County of Orange

Water Quality Department

Quality Assurance/Quality Control Manual

February 2004

County of Orange
Health Care Agency
Public Health Laboratory
Water Quality Department

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Quality Assurance/Quality Control Project Plan

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INTRODUCTION

1.1 Bacteriological Monitoring Quality Assurance/Quality Control Goals

The Orange County Health Care Agency (OCHCA) has been monitoring water quality at numerous locations for over ten years. The Water Quality Department Laboratory (WQDL) Quality Assurance and Quality Control (QA/QC) Plan was developed in compliance with the QA/QC requirements set by Orange County Public Health Laboratory, Standard Methods (1020.A-C, 1030.A-D, 1040.A-C, 1050.B, 1060.A-C, 1080.A-C, 1090.A-H, 1090.J, 1100.A-C, 9030.B, (20th ed., American Public Health Association)) and Environmental Laboratory Accreditation Program (ELAP) requirements. The laboratory QA/QC plan consists of strict adherence to the (1) Quality Assurance and Quality Control manual and (2) Standard Operating Procedures (SOP) manual; training manuals; maintenance of QC records; ongoing review of QC procedures; and implementation of QA/QC improvements to provide quality results. California Department of Health Services (CDHS) also verifies the QA/QC plan by means of a laboratory inspection and annual requirement for acceptable analytical performance on performance evaluation (PE) studies. There is a 32-page ELAP on-site inspection list that is used by DHS to confirm laboratory compliance with ELAP required QA/QC. Additional information regarding ELAP requirements can be found at http://www.dhs.cahwnet.gov/.

Due to the large size of the electronic versions of the laboratory SOP and QA/QC manuals that also contain Excel spreadsheets, tables, photos and figures, the body of this QAPP contains limited sections of the manuals that are specific to meeting the objectives of this project. The laboratory SOP, QA/QC manuals and QC records or notebooks are available to project managers for review. References to sections of Standard Methods (SM) for the Examination of Water and Wastewater used by the WQD laboratory have been included. For example, analytical procedures that are detailed in the SOP, such as the membrane filtration technique to enumerate bacterial densities in water are not included in this document but referenced with the SM number. In addition, selections of QA/QC procedures from the WQDL QA/QC manual are located in Appendix A. This QAPP includes a general overview of the WQDL QA/QC practices.

1.2 Organizational Scheme

Title/Responsibility	Name	Phone number	E-mail
Laboratory Director	Douglas Moore, Ph.D.	(714) 834-8385	Dmoore@ochca.com
Laboratory Supervisor	Donna Ferguson	(949) 219-0424	DFerguson@ochca.com
Laboratory Staff	Martin Getrich	(949) 219-0423	Mgetrich_labhca@sbcglobal.net
Laboratory Staff	Mariam Zhowandai	(949) 219-0428	Mzhowandai_labhca@sbcglobal.net

2.0 SAMPLE COLLECTION, HOLDING TIME, AND TRANSPORT

2.1 SAMPLE COLLECTION

2.1.1 When collecting the sample, leave enough air space in the bottle to allow for proper mixing before examination. Collect samples that are representative of the water being tested. Flush or disinfect sample ports, and use aseptic techniques to avoid sample contamination. Keep the sample bottle closed until it is to be filled. Remove cap carefully to avoid contaminating the inner surface of the cap and neck of the bottle. Fill container without rinsing. Replace cap immediately. The volume of sample should be sufficient to carry out all tests required (not less than 100 ml). Provide complete and accurate sample identification information as specified on the sample collection sheet (chain of custody form).

2.1.2 Sample Containers

2.1.2.1 For bacteriological samples, use sterilized bottles of glass or plastic of appropriate size and shape. Bottles must be capable of holding a sufficient volume of sample for all required tests, while allowing for air space. Commercially available, wide-mouthed, autoclavable or pre-sterilized Polypropylene bottles of suitable size are satisfactory. Pre-sterilized plastic bags, with or without a de-chlorinating agent, may also be used. Water samples that may have residual chlorine or another halogen require sample bottles containing a reducing agent, such as sodium thiosulfate. Sodium thiosulfate neutralizes any residual halogen and prevents the continuation of bactericidal action during sample transit.

2.1.2.2 OCHD water collection bottles are quality controlled by batch (See Media QC Notebook). Sample bottles are stored in the water laboratory for use by Environmental Health personnel and other governmental agencies upon request.

2.1.3 Sample Types

2.1.3.1 Potable Water

- For drinking water analysis, collect samples consisting of finished water.
- Open tap fully and flush water for 2 - 3 minutes, or until a time sufficient to permit clearing the service line.
- Reduce water flow to permit filling bottle without splashing.
- Do not sample from leaking taps that allow water to flow over the outside of the tap.
- When sampling from a mixing faucet remove faucet attachments, run hot water for 2 minutes, then cold water for 2 to 3 minutes, and collect sample as indicated above.

- If the sample is taken from a well fitted with a hand pump, pump water for 5 minutes before collecting sample. .
- If the well is equipped with a mechanical pump, collect sample from a tap on the discharge.
- If there is no pumping machinery, collect a sample directly from the well.

2.1.3.2 Raw Water

For collecting samples directly from a river, stream, lake, reservoir, spring, or shallow well, obtain samples representative of the water that is the source of supply to consumers.

2.1.3.3 Surface Waters

Select sampling locations to include a baseline location upstream from the study area. Where a tributary stream is involved, select the sampling point near the confluence with the mainstream points. To monitor stream and lake water quality, establish sampling locations at critical sites. Sampling frequency may be seasonal.

2.1.3.4 Bathing Beaches

Sampling locations for recreational areas should reflect water quality within the entire recreational zone. Collect samples in the swimming area from a uniform depth of approximately 1 m. To obtain baseline data on marine and estuarine bathing water quality include sampling at low, high, and ebb tides. Relate sampling frequency directly to the peak bathing period.

2.1.3.5 Swimming Pools

A swimming pool is a body of water of limited size contained in a holding structure. The water is generally chlorinated potable water but may also be derived from thermal springs or saltwater. Collect samples in the area and time of maximum bather density. Collect samples where water is 1 m. (See Std. Methods17th Edition Section 9213 B. for further information.)

2.1.3.6 Sediment and Sludge

Sediments provide a stable index of the general quality of the overlying water. Sampling frequency may be related to seasonal changes in water temperatures and storm water runoff. Sludge monitoring may indicate the effectiveness of wastewater treatment processes. Bottom sediment sampling requires special apparatus. (See Std. Methods 9060 A. 3. g.)

2.2 HOLDING TIME AND TEMPERATURE

2.2.1 General

Start microbial analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population. Keep samples cold during transport to the laboratory (4-10ºC), if they cannot be processed within 1 hour after collection. Blue ice packs are preferred over ice. If using ice, avoid direct contact of samples with ice using plastic packing material.

2.2.2 Drinking Water for Compliance Purposes

Hold samples at 4-10ºC during transit to the laboratory. Analyze samples on day of receipt whenever possible and refrigerate overnight if arrival is too late for processing on same day. Do not exceed 30-hour holding time from collection to analysis for coliform bacteria. Do not exceed 8 hour holding time for heterotrophic plate counts.

2.2.3 Non-potable Water for Compliance Purposes

Hold source water, stream pollution, recreational water, and wastewater samples below 4-10ºC during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. When transport of samples is longer than 6 hours consider using delayed incubation procedures.

2.2.4 Water for Non-compliance Purposes

Hold samples between 4-10ºC during transport and until time of analysis. Do not exceed 24-hour holding time.

3.0 SAMPLE MANAGEMENT AND DOCUMENTATION

3.1 Chain-of-Custody Procedures

3.1.1 Samples that are transferred from one agency to another agency for analysis require the use of Chain-of-Custody (COC) procedures that include the following requirements for the laboratory to accept custody of samples:

3.1.1.1 Sample Label
Samples must be properly labeled using waterproof ink to record the sample number/description, date and time collected.

3.1.1.2 Chain-of-Custody Forms
Orange County WQD Laboratory provides COC forms to field sampling personnel for detailed record keeping. There are two separate documents that make up the COC form. The field data sheet, referred to as the "Bacteriological Examination of Waters (BEW)" worksheet is the first COC form used and contains information such as the project name, sample identification, water type (marine, freshwater or other), weather, date and time of collection, sample location, field sampler name, field bottle number and tests requested. The BEW worksheet must accompany the sample during sample collection and transport to the laboratory. The second form is the "Water Lab Sign-In Sheet (WLSIS)" which is filled out at the lab upon sample delivery.

3.1.1.3 Transfer of Custody

Immediately following receipt of water samples to the laboratory, a laboratory assistant or microbiologist will conduct inventory and document information regarding sample transport and laboratory processing according to the "Logging in Membrane Filtration Samples" SOP. The inventory consists of checking the samples for proper labeling, cross-referencing sample labels with the BEW worksheet and reading the cooler thermometer to ensure proper transport conditions. Laboratory personnel receiving samples will record the date and time of sample receipt, number of samples, type of samples and cooler temperature and other pertinent comments on the "Water Lab Sign-In Sheet". The sample deliverer and the analyst receiving the samples must initial the WLSIS. Samples received leaking, broken, containing insufficient volumes, exceeding holding times or stored in coolers with temperatures above 10ºC will not be accepted. The sample anomalies will be pointed out to the sample deliverer and also noted on the WLSIS and BEW worksheet. The time of sample receipt will be recorded on the WQD "Bacteriological Examination of Waters (BEW)" worksheet that serves as the field data sheet and accompanies the samples. The BEW worksheet will be then be checked for completeness of field data information. Corrections to worksheets will be made by crossing out the incorrect information, recording the change and recording the date and initials of the analyst making the change. When the COC forms are completed, copies may be provided to the appropriate party(s).

3.2 Sample Log-In

3.2.1 Following transfer of custody, the samples are logged in with a laboratory number, which is used to track the sample throughout the analytical process. Laboratory numbers are assigned in sequential numeric order of receipt. Water samples for bacteriological testing are either tested immediately or refrigerated at 5 ºC to maintain sample integrity. Samples for compliance testing are tested within 2 hours of receipt in the laboratory and less than 6 hours from sample collection or up to 24 hours for noncompliance purposes.

3.2.2 Upon completion of bacterial analysis, the samples are stored for a minimum of 24 hours at 5ºC until they are either archived in storage areas or properly disposed.

3.3 Documentation of Field Data

Field data will be recorded in field notebooks and field data sheets. Field notebooks should be used to document field observations that are supplemental to field data recorded on the field data sheet.

4.0 DATA QA/QC OBJECTIVES

To produce acceptable testing results, the general data quality objectives for this QA/QC Manual are to ensure that the data is representative, comparable, complete, accurate, and precise. Acceptable results are those values that fall within the acceptable range specified. Corrective actions for unacceptable results for specific testing methods are detailed in the SOP and QA/QC manuals. All corrective actions taken are documented in the QA/QC manual. The laboratory will notify the Project Manager of any samples that are impacted by unacceptable QA/QC results.

4.1 Representativeness

Representativeness is the degree to which the data represent the actual condition of a sampling site. The following factors determine the representativeness of the data: sampling location, sampling frequency, sample type, sample collection methods, sample preservation, sample holding times and analytical methods used. These factors are critical components of a sampling plan designed to maximize representativeness of the data to the extent practicable.

4.2 Comparability

Comparability of data is the degree to which the data produced by one laboratory or study can be compared to another. The WQD laboratory uses EPA approved analytical methods where possible or methods that have been determined to produce measurement data of known and quality sufficient to meet the objectives of this project. The data will be reported in commonly used units.

4.3 Completeness

The completeness of data is the percentage of planned data that will be used to meet statistical criteria needed to reach study conclusions. Acquiring 100% of the data planned is difficult due to unexpected circumstances, adverse weather conditions, equipment problems, laboratory error, loss of samples or samples that are invalid because they do not meet all of the laboratory sample acceptance criteria. The goal of this project is to obtain 80% of data completeness. Percent completeness is the number of data values generated/number of samples collected multiplied by 100.

4.4 Accuracy

Accuracy is the degree to which the measurement is to its true value. Accuracy of the WQD laboratory methods is determined by means of testing the following: (1) performance evaluation (PE) samples consisting of known quantities of bacteria, (2) performance of culture media, (3) laboratory and field blanks and (4) split samples. In addition, equipment calibration checks are routinely done to ensure accuracy of measurements.

4.4.1 Performance evaluation studies

To assess laboratory accuracy and comparability of bacterial density estimation, the laboratory participates in performance evaluation (PE) studies once a year. Certified PE materials or challenge samples are purchased from vendors approved by the CDHS. These include samples spiked with known amounts of bacteria provided from the vendor. The vendor evaluates the PE sample testing results using target and range values generated from data produced by several laboratories using the same analytical methods. The results must fall within 3 standard deviations of mean bacterial counts obtained from participating laboratories. The results are sent to the laboratory and to CDHS. If the laboratory receives a “not acceptable” rating for a method, they must immediately review their work, implement the necessary corrective action and send a summary of the correction action to the CDHS. ELAP certified laboratories must receive an acceptable rating for ELAP approved fields of testing on an annual basis to meet the certification requirements.

4.4.2 Culture media QC

The performance of culture media used to enumerate bacterial densities is also tested to ensure accuracy of bacterial enumeration using membrane filtration. Refer to the "Membrane Filtration Media Quality Control" procedure in Appendix A. Briefly, a known amount of indicator bacteria is spiked into phosphate buffered water and filtered as routine samples. The membrane is placed on culture media, incubated overnight and the colonies on membrane filters are enumerated. The media is also tested using negative control bacteria that should not grow on selective media. The number and appearance of bacterial colonies is recorded. Each new lot of media is checked in parallel with the old lot.

4.4.3 Field blanks

Field blanks are used to check for background contamination as well as handling and storage problems that may affect the results. A field blank for bacterial analysis should be tested for each sampling event or at least once a month for each storm drain sampling site. The blank may be prepared using reagent water (i.e., double distilled water or sterile de-ionized water) that has been bottled in a laboratory or is commercially available. The field sampler or personnel from an agency external to the lab should prepare the blank in the field by pouring reagent water into the sample bottle using sterile techniques i.e., wearing sterile gloves and avoiding aerosol production/exposure. The field blank should be included with the routine samples delivered to the WQD laboratory. The blank should not yield a value higher than that allowed by the acceptance limits (Table X). In the case of membrane filtration of reagent water, the counts should be below detection limits i.e., no colony forming units should be detected.

4.4.4 Laboratory blanks

The membrane filtration equipment, membrane filters and dilution buffers used to process the samples are tested for possible bacterial contamination that can occur from carryover contamination due to insufficient sterilization of the filtration apparatus between samples. The WQDL uses sterile, disposable pipettes, funnels, and forceps to minimize potential bacterial contamination and to increase sample throughput. To test the sterility of supplies and equipment, sterile dilution buffer is processed similarly as a water sample at the beginning, middle and end of the membrane filtration test run (uninterrupted series of analyses). If any contamination is found, the supervisor is notified immediately. An investigation of the source is initiated and the analytical data from samples tested with these materials is rejected (SM 9020B.8.a.5). Refer to "Membrane Filter Manifold Sterility Check" and "Sterility Testing" sections in Appendix A.

4.4.5 Calibration checks

Laboratory instruments are inspected and calibrated by laboratory personnel or equipment maintenance contractors using standards as per manufacturers instructions or the SOP. Electronic instruments for water analyses performed in the field will be calibrated once a day, prior to use.

4.5 Precision

Precision is the degree of agreement of repeated measurements of the sample, usually reported in standard deviation or relative percent difference (RPD). The precision of bacterial enumeration for the membrane filtration method is determined using the "Precision of Quantitative Methods" and "Duplicate Analyses" procedures.

4.5.1 Precision of Quantitative Methods

Initial demonstration of capability is conducted by each analyst before performing any analysis of samples and annually thereafter. The precision in performing the membrane filtration method is determined as outlined in "Method of Precision" in Appendix A. Briefly, analysts perform duplicate analyses on the first 15 positive samples tested for indicator bacteria testing. Since the laboratory currently analyzes over 200 samples per week, the precision criterion for each analyst can easily be determined. The precision criterion is calculated as 3.27 times the mean relative range value, "R" of duplicate results and is determined for each analyst of the WQD laboratory. The precision criterion for each analyst is determined annually. Precision between analysts in counting colonies on culture media plates is also assessed monthly following the "Analyst Comparison of Plate Counts" in Appendix A.

4.5.2 Duplicate Analyses

Duplicate samples are analyzed to assess the reproducibility of the sampling and analysis methods.

4.5.2.1 Laboratory Duplicates
To asses precision of the membrane filtration method, duplicate analysis is performed on 10% of all samples or at least one sample per test run. Refer to "Method of Precision" in Appendix A. The duplicate analyses is also used to determine the precision criterion for each analyst performing water testing and the results are compared for all analysts using an Excel spreadsheet.

4.5.2.2 Field Duplicates
Field duplicates will be tested to assess the repeatability of sampling. A field duplicate is a second sample that is collected at the same time or immediately following collection of the first sample. At least one field duplicate per sampling event or a minimum of one per month from each storm drain sampling site will be tested for indicator bacteria.

4.6 Sensitivity (Method Detection Limits)

Method Detection Limit is the lowest possible concentration that the equipment or analysis can detect. In the case of bacterial enumeration by membrane filtration, the "minimum detection limit" of bacterial colony forming units (CFU) using membrane filtration is based on the volume of water tested. Fecal indicator bacterial standards are based on bacterial counts per 100 ml of water. If 100 ml of sample is filtered and no bacteria are detected, the count is reported as "less than" (<) 1 CFU/100 ml because bacteria may have been present but not detected. Thus, "1 CFU/ 100 ml" is also the minimum detection limit. If only 10 ml of the 100 ml sample is filtered and no bacteria are detected, the count is reported as "less than 10 CFU/100 ml". The minimum

detection limit in this case is also 10 CFU/100 ml. For membrane filtration of stormdrain water samples, a minimum of 3 dilutions or volumes of water will be filtered to optimize detection of bacteria. Testing volumes should result in bacterial counts in the "countable range" as per Standard Methods to ensure accuracy of results. If the total number of bacterial counts exceeds 200 per membrane, or if the colonies are not distinct enough for accurate counting, the results will be reported as greater than or equal to (≥) the maximum number countable.

5.0 DATA MANAGEMENT AND HANDLING

5.1 Bacteriological Examination of Waters Worksheet (BEW)

5.1.1 Laboratory results include the following information documented on the BEW worksheet as described in the "Reporting Water Lab Results" section of the SOP:

- Date and time that the samples are processed
- Date and time analysis was completed
- Testing results
- Corrective actions

5.1.2 The laboratory results are checked by a second analyst (Microbiologist) for accuracy of the calculations (colony forming units (CFU)/100 ml) and completeness of the worksheet. Both the analyst that records the results and microbiologist who confirms calculations sign off on the results.

5.1.3 Corrective actions regarding sample collection, preservation and transport are documented on the worksheet in the "Field or Lab Remarks" section. This documentation includes the date, analyst, sample affected, problem and resolution.

5.2 Data Management

5.2.1 The laboratory results will be entered into an electronic spreadsheet (MS Excel) that is available to the project manager after the laboratory has reviewed the database for correctness of data entry. The BEW worksheets and back-up disks are kept at the laboratory for five years and also are made available to the Project Manager upon request.

5.3 Data Handling

5.3.1 Distribution of analytical results

Bacterial densities in environmental waters are highly variable, ranging from many values below detection to a few high ones. In such cases the data would be positively skewed and not normally distributed about the mean. Since statistical analysis assumes a normal distribution of data, the numbers must be converted to their logarithms to approximate a symmetrical distribution. The best estimate of central tendency of log-normal data is the geometric mean or antilog of the arithmetic mean of the logarithms.

5.3.2 "Less than" (<) values

Water samples with no indicator bacterial detected are reported with "less than" (<) values using the calculations in Standard methods (9222B.6). If no colonies are detected, the calculation is done as if there was 1 colony detected and reported with the "less than" qualifier "<". With the exception to <1/100 ml values reported for testing 100 ml volumes of, test volumes should be adjusted, when possible to avoid the use of qualifiers.
If there are large numbers of values with the "less than" or "greater than" qualifiers, the qualifier can be omitted so that the values can be included in the data analysis. However, values with "less than" qualifiers may also be converted to 1, 0 or ½ (values halfway between zero and the "less than" value) depending on the value, the detection limit and distribution of values in the data set.

5.3.3 "Greater than or equal to" (≥) values, TNTC and Confluent Counts

Water samples with high levels of indicator bacteria, particularly total coliforms, may result in counts reported as "greater than or equal to" values, "too numerous to count" (TNTC) or "confluent" (CONF). Such samples usually contain bacteria other than the specific indicator bacteria being tested, such as non-coliforms that can also grow on the membranes. High levels of non-coliforms or atypical bacteria may interfere with the detection of typical coliform bacteria such that the actual number of total coliforms in the sample may be greater or equal to the number detected. In this case, the number of typical coliform colonies is reported but with the " ≥ "qualifier. If no total coliforms are detected and there are greater than 200 colonies, the results are reported as "TNTC". If the total number of bacteria colonies (coliforms plus non-coliforms) exceeds 200 per membrane, the number of total coliforms detected is reported with the notation "(TNTC)". For example, if 10 total coliforms are detected, but there are greater than 200 total coliforms and non-coliforms on the membrane, the results are reported as "10 (TNTC)". If colonies cover the entire membrane and are not discrete enough for accurate counting, the results are reported as "confluent growth with coliforms present" or "confluent growth without coliforms detected". In each of these situations, additional samples should be collected. However, this may not be possible for samples collected during special events or circumstances. For noncompliance samples, the analysis can be repeated using additional volumes to obtain countable colonies or an "endpoint". Since the analysis is performed beyond 24 hours of sample collection, the results will not be highly accurate. Therefore, the project manager and laboratory should assess the significance of including these results in the data analysis.

6.0 ANALYTICAL METHODS

The WQDL uses EPA compliance methods for detection of total coliform, fecal coliform, *E. coli* and enterococci indicator bacteria. The total coliform and fecal coliform methods are described in the most current EPA approved versions of Standard Methods for the Examination of Water and Wastewater (20th edition). The *E. coli* and enterococci methods are described in "Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli* " (USEPA). The membrane filtration methods used by the WQDL are well established and

have known levels of bias and variability. These methods have been combined into a single document in the SOP entitled "Membrane Filtration Technique".

6.1 SOP Overview

The SOP describes all operating procedures and analytical methods used at the WQDL and are inclusive of compliance methods as well as research methods. Compliance methods are published by the USEPA in the Federal Register and are used to determine compliance with EPA water quality standards for drinkable or swimmable waters. Research methods may include non-approved EPA methods that involve measurements that are considered developmental. Research protocols developed or used by the laboratory are generally methods that have been published in scientific peer reviewed journals. Prior to implementation, the laboratory establishes the precision, accuracy and quality control of experimental methods. Any modifications to these methods are discussed between laboratory director, laboratory supervisor and project managers prior to implementing changes.

The SOP and QA/QC manual are updated on a regular basis and reviewed by the laboratory director. Strict adherence to the SOP is required of sample collectors and laboratory analysts to ensure consistency of sampling procedures and to produce data of high quality.

The SOP includes the following information:

- Analytical methods to be used in the laboratory
- Description of materials, reagents and culture media
- Description of sample types
- Sample collection
- Sample transport
- Procedures used to estimate bacterial densities
- Spreadsheets for calculation purposes
- References to methods
- Safety considerations
- Waste management

7.0 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

The Quality Assurance/Quality Control (QA/QC) procedures used at the Water Quality Department Laboratory to meet the project data quality objectives are listed below. The purpose for each QA/QC procedure and the method references are provided. A description of each procedure is also included following this list.

7.1 QA/QC Manual Overview

Quality Assurance and Quality Control requirements and procedures contained in the WQL Quality Assurance/Quality Control Manual are inclusive of the following information:

- Organizational structure
- Laboratory floor plan
- Personnel qualifications
- Personnel responsibilities and duties

- Analyst training, performance and competency record
- Procedures for handling and receiving samples
- Sample control and documentation procedures
- Equipment, instrumentation and reference standard measurements used
- Equipment preventive maintenance procedures
- Internal quality control activities
- Procedures and documentation for calibration, verification, maintenance of instrumentation and equipment
- QA/QC charts and forms
- Data verification practices, including intra-laboratory comparison and proficiency testing programs
- ELAP accreditation information
- Procedures and documentation of proficiency evaluation (PE) sample testing
- Corrective actions procedures
- Procedures for assessing data precision and accuracy
- Procedures for data reduction, validation and reporting
- Procedures for records archiving

7.2 Analytical QA/QC Procedures

7.2.1 Membrane filtration verification (*Standard Methods* 20th Edition, 1998; 9020B.9) or confirmation of total coliform, fecal coliform, *E. coli* and enterococci is performed monthly. (Refer to Membrane Filtration procedure in SOP).

7.2.1.1 The identification of bacterial colonies isolated from positive samples is confirmed using biochemical reactions and growth characteristics.

7.2.2 Verification of Membrane Filtration Manifold Sterility (*Standard Methods* 20th Edition; 1998; 9020B.8.a.5)

7.2.2.1 The sterility of media, membrane filters, buffered dilution and rinse water, pipettes, flasks and dishes, and equipment used during the membrane filtration procedure is determined.

7.3 Analyst QA/QC Procedures

7.3.1 Analyst Comparison of Plate Counts (*Standard Methods* 20th Edition, 1998; 9020B.8.a.2)

7.3.1.1 The ability to accurately enumerate bacterial colonies on solid media is determined for each analyst. The count differences between analysts must not exceed 10%.

7.3.2 Measurement of Method Precision (*Standard Methods* 20th Edition, 1998; 9020B.8.b) and Duplicate Analysis (*Standard Methods* 20th Edition, 1998; 9020B.8.a.4)

7.3.2.1 The precision of analyzing duplicate samples is determined for each analyst.

7.4 QA/QC of Laboratory Equipment

7.4.1 Sample Preservation and Storage (*Standard Methods* 20th Edition, 1998; 9060B.1).

7.4.1.1 Samples that are transported to the laboratory are held at 10ºC until they can be refrigerated or analyzed.

7.4.2 Sample Collection Bottle Sterility

7.4.3 Inhibitory Residue Testing (*Standard Methods* 20th Edition, 1998; 9020B.4.a.2)

7.4.3.1 The presence of bacteriostatic residues on glassware and plasticware from wetting agents or detergents is determined.

7.4.4 pH Check of Glassware (*Standard Methods* 20th Edition, 1998; 9020B.4.a.1)

7.4.4.1 The presence of alkaline or acid residue on glassware is determined.

7.4.5 Sample collection (*Standard Methods* 20th Edition, 1998; 9060A)

7.4.5.1 Standardized collection methods as described in Standard Methods are followed.

7.4.6 Equipment Temperature Checks (*Standard Methods* 20th Edition, 1998; 9020B.3)

7.4.6.1 Incubators, refrigerators, freezers, waterbaths, autoclave.

7.4.6.1.1 Temperatures of incubators and refrigerators are monitored on a regular basis using calibrated thermometers to maintain required temperatures for sample storage and bacterial growth. The required temperature ranges for all equipment are recorded on temperature charts at least once every day of laboratory use and/or operation. Autoclave temperature is checked with every use.

7.4.7 Thermometer Calibration (*Standard Methods* 20th Edition, 1998; 9020B.3.a)

7.4.7.1 Thermometers are calibrated annually using a certified National Institute of Standards and Technology (NIST) thermometer.

7.5 QA/QC of Media, Buffer, and Reagents

7.5.1 Membrane Filtration Media Quality Control (*Standard Methods* 20th Edition, 1998; 9020B.4.i.)

7.5.1.1 The performance and sterility of the membrane filtration media performance is determined using positive and negative control organisms.

7.5.1.2 Media tested: m-Endo (Total Coliforms), m-FC (Fecal Coliforms), mEI (Enterococci), m-TEC (*E. coli*).

7.5.1.3 Media that fails media QC procedures are not used.

7.5.2 Membrane Filtration Manifold Sterility Check

7.5.2.1 To determine the sterility of the manifold at set points throughout the Membrane Filtration process.

8.0 SAFETY

General laboratory safety procedures and work practices are detailed in the Orange County Public Health Laboratory (1) Exposure Control Plan manual, (2) Chemical Hygiene Plan manual and (3) Basic Safety Rules (SOP). Safety training, including the use of universal precautions and sterile techniques is provided to employees prior to working in the laboratory and on an ongoing basis. Laboratory personnel receive immunizations to pathogens, including Hepatitis B virus and tetanus prior to working with these organisms or environmental samples that may contain such pathogens. The laboratory microbiologists and laboratory assistants wear personal protective equipment. The laboratory is equipped with safety eyewashes, first aid kits and fire extinguishers that are tested annually.

Table 1 Quality Control Tests, Frequencies, and Acceptable Limits.

Quality Control Test	Testing Frequency	Acceptable Limits
Performance Evaluation (PE) Testing for Bacterial Water Quality	1x/year	Within 3 std. dev. of mean
Membrane Filtration Media	Each new lot number or shipment of media	UUPositive control organism: Counts within countable range Typical colonial morphology Negative control organism & Sterility: No Growth
Indicator Bacteria Confirmation/Verification	1x/month	Confirmation of at least 10 colonies per positive sample.
Inhibitory Residue on Glassware	1x/year or when using a new supply of glassware detergent	Refer to SM 9020B.4.a
QC Reagent Grade Water (In-house Milli-Q or Type I water)	Conductivity: each use pH: each use Heterotrophic plate count (HPC): monthly Total chlorine residual : monthly Heavy metals: annually	Conductivity: < 2µmhos/cm at 25ºC pH: 5.5 – 7.5 HPC: <1,000 CFU/ml Total chlorine residual: <0.01 mg/L Heavy metals: <0.05 mg/L
Test for the Bacteriological Quality (bactericidal properties) of Reagent Grade Water (Milli-Q or Type I)	1x/year	Growth ratio between 0.8 and 3.0 to control water
Test pH of glassware	2x/year	Neutral pH
Field Blank	Each sampling event or at least 1x/month	< 1 CFU
Membrane Filtration Sterility Check (Laboratory Blank)	Dilution buffer blank every 10 samples each day of use	< 1 CFU
Sterility Check of Labware (including sample bottles), Media and Reagents	Each new lot number.	< 1 CFU
Microbiological Media	Each new lot number	Typical growth or biochemical reaction for positive and negative organisms (Refer to SOP)
Analyst Precision Criterion	1x/year	3.27 R (R = mean of the range of the logarithms of duplicate counts for 15 samples)
Analyst Plate Count Comparison	Monthly	< 10% difference

Duplicate Samples	**Lab Duplicates:** Duplicate analysis: 10% of all samples Duplicate plate counts : monthly **Field Duplicate:** Each sampling event or at least 1x/month	Lab Duplicates: Within 5% agreement for the same analyst or 10% between analysts Field Duplicate: Within 95% confidence limit (SM Table 9222:II)

Table 2 Water Sample Parameters, Methods, Sampling Requirements, and Units.

Parameter	Method	Sample bottle type/ preservative	Maximum Holding Time/Temp	Units
Total Coliforms	SM 9222B	IDEXX 120 ml with sodium thiosulfate	6 h/ 2 - 10°C in dark	Colony forming units (CFU)/100 milliliter (ml)
Fecal Coliforms	SM 9222D	IDEXX 120 ml with sodium thiosulfate	6 h/ 2 - 10°C in dark	CFU/100 ml
Enterococcus spp.	EPA Modified Enterococci Method 1600	IDEXX 120 ml with sodium thiosulfate	6 h/ 2 - 10°C in dark	CFU/100 ml
E. coli	EPA Modified *E. coli* Method	IDEXX 120 ml with sodium thiosulfate	6 h/ 2 - 10°C in dark	CFU/100 ml

APPENDIX A: SAMPLE CHAIN OF CUSTODY FORMS

BACTERIOLOGICAL EXAMINATION OF WATERS (BEW)

County of Orange, Health Care Agency
Water Quality Laboratory
700 Shellmaker Rd.
Newport Beach, CA 92660 ELAP #1275
Phone:(949)219-0423 FAX:(949)219-0426

STUDY: _____
SAMPLE TYPE: _____
☐ Marine ☐ Freshwater ☐ Other_____
WEATHER:_____

FIELD DATA

Date Collected
Sampler

Field Bottle # | Time | Station Number / Location | Sand Present

LABORATORY REPORT

Date Received_____ Received by_____
Time In_____ Time Run_____ Date/Time Read_____

	Total Coliforms		Fecal Coliforms		*E. coli*		*Enterococcus*		
	m-Endo Agar LES		m-FC Agar		modified M-TEC		m-EI Agar		Report Date
O / Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
1 / 100.0									Micro Initial
10.0									
2 / 1.0									
0.5									
3 / 0.1									
0.01									
0.001									

☐ Total Coliforms
☐ Fecal/E.coli
☐ Enterococcus
☐ Inc.Dil _____
Laboratory No.

	m-Endo Agar LES		m-FC Agar		modified M-TEC		m-EI Agar		Report Date
O / Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
1 / 100.0									Micro Initial
10.0									
2 / 1.0									
0.5									
3 / 0.1									
0.01									
0.001									

☐ Total Coliforms
☐ Fecal/E.coli
☐ Enterococcus
☐ Inc.Dil _____
Laboratory No.

	m-Endo Agar LES		m-FC Agar		modified M-TEC		m-EI Agar		Report Date
O / Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
1 / 100.0									Micro Initial
10.0									
2 / 1.0									
0.5									
3 / 0.1									
0.01									
0.001									

☐ Total Coliforms
☐ Fecal/E.coli
☐ Enterococcus
☐ Inc.Dil _____
Laboratory No.

	m-Endo Agar LES		m-FC Agar		modified M-TEC		m-EI Agar		Report Date
O / Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
1 / 100.0									Micro Initial
10.0									
2 / 1.0									
0.5									
3 / 0.1									
0.01									
0.001									

☐ Total Coliforms
☐ Fecal/E.coli
☐ Enterococcus
☐ Inc.Dil _____
Laboratory No.

SUBMITTER INFORMATION / SUBMITTER NUMBER

Field or Lab Remarks:

WATER LAB SIGN-IN SHEET (LSIS)						
DATE	TIME IN	AMOUNT OF SAMPLES		TEMPERATURE <10°C	INITIALS	COMMENTS
		RECREATIONAL	DOMESTIC			

APPENDIX B: MEMBRANE FILTRATION MEDIA QC PROCEDURE

Principle

For membrane filtration, QC procedures are followed when a new batch or lot of m-Endo, M-FC, m-EI or m-TEC media is received. The following protocol establishes which positive and negative control organisms to use, how to dilute out the organism suspensions to get countable plates, and how to record the results.

Equipment

Refer to Membrane Filtration SOP (Recreational)

Reagents

Media: m-Endo, m-FC, m-EI, m-TEC
95 % Ethanol
Phosphate buffered saline (PBS)
15-ml Centrifuge tubes
2ml Sterile PBS (4 tubes)
9.9ml Sterile PBS (12 tubes)

Quality Control Organisms for Media

Media	Control organisms
1. m-Endo	
Positive Control -	*Escherichia coli* ATCC 25922
Negative Control -	*Sal. typhimurium* ATCC 14028
Negative Control -	*Staph. aureus* ATCC 25923
2. m-FC	
Positive Control -	*Escherichia coli* ATCC 25922
Negative Control -	*Enterococcus faecalis ATCC* 29212
3. m-EI	
Positive Control -	*Enterococcus faecalis* ATCC 29212
Negative Control -	*Escherichia coli* ATCC 25922
4. m-TEC	
Positive Control-	*Escherichia coli* ATCC 25922
Negative Control-	*Enterococcus faecalis* ATCC 29212

Procedure

1. The membrane filtration (MF) media, m-Endo, m-FC and m-EI can be made in-house, in the Media Room located at the Orange County Public Health Laboratory. Currently, all MF media are purchased from outside vendors. Set up the QC for each new lot number received.

2. The media prepared in-house will be transported to the Water Quality Department in large plastic Tupperware® with bubble-wrap to keep plates from moving. The person receiving the media must also initial the requisition form.

3. Select 3 plates of m-Endo media for the control organisms and 1 plate from each Tupperware® container or 5% of the total shipment to set up sterility controls. Select 2 plates of M-FC media for the control organisms and 1 plate from each Tupperware® container or 5% of the total shipment to set up sterility controls. Select 2 plates of m-EI media for the control organisms and 1 plate from each Tupperware® container or 5% of the total shipment to set up sterility controls. Select 2 plates of m-TEC media for the control organisms and 5% of the total shipment for sterility controls. Allow the plates to reach room temperature and label them with the control organism and date.

4. Label 4 tubes of 2ml Sterile PBS with each of the control organisms (*E. coli, S. typhimurium, S. aureus, and E. faecalis*).

5. For each of the control organisms, use three tubes of 9.9-ml of Sterile PBS dilution blanks (12 total). Label them with the control organism and the three serial dilutions that will be made (1.5×10^6, 1.5×10^4, and 1.5×10^2).

6. Use a sterile swab to make a suspension for each of the control organisms to 0.5 McFarland turbidity, using the 0.5 McFarland turbidity standard for comparison. The suspension should have approximately 1.5×10^8 bacteria/ml.

7. Tightly cap the suspension and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.1ml of the 0.5 McFarland suspension to the 9.9-ml of Sterile PBS dilution blank labeled as the 1.5×10^6 dilution.

8. Tightly cap the 1.5×10^6 dilution and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.1ml of the 1.5×10^6 dilution to the 9.9-ml of Sterile PBS dilution blank labeled as the 1.5×10^4 dilution.

9. Tightly cap the 1.5×10^4 dilution and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.1ml of the 1.5×10^4 dilution to the 9.9-ml of Sterile PBS dilution blank labeled as the 1.5×10^2 dilution.

10. Tightly cap the 1.5×10^2 dilution and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.5ml of the 1.5×10^2 dilution to a funnel containing 10ml buffered water and filter. Refer to the diagram.

11. Filter the *E. coli* suspension 4 times on 4 separate filters. Transfer the first filter to the m-Endo positive control plate, the second to the M-FC positive control plate, the third to the m-EI negative control plate, and the fourth one to the m-TEC positive control plate (as needed).

12. Filter the *E. faecalis* suspension 3 times on 3 separate filters. Transfer the first filter to the m-EI positive control plate, the second to the M-FC negative control plate, and the third one to the m-TEC negative control plate.

13. Filter the *S. typhimurium* and the *S. aureus* suspensions once for each organism. Transfer the filters to the m-Endo negative control plates.

14. Incubate the plates within 30 minutes as follows:
    m-Endo at 35 ± 0.5ºC for 22-24 hours
    m-FC at 44.5 ± 0.2ºC for 24 hours
    m-EI at 41 ± 0.5ºC for 24 hours
    m-TEC at 35 ± 0.5ºC for 2 hours and 44.5 ± 0.2ºC for 22 hours

Reporting

Record results in the QC notebook. Record positive, negative, and sterility results. Indicate the date tested and the date the results were read. Record lot numbers and expiration dates. If QC results are atypical notify the Micro and do not use the media from that lot until discrepancy has been resolved.

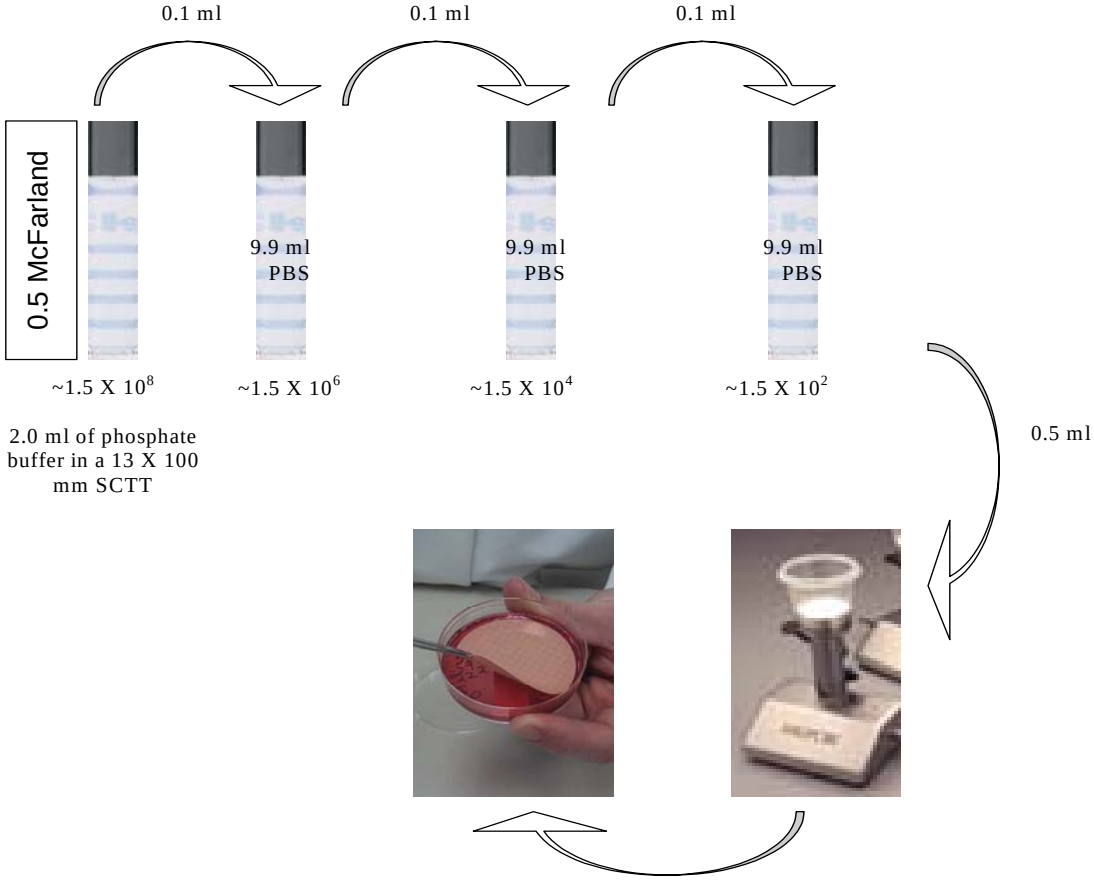

Diagram for setting up MF media quality control