needed. Zaira has participated in the Epidemiological Study in Mission Bay and Southern California Bight projects, Bacterial Monitoring and Source Tracking for Pacific Beach Point.

Rumana Shahzad
Laboratory Technician

Rumana Shahzad is a Laboratory Technician in the Marine Microbiology and Vector Management section. Her main duties include media, glassware and buffer water preparations, QA analysis, field sampling of ocean and bay waters, and participation in special projects and regional studies. Rumana Shahzad has a Masters degree in Clinical Microbiology from the University of Karachi in Pakistan, and her Professional Certificate in Occupational Health and Safety from the University of California San Diego (1997), Rumana worked as a Laboratory technician in the Industrial Waste laboratory from 1994-2003, where she entered Organic chemistry and field sampling data into the LIMS system, designed Chain Of Custody forms, reviewed SOPs, and conducted hazardous materials disposals of solvents under ISO protocol. She also performed tests on wastewater for flash point, pH, conductivity, sulfides, Organic extractions/Concentrations for Base neutral Acids, Pesticides and Voc. She also has experience with field sump inspections and industrial wastewater sampling. Rumana has four years of experience working as an Analytical Chemist at Analytical Technologies in San Diego. She also has extensive Medical Technology background, and worked at Sharp Rees Stealy for four years as well as with leading Doctors offices.

Zakee Shabazz
Assistant Laboratory Technician

Zakee is as Assistant Laboratory Technician in the in the Marine Microbiology and Vector Management Section. He served as a Hospital Corpsman and Preventive Medicine Technician in the U.S. Navy for seventeen years. His areas of expertise included microbiology, environmental health, occupational health, and water/waste water treatment. As a Hospital Corpsman, Zakee collected water samples from shore and ship stations, sterilized surgical instruments, and prepared membrane filtration equipments and supplies. He started his career with the City in 1997. Currently, his main duties include field sampling of ocean, bay and creek waters, glass ware and media preparation, supplies and samples transportation.

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Appendix C

Certifications

Environmental Laboratory Accreditation Program (ELAP),
ISO 140001 Certification

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STATE OF CALIFORNIA
DEPARTMENT OF HEALTH SERVICES
ENVIRONMENTAL LABORATORY ACCREDITATION PROGRAM

ENVIRONMENTAL LABORATORY CERTIFICATION

Is hereby granted to

CITY OF SAN DIEGO - MARINE MICROBIOLOGY LABORATORY
ENVIRONMENTAL MONITORING & TECHNICAL SERVICES

2392 KINCAID ROAD
SAN DIEGO, CA 92102-1811

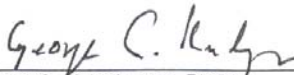
Scope of certification is limited to the
"Accredited Fields of Testing"
which accompanies this Certificate.

Continued certification status depends on successful completion of site visit,
proficiency testing studies, and payment of applicable fees.

This Certificate is granted in accordance with provisions of
Section 100825, et seq. of the Health and Safety Code.

Certificate No.: **2185**
Expiration Date: **11/30/2008**
Effective Date: **11/01/2006**

Richmond, California
subject to forfeiture or revocation



George C. Kulasingam, Ph.D.
Program Chief
Environmental Laboratory Accreditation Program

CALIFORNIA DEPARTMENT OF HEALTH SERVICES
ENVIRONMENTAL LABORATORY ACCREDITATION PROGRAM
Accredited Fields of Testing

CITY OF SAN DIEGO - MARINE MICROBIOLOGY LABORATORY
ENVIRONMENTAL MONITORING & TECHNICAL SERVICES
2392 KINCAID ROAD
SAN DIEGO, CA 92102-1811

Lab Phone (619) 758-2311

Certificate No: 2185 Renew Date: 11/30/2006

Field of Testing: 107 - Microbiology of Wastewater

107.010	001	Heterotrophic Bacteria	SM9215B
107.020	001	Total Coliform	SM9221B
107.040	001	Fecal Coliform	SM9221C,E (MTF/EC)
107.060	001	Total Coliform	SM9222B
107.080	001	Fecal Coliform	SM9222D
107.245	001	E. coli	SM9223

Field of Testing: 126 - Microbiology of Recreational Water

126.010	001	Total Coliform (Enumeration)	SM9221A,B,C
126.020	001	Total Coliform (Enumeration)	SM9222A,B
126.030	001	Fecal Coliform (Enumeration)	SM9221E
126.040	001	Fecal Coliform (Enumeration)	SM9222D
126.050	001	Total Coliform and E. coli	SM9223
126.070	001	Enterococci	EPA 1600
126.080	001	Enterococci	IDEXX

ABS Quality Evaluations

CERTIFICATE OF CONFORMANCE

This is to certify that the Environmental Management System of:

City of San Diego - EM&TS Division Wastewater Laboratory

(WITH FACILITIES LISTED ON ATTACHED ANNEX)

has been assessed by ABS Quality Evaluations, Inc. and found to be in conformance with the requirements set forth by:

ISO 14001:2004

The Environmental Management System is applicable to:

SAMPLING AND ANALYSIS OF WASTEWATER

Certificate No:
Original Certification Date:
Effective Date:
Expiration Date:
Issue Date

37040
03 June 2002
05 June 2006
02 June 2008
05 June 2006



Charles D. Russo
Charles D. Russo, President

Validity of this certificate is based on periodic audits of the management system defined by the above scope and is contingent upon prompt, written notification to ABS Quality Evaluations, Inc. of significant changes to the management system or components thereof.

ABS Quality Evaluations, Inc. 16800 Greenpoint Park Drive, Suite 300 South, Houston, Texas 77060 U.S.A.

Validity of this certificate may be confirmed at www.abs-qe.com/cert_validation.

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Appendix D

Miscellaneous QA Test Results

Test for Bacteriological Quality of Reagent Water Results, Test for Inhibitory Residue,
Split/ Duplicate Sign Test Analyses Results

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TEST FOR BACTERIOLOGICAL QUALITY OF REAGENT WATER RESULTS

Effective date: December 26, 2007

Final Count

The bacterial suspensions (*E. aerogenes* ATCC# 13048) with the dilution of 10^{-7} were chosen for final inoculations.

Test Water	Sample Volume	Plate Count			Average	Ratio Test/Control
		Rep 1	Rep 2	Rep3		
Control (Redistilled)	10^{-3}	>300	>300	>300	127	1
	10^{-4}	140	130	110		
	10^{-5}	12	13	16		
Milli-Q #1	10^{-3}	>300	>300	>300	110	0.87
	10^{-4}	110	120	100		
	10^{-5}	2	0	1		
Milli-Q #2	10^{-3}	>300	>300	>300	109	0.86
	10^{-4}	98	120	110		
	10^{-5}	12	10	13		

Interpretation: Pass. The ratio of test water to control water is within the accepted range of 0.8-1.2%.

Air Plate: 0

Media: 0

Buffer: 0

Air Plate: 0

Set-up Date/Time: December 27, 2007 1030

Read Date/Time: December 28, 2007 0900

Analyst: J. Toctocan/ S. Romero/ G. Alfonso

TEST FOR BACTERIOLOGICAL QUALITY OF REAGENT WATER RESULTS

Effective date: January 10, 2008

Final Count

The bacterial suspensions (*E. aerogenes* ATCC# 13048) with the dilution of 10^{-7} were used for final inoculations.

Test Water	Sample Volume	Plate Count		Average	Ratio Test/Control
		Rep 1	Rep 2		
Control (Redistilled)	10^{-5}	2	0	N/A	1.0
	10^{-4}	59	51	55	
	10^{-3}	>300	>300	N/A	
Purelab	10^{-5}	6	4	N/A	0.9
	10^{-4}	57	46	52	
	10^{-3}	>300	>300	N/A	

Interpretation: Pass. The ratio of test water to control water is within the accepted range of 0.8-1.2%.

Air Plate: 0

Media: 0

Buffer: 0

Set-up Date/Time: 01-09-08/1020

Read Date/Time: 01-10-08/0730

Analyst: GA/AR

CITY OF SAN DIEGO MARINE MICROBIOLOGY LABORATORY
INHIBITORY RESIDUES ON GLASSWARE

EMTS Facility
2392 Kincaid Dr.
San Diego, CA 92101

Date: February 3, 2004

Analysts: R.Davis, J. Toctocan, G. Alfonso

Detergent: Det-O-Jet

Bacterial Culture: *E. aerogenes* (ATCC # 13048)

Plate	Analysis Volume	Plate Count			Total	Ave.
A	1 mL	227	207	211	645	215
	0.1 mL	33	42	22	97	32
B	1 mL	230	208	163	601	200
	0.1 mL	18	16	20	54	18
C	1 mL	204	186	238	628	209
	0.1 mL	33	17	29	79	26
D	1 mL	201	189	189	579	193
	0.1 mL	18	24	17	59	20

Control Plate Counts:

Buffer Water: ≤1

Media : ≤1

15min.Air Plate:1

CALCULATIONS:

1. Routine rinsed (A) and well-rinsed (B)

$$\frac{B-A}{B} \times 100 = \frac{200-215}{200} \times 100 = 7.5 \%$$

2. Well rinsed (B) and unrinsed (C)

$$\frac{B-C}{B} \times 100 = \frac{200-209}{200} \times 100 = 4.5\%$$

3. Well rinsed (B) and plastic (D)

$$\frac{B-D}{B} \times 100 = \frac{200-193}{200} \times 100 = 3.5\%$$

4. Routine rinsed (A) and unrinsed (C)

$$\frac{A-C}{A} \times 100 = \frac{215-209}{215} \times 100 = 2.8\%$$

**CITY OF SAN DIEGO MARINE MICROBIOLOGY LABORATORY
INHIBITORY RESIDUES ON GLASSWARE**

**EMTS Facility
2392 Kincaid Dr.
San Diego, CA 92101**

INTERPRETATION:

1. There was no inhibitory residue when the dishes were washed using the routine washing procedure.
2. The detergent does not have inhibitory properties.
3. The plastic petri dishes are acceptable.
4. The cleaning detergent is eliminated during routine washing.

Split/ Duplicate Sign Test Analyses Results

Table 9

Summary of duplicate bacteriological analyses for the Point Loma and South Bay Ocean monitoring programs conducted from January through December 2007. The paired duplicate samples were compared using the sign test (see Gilbert, 1987) at a $p=0.05$ level of significance.

Duplicate samples

Parameter	N	B	Zb	P	Accept H_0
Total	153	80	0.57	>0.05	Accept
Fecal	128	68	0.71	>0.05	Accept
Enterococcus	110	51	-0.76	>0.05	Accept

Split samples

Parameter	N	B	Zb	P	Accept H_0
Total	19	9	-0.23	>0.05	Accept
Fecal	18	9	0.00	>0.05	Accept
Enterococcus	16	5	-1.50	>0.05	Accept

H_0 = The probability of observing positive and negative differences in plate counts between paired samples is equal (see text).

N = Number of sample pairs with different colony counts; samples without differences are not considered.

B = The number of positive differences between pairs.

Zb = Sign test outcome.