Laboratories and Consulting Group

QUALITY ASSURANCE MANUAL
FOR
IEH-EEL LABORATORY

A Commercial Water, Wastewater and Food Testing Laboratory

Microbiological and Chemical Consulting Services
Water and Pollution Studies

Chemical and Microbiological Laboratory Services
Approved by California Department of Health Services

Plan Reviewed and Approved

Hojabr Dezfulian, Ph.D. Date
Managing Director,
Microbiology Laboratory Director,
& Quality Manager

Xzayla Zabiti Date
Deputy Director & QA/QC Officer

Michael Harris, Ph.D. Date
Chemistry Laboratory Director

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MANAGEMENT QUALITY POLICY STATEMENT AND ORGANIZATION

IEH Laboratories is a privately owned, independent laboratory. IEH corporate headquarters in Seattle, WA is an A2LA accredited laboratory and meets the requirements of ISO/IEC17025:2005 and cGMP regulations. IEH Laboratories & Consulting Group is a full service testing laboratory and consulting firm specializing in food, pharmaceutical and environmental safety since 2001. IEH (Institute for Environmental Health) –EEL (Environmental Engineering Laboratory) are business units of the IEH. The managing director of EEL reports directly to the president of IEH. IEH and EEL are in essentially the same business and, as such, IEH exerts no influence over EEL, with respect to the results or reporting of analytical results.

Our major focus is providing expert analytical and technical support to the food industry through consultation, research, product-development, and the investigation of microbiological issues in food. IEH-EEL is also participating in shellfish sanitation program. We evaluate product-specific problems, design and conduct experiments, collect and interpret data, and implement solutions for the food industry.

At IEH-EEL, we are committed to serving our clients with the utmost integrity and accuracy of environmental and food analysis.

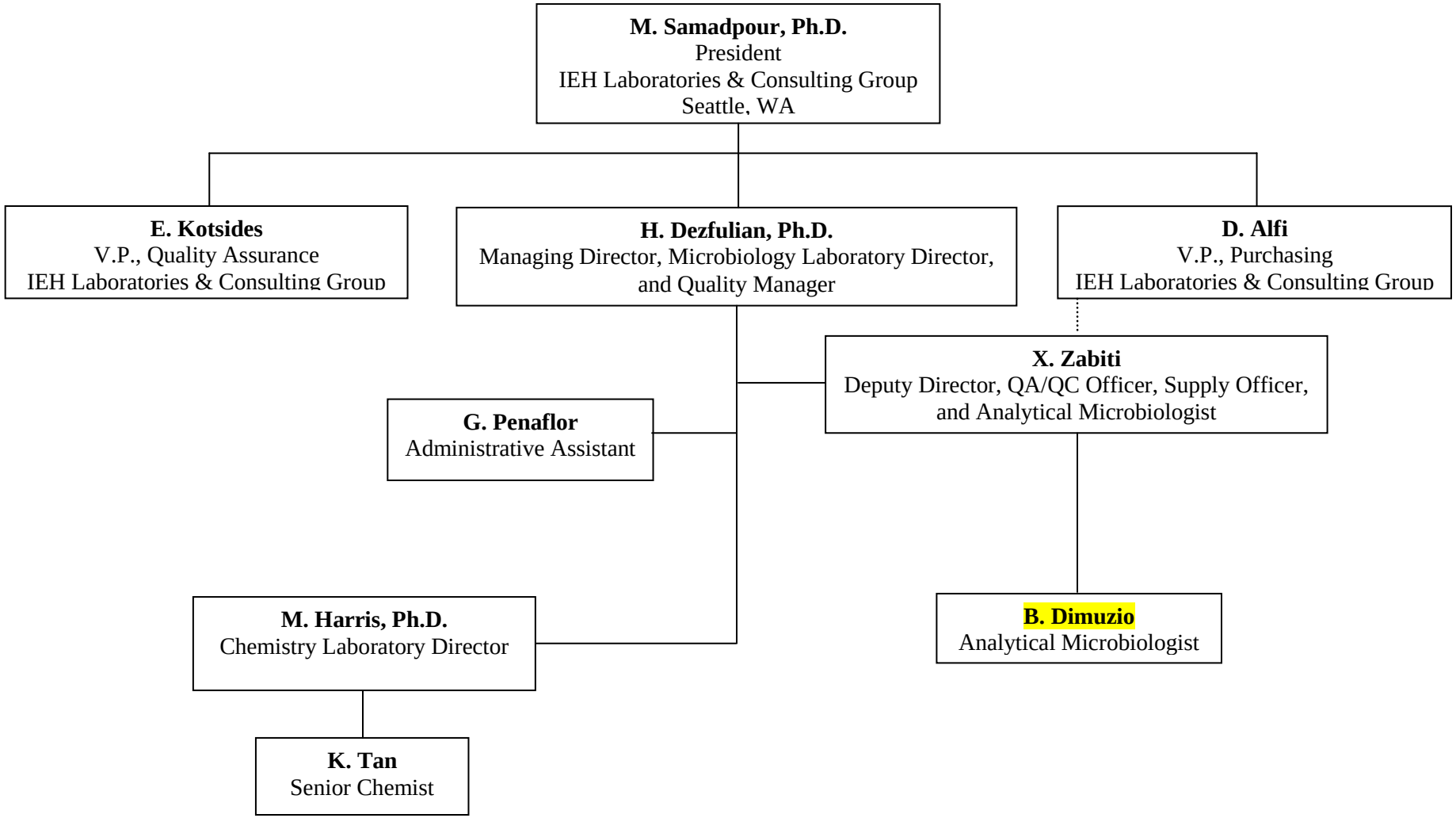
It is the policy of IEH-EEL Laboratories to provide all clients with test results that are accurate and legally defensible. IEH-EEL Laboratories management is committed to good professional practices and quality in environmental testing and calibration as documented in the Quality Assurance Manual and all applicable ELAP (Environmental Laboratory Accreditation Program) standards.

This policy has the full support of Management and must be accomplished with the cooperation of all employees. All personnel concerned with environmental testing and calibration activities within the laboratory are required to familiarize themselves with the quality documentation and implement the policies and procedures in their work.

LABORATORY ORGANIZATION

IEH has nationwide representation and is constantly growing. Our corporate office is located in Seattle, WA as well as a Research and Development Center and a Commodity Analytical Laboratory. Other locations include Warren Analytical in Greeley, CO; JL Analytical in Modesto, CA; and Environmental Engineering Laboratories in San Diego, CA. Environmental Engineering Laboratory, Inc. is a commercial laboratory established in 1962 to provide laboratory services for the sanitary engineering community in Southern California. It is the oldest state certified water and wastewater laboratory in San Diego.

IEH-EEL ORGANIZATION CHART



JOB DESCRIPTION OF THE IEH-EEL PERSONNEL

Job Description of Managing Director

- The Managing director is responsible for both the day-to-day running of the laboratory and developing business plans for the long term future of the organization.
- Will Carry out responsibilities assigned by the IEH Principal, VP's and Directors in their area.
- Will convey information to scientists, microbiologists and other employees in their area, and from their area to the IEH Principal or VP's and Directors in their area.
- Responsible for the overall technical quality of the work performed in the Laboratory and for assuring the use of standard methods.
- Supervises all personnel employed by the Laboratory. Assures that the Laboratory has sufficient personnel having the necessary education, training and technical knowledge and experience for their assigned duties.
- Assures that the Laboratory has appropriate equipment and supplies.
- Assures that the Laboratory has the capacity, facility and resources to perform new work.
- Responsible for ensuring that Laboratory employees are compliant with ELAP, ISO 17025, and other standards.
- Oversees the Scheduling of projects and the completion of tasks within the required time schedule and sample hold times.
- Monitors progress of projects and communicates with Laboratory staff and users as required.
- May execute any of the responsibilities of microbiologists, scientists and other employees.
- Responsible for issuing "stop work orders" in analytical areas when significant quality requirements are not met.
- Authorized to issue report.

Job Description of Deputy Managing Director

- In the absence of the managing director, the deputy managing director is responsible for all the job description of managing director.

Job Description of Laboratory Director(s)

- The laboratory director(s) have over-all responsibility for laboratory technical management in their own field of work. The technical management includes supervision of sample collection, preservation, laboratory analysis, data reduction, computation of results and preparation of reports.
- Performs Laboratory Supervisor duties in the absence of managing director.
- It is laboratory director(s) responsibility to train all other personnel in their field of work.
- Will write and / or oversee the writing of all SOPs as well as ensure that these SOPs are diligently executed.
- Provides technical assistance to laboratory staff regarding Quality Assurance (QA) problems and method and instrument selection.
- Acts as liaison between Laboratory and regulatory agencies and Laboratory users.
- Provides technical assistance to Laboratory users in regard to the selection of appropriate analytical and/or sampling methods.
- Reviews Laboratory standard operating procedures and may review QA project plans submitted to the Laboratory.
- Oversees the transformation of analytical data which may be necessary to meet program needs.
- Maintains sample custody and tracking system within the Laboratory and oversees the Chain of Custody (COC) sample transfer into the Laboratory and assures that data handling and COC records are organized and accessible.
- Evaluates periodic summaries of quality assurance data provided by the QA officer and determines when data quality is unacceptable.
- Assures that the quality of all data reported by the Laboratory is documented. May participate in internal bench audits initiated by the quality manager.
- Assures that results from different parameters of a sample correlate.
- Will ensure that :
    - all validation data are recorded and dated to ensure compliance.
    - all data and final reports are verified and validated.
    - all reports generated from this data are complete and not lacking pertinent information.
- Will confirm final report before submitting to IEH president and customers.

- Will follow through with customers regarding content of report and discuss any irregularities or concerns.
- will ensure that:
    - the laboratory is equipped with all necessary equipment and accompanying materials and consumables.
    - all the equipment, materials and consumables will meet the specifications of the analytical methods employed to conduct the SOPs.
    - all equipment and process validations are performed as per SOP.
    - all laboratory data is reported in a timely manner.
    - all data are dated to conform with sample accession number.
    - all data are accurate and validated consistent to assigned SOPs.
    - all analyses performed by the staff and supervisor have been dated and tracked for authenticity.
    - all experimental details and resulting data entries in laboratory notebooks and/or forms are authentic and consistent with samples and SOPs.
    - all equipment is maintained and calibrations conducted on a set routine. basis to ensure that the SOPs can be performed to manufactures as well as validated specifications.
    - all materials and consumables necessary to conduct all procedures are in ready supply as well as have a routine re-supply system.
- Troubleshoot and report irregularities and/ or discrepancies in SOPs and data reported to the VP's/Directors.
- Will supervise equipment and process validations.
- Will provide support for all non-routine repair and necessary replacement part orders when needed.
- Will assign equipment monitors as needed to perform periodic equipment checks.
- Will check the routine maintenance and calibration records and address any variations in equipment performance.
- Will analyze all data.
- Will verify quality of all final data generated and reported.
- Will provide summary for report generation.
- Will review Data including QC documentation to ensure that:

- all analytical data and information provided to them by the scientist/microbiologist and other employees is complete and accurate.
- the data gathering is completed in a timely manner consistent with the demands of the customers.
- the data is procured and recorded in a manner that is consistent with all recording procedures that are established and documented, including data log books and electronic files.
- all sample classification codes are correctly noted relative to final data files.
- all analytical findings are reviewed and the relevant conclusions drawn and recorded for final report generation.

- Reviews all data before it is reported as final.
- Responsible for ensuring that Laboratory employees are compliant with ELAP, ISO 17025, and other standards.
- Responsible for issuing "stop work orders" in analytical areas when significant quality requirements are not met.
- Authorized to issue report.

Job Description of Quality Manager

- Responsible for ensuring that Laboratory employees are compliant with ELAP, ISO 17025, and other standards.
- Promoting quality achievement and performance improvement throughout the organization.
- Setting QA compliance objectives and ensuring that targets are achieved.
- Maintaining awareness of the business context and company profitability, including budgetary control issues.
- Assessing suppliers' and own company's product specifications and customer requirements.
- Working with purchasing staff to establish quality requirements from external suppliers.
- Ensuring compliance with national and international standards and legislation.
- Considering the application of environmental and health/safety standards.
- Agreeing standards and establishing clearly defined quality methods for staff to apply.
- Defining quality procedures in conjunction with operating staff.
- Setting up and maintaining controls and documentation.

- Identifying relevant quality-related training needs.
- Collating and analyzing performance data and charts against defined parameters.
- Ensuring tests and procedures are properly understood, carried out and evaluated and that product modifications are investigated if necessary.
- Supervising technical staff in carrying out tests and checks.
- Writing technical and management system reports.
- Bringing together staff of different disciplines and driving the group to plan, formulate and agree comprehensive quality procedures.
- Persuading reluctant staff to change their way of working to incorporate quality methods.
- Liaising with customers' auditors and ensuring the execution of corrective action and compliance with customers' specifications.
- Establishing standards of service for customers or clients.
- Preparing clear explanatory documents such as customers' charters.
- Monitoring performance through gathering relevant data and producing statistical reports.
- Responsible for issuing “stop work orders” in analytical areas when significant quality requirements are not met.
- Evaluates periodic summaries of quality assurance data provided by the QA Officer and determines when data quality is unacceptable.
- Maintains quality assurance documentation on procedures, equipment, reagents and standards.
- Assures that all SOPs are appropriately detailed for personnel performing a method or step of a method and SOP protocol is followed.
- Authorized to issue report.

Job Description of QA/QC Officer

- Assisting quality manager to oversees quality assurance and quality control measures taken to assure the quality of the tests.
- Responsible for issuing “stop work orders” in analytical areas when significant quality requirements are not met.
- Recommends resolutions for ongoing or recurrent nonconformance within the laboratory.
- Assisting quality manager for ensuring and improving quality within facility operations.

- Assisting quality manager for ensuring that Laboratory employees are compliant with ELAP, ISO 17025, and other standards.
- Assists in maintaining regulatory analytical compliance.
- Assisting quality manager to generates and maintains current Standard Operating Procedures (SOPs) for Laboratory operation within his/her work area and assures that all referenced method requirements are part of the SOP.
- Assures that all SOPs are appropriately detailed for personnel performing a method or step of a method and SOP protocol is followed.
- Assisting quality manager to maintain quality assurance documentation on procedures, equipment, reagents and standards. Initiates corrective action when quality assurance data does not meet pre-established control and warning limits.
- Verifies laboratory results and signs laboratory reports prior to the final approval of Laboratory Director(s).

Job Description of Safety Officer

- Promote occupational health and safety within the organization and develop safer and healthier ways of working.
- Make sure that personal protective equipment, such as dust masks, safety glasses, footwear and safety helmets, is being used in workplaces according to regulations.
- Make sure that dangerous materials are correctly stored.
- Identify and test work areas for potential accident and health hazards, such as toxic fumes and explosive gas-air mixtures, and implement appropriate control measures.
- Conduct training sessions for management, supervisors and workers on health and safety practices and legislation.
- Maintains and implements a laboratory safety plan and material safety data sheets.
- Orients all new laboratory employees and users to laboratory safety plan.
- Monitors and maintains or oversees the maintenance of safety systems within the building.
- Responsible for the management of hazardous waste storage and disposal.
- Develop occupational health and safety systems, including policies, procedures and manuals.

Job Description of Supply Officer

- Responsible for ordering all consumables needed for methods performed and assuring they meet standards.
- Responsible for tracking outstanding orders.
- Maintains electronic standard/reagent electronic inventory program.
- Maintains the supply of sample bottles used for sample collection.
- Responsible for equipment maintenance and maintenance contract oversight and acts as a liaison with service engineers to troubleshoot equipment problems.

Job Description of Administrative Assistant

- Administrative assistant is responsible for ordering all consumables needed as office supplies and equipment.
- Responsible for maintaining chain-of-custody and other pertinent data tracking forms within the laboratory.
- Logs in and labels samples delivered to the Laboratory by couriers when technical positions are not available.
- Responsible for the accurate and timely entering of analytical results generated by field staff into the data management system and the generation and distribution of Laboratory final reports after the reports have been approved by Laboratory director(s).
- Responsible for the daily operation of the Laboratory data management system. Oversees the day to day operation of the LIMS and trouble shoots all aspects of the LIMS. Data processing responsibilities include maintaining/archiving all current and historical Laboratory Standard Operating Procedures and Laboratory Quality Assurance Plan.
- Assures that all computer files are backed-up on a routine basis and electronic back-ups are properly documented and stored.

Job Description of Analytical Chemist(s) and Analytical Microbiologist(s)

- Responsible for the quality of analytical results of analysis performed. Works under the supervision of a permanent staff scientist. They are responsible for sample analysis from glassware preparation (when special preparation is required) through computation of results and recording data.

Each technician specializes in a particular series of analytical procedures, but the responsibilities are all rotated so that all technicians are familiar with all the routine laboratory procedures.

- Pick up, Log in, and label samples.
- Follow SOPs and QA/QC requirements of methods and the Laboratory.
- Informs immediate supervisor when precision and accuracy values are beyond established warning and control limits. Maintains QA/QC records for tests performed.
- Assists in data review for his/her analytical area.
- Are responsible for providing clean glassware and sample containers according to specific instructions.
- Monitors the temperatures of refrigeration units, calibrates analytical balances and monitors indicator lights on the Laboratory water system on a daily basis.
- Prepares containers and other sampling items needed by samplers.

No other personnel are allowed in the laboratory for security and safety reasons except supervised equipment and maintenance personnel. All visitors must sign in and out, wear limited duration visitor badges and wear a laboratory coat when entering the laboratory.

PHYSICAL FACILITIES

The laboratory consists of a single level building with 4,500 sq. ft. located at 3538 Hancock Street, San Diego, CA 92110. The building is without ventilating windows but has three air conditioning systems, which maintains the laboratory at a constant temperature throughout the year. Makeup air is circulated through the air conditioners and discharged through six exhaust hoods. The laboratory consists of a reception area, Directors' and quality assurance officer office, canteen and conference room, chemistry lab, water and waste water bacteriological lab, food microbiology lab, Virology and Radiochemistry lab, PCR room, restrooms and storage room. There are 400 linear feet of bench space, including 56 linear feet of exhaust hood bench space. The bench tops are plywood coated with acid resistant paint, acid resistant Formica, epoxy resin or high density polyethylene. Lighting is provided by seventy-four six-foot cool-daylight fluorescent lights of 75 watts each. They are placed to eliminate most shadows while providing adequate light intensity. The building is serviced by a 400 amp, 220-volt power supply which is distributed to the laboratory equipment by forty six duplex 110 volt, 20 amp grounded outlets, nine duplex 220 volt 30 amp grounded outlets and special circuits to some specific equipment. This is more than an adequate amount of power. There are six sinks located throughout the laboratory that are supplied

with hot and cold tap water and deionized water. The basic building security rules are described in Standard Operating Procedure (SOP) number; General Quality Assurance (GQA 005).

STAFF TRAINING REQUIREMENTS

On-going training is essential for the laboratory to maintain the level of proficiency needed. Therefore, IEH-EEL has a program that specifies the appropriate training required for employees to perform their assigned tasks. IEH-EEL's Quality Policy, 2.2.1 states "Appropriately educated and trained analysts perform all testing. Analysts do not work on client samples until competence has been demonstrated and recorded." Definitions of, and procedures for, training are described in SOP number; GQA003 as follow: Training falls into several categories. The first is education and training achieved before employment at IEH-EEL Laboratory. This is documented by the use of diplomas, training certificates where available and appropriate, and resumes or curriculum vitae. The second is training received after employment at IEH-EEL Laboratory and is provided by outside contractors. Certificates from the courses document this type of training or other appropriate documents can be used. They are placed in the individual's folder in the Q. A. files. The third type of training is IEH-EEL SOP, Equipment Performance and Operating Instruction (EPOI), or Test Method (TM) specific. The director(s) or appropriate technical personnel appointed by director(s) will provide this training. The form used to record evidence of training is attached to SOP GQA003 as the Appendix. It is filled out for the specific SOP, EPOI, or TM as the training is provided, to approve the person as trained and competent for that SOP. Training is required when: (1) a new SOP (QA, Test Method or EPOI) is introduced; (2) a SOP is revised; (3) laboratory control sample performance indicates a failure or trend: (4) proficiency samples are failed. Competence is determined by: (1) interviewing the analyst to ascertain their understanding; (2) observing the analyst performing the method: (3) the successful analysis of appropriate Q. A. or proficiency samples; (4) trend analysis of Laboratory Control Sample (LCS) data. All records used to demonstrate successful completion of the training are kept in the Q. A. files. The department supervisors may authorize additional individuals as competent to provide the training on each method on a case-by-case basis. The Quality Manager (Q. M.) will provide training to the department supervisors and other key personnel as needed for the IEH-EEL Quality Manual SOP's. After they are trained the Q. M., may authorize the department supervisors to train their direct personnel. Records of training completion are kept in the corresponding department and in the electronic SOP database.

STANDARD OPERATING PROCEDURE

There are three forms of documentation that IEH-EEL uses in its good laboratory practice (GLP) efforts: Equipment Performance and Operating Instructions (EPOI's), Test Methods (TM's), and Standard Operating Procedures (SOP's). The TM is a document or set of documents that thoroughly describes the Method's Scope and Significance, Summary of Method, Definitions, Precision and Bias, Safety Precautions, Sources of Uncertainty, Apparatus, Reagents and Materials, Calibration and Standardization, Sample Preparation, the Procedure, Quality Assurance, Calculations, Report, Bibliography. IEH-EEL GLP requirements are that every type of analysis and/or every sample type require a TM. That is to say that every sample that comes to the laboratory should have an appropriate TM before the sample is analyzed. The TM's are to be used by both experienced and new personnel. All personnel learn from the detailed instructions and then perform the method uniformly in conformance with the TM as they get more experienced. As with the other two IEH-EEL GLP document type, the original will be filed in the QA/QC office. A working copy of the document will be in the laboratory area near the work area. These are the only two controlled copies of the TM. The TM shall be a typed and approved document. The format will follow that of the SOP format guidelines, number GQA006. The "Summary Info" in Microsoft Word must be filled out with meaningful keywords and a meaningful title. The DOS name for the document shall be its IEH-EEL SOP number. The body of the TM will contain the sections detailed below. In addition to the titles the minimum amount of information for each section is given. Each section must appear in the method with its correct section number and with at least its minimum statement.

SCOPE AND SIGNIFICANCE

This section describes the type of sample(s) (matrix and analyte), the general type of method used, and the working range of the method.

SUMMARY OF METHOD

This is a brief (normally less than 5 lines) technical description of how the method measures the analyte in the specified matrix (matrices).

DEFINITIONS

Any words or phrases that would not be easily recognizable by an experienced chemical/microbiological technician should be listed here. The minimum statement would be:

"The terms employed in this method are commonly used in normal laboratory practice and require no special comment."

PRECISION AND BIAS

Each method must have its precision and bias determined. For an estimate of precision (repeatability and reproducibility) a minimum number of determinations would be 4 full determinations on each of 5 days utilizing as many analysts as possible for the separate days (within a day determinations should be run by the same analyst). For bias the mean of the data would be compared to the known value of the starting material. For quantitative methods a detection limit and practical quantitation limit as defined by the EPA (CFR 40, Part 136, App. B) and the FDA should be given. In the rare instance that these criteria cannot be met, a statement of which items were not determined and why must be given. The statement should also include whenever possible the best guess when the situation will be corrected.

SAFETY PRECAUTIONS

The minimum Statement for this section is:

"Normal safety precautions and safe handling practices should be observed. Consult the appropriate Material Safety Data Sheets for full hazard details."

If there are any hazards beyond the normal of a chemical/microbiological lab they should be enumerated. Any personal protective equipment beyond safety glasses must be specifically listed.

SOURCES OF UNCERTAINTY

The most common sources of uncertainty for the method or type of method need to be listed. There is no minimal statement because **all** methods have sources or potential sources of uncertainty, which need to be listed.

APPARATUS

All instruments, equipment, glassware, etc. used in the procedure shall be listed. They will be listed in directory style, e.g.: Beaker, PYREX, 250 ml or Meter, pH, VWR, model 8015 or equivalent.

REAGENTS AND MATERIALS

All reagents, chemicals, standards, reference materials shall be listed. They will be listed in directory style, e.g.: Water, deionized, 16 MOHM or better or NaOH, 0.010 N ±0.003. Items that need to be made

up in the lab can have detailed instructions listed here, can have separate appendices with complete details, or if the same items are used in several different TM's in a separate document (TM) may be in order. Items that are purchased from outside vendors should list the strength, purity, name, vendor, and catalog number. The statement "or equivalent" should follow this.

CALIBRATION AND STANDARDIZATION

All items that need calibration or standardization should be listed along with instructions on how it is to be accomplished. The exception to this would be a piece of equipment with its own EPOI.

The appropriate EPOI would be cited here. However, any standardization that is done on a frequent or daily basis needs to be detailed in this section or an appendix. Examples of this would include specific standards used for checking or setting the "calibration curve" of a GC or AA on that day. Standards that need to be made up in the lab can have detailed instructions listed here, can have separate appendices with complete details, or if the same items are used in several different TM's in a separate document (TM) may be in order.

SAMPLE PREPARATION

All steps of sample preparation between entry into our lab area and the actual analysis will be given here. They will be numbered starting with 10.1. If sub steps are used, they will be separately numbered using a third set; e.g., 10.1.1. All steps will be given as simple imperative sentences. There will be one action for each statement. Important supplemental information can be listed on a separate line between numbered steps.

PROCEDURE

All steps of the actual analysis will be given here in complete detail. No steps will be skipped and no assumptions will be made. All apparatus and chemicals will be stated precisely and unequivocally. The steps will be numbered starting with 11.1. If sub-steps are used, they will be separately numbered using a third set; e.g., 11.1.1. All steps will be given as simple imperative sentences. There will be one action for each statement. Important supplemental information can be listed on a separate line between numbered steps.

QUALITY ASSURANCE

Controls will be specified for all methods. Typical minimums include duplicate full analysis of the sample, blanks on each lot of each material used, standards run as samples, and sample spikes.

The quality manager must approve any deviation from these minimums. All controls are to be charted and carefully watched. Problems should be reported to the area supervisor and QA officer.

CALCULATIONS

All calculations used must be given in full detail including units, factors, conversions, etc. A simplified version with these items condensed into a single number may follow if desired.

REPORT

Exact details about what is to be reported, how, and by who is to be listed in simple, numbered imperative sentences.

BIBLIOGRAPHY

All references used listed in order of relative importance. They will be sequentially numbered.

APPENDICES

Appendices are to be added as needed.

APPROVAL

The author's name, effective date and a "CONTROLLED COPY" watermark on each page indicate the approval of a TM. The most recent approved version is listed in the SOP database.

The TM's shall be reviewed and/or updated every three years or sooner if changes are made to the equipment and/or its intended use. Evidence of the review is recorded in the SOP database.

The reviewer will, at the time of review, decide if the TM can be used as written, needs revision, or is obsolete. If any changes are noted on the working copy, the TM must be rewritten.

Essential points to consider in the review include:

Does the procedure conform to the lines described in this document?

Does the procedure conform to the current methodology?

Does it accurately reflect what is being done in the laboratory?

Differences need to be reconciled.

QUALITY ASSURANCE PROCEDURES

Quality Control samples are normally analyzed with each batch of samples for each analysis or environmental samples. The Quality control samples include a method Blank (MB), Laboratory Control Sample (LCS) and a Matrix Spike and/or Matrix Spike Duplicate. These QC samples are included in each batch of twenty samples or less for each matrix (frequency equivalent to 5% of all samples analyzed). If spike analyses are not feasible, a duplicate sample analysis is generally performed (e.g. TDS, dissolved oxygen, turbidity). For more information please refer to GQA013. The method blank is used to assess the preparation batch for possible contamination during the preparation and processing steps. The method blank is processed along with and under the same conditions as the associated samples to include all steps of the analytical procedure. Procedures are included in the method to determine if a method blank is contaminated. Any affected samples associated with a contaminated method blank are reprocessed for analysis or the results reported with appropriate data qualifying codes. The LCS is used to evaluate the performance of the total analytical system, including all preparation and analysis steps. Results of the LCS are compared to established criteria and, if found to be outside of these criteria, indicate that the analytical system is "out of control". Any affected samples associated with an out of control LCS are reprocessed for re-analysis or the results reported with appropriate data qualifying codes. The LCS is run at the same frequency as QC samples for each type of matrix. The LCS is obtained when possible, from a source external to the laboratory. The LCS may be prepared by the laboratory using standards from a different source or a different lot number from the source used for calibration standards.

A matrix spike and/or matrix spike duplicate sample are normally analyzed with each batch of twenty samples or less. Matrix spikes are duplicate aliquots of a sample which are spiked with the analytes of interest and taken through the same analytical procedures. The recovery of the analyte concentration is calculated to indicate the accuracy of the analysis in the sample matrix. The relative percent difference between the matrix spike and/or matrix spike duplicate sample provides a measure of precision of the analyses in the sample matrix. Surrogate spike analyses are performed for all organic analyses when required by the method. Surrogates are used most often in organic chromatography test methods and are chosen to reflect the chemistries of the targeted components of the method. Added prior to sample preparation/extraction, they provide a measure of recovery for every sample matrix. The surrogate spike solution is added to all samples, standards and blanks. The results are compared to the acceptance criteria as published in the mandated test method or laboratory generated acceptance criteria. Results reported from analyses with surrogate recoveries outside the acceptance criteria should include appropriate data qualifiers. All other QC requirements (tuning, multiple points' calibration, daily calibration check, etc)

are performed as specified in the test method. All QC data are to be recorded on the appropriate forms and kept on file by each department. Accuracy and precision data may be used to generate control charts. Acceptance limits for QC samples are detailed in the SOP for each test method, and may be established by the original reference source or statistical analysis of the historical data for each type of QC sample, method and matrix using control charts. For more information please refer to GQA014. When QC acceptance criteria are exceeded, corrective actions are to be taken as specified in the test method or as instructed by the department director. Non-conformances such as QA limit failures which cannot be corrected by re-analyses, client requirements which cannot be met or standard method modifications are documented by initiating a feedback, complaint, departure, preventive & corrective action form (FCDPCA), as described in GQA018.

TRACEABILITY OF MEASUREMENTS

Traceability of measurements is achieved by using standards for calibration and calibration checks which are traceable to primary National Institute of Standards and Technology (NIST) standards. Certificates of Analysis or purity are kept on file for each standard purchased, showing the traceability of the standard to a primary NIST standard. All balances are calibrated and certified annually using NIST certified weights. Thermometers are also calibrated at least annually using a thermometer certified against an NIST temperature standard or will be send to the manufacturer for the calibration. All commercially prepared standards have a maximum expiration date of one year from the date of receipt or other expiration date as established and documented by the supplier (GQA030). Reagents are purchased from established commercial suppliers as specified by the laboratory standard methods (GQA035). Reagents are stored at the appropriate temperature (refrigeration, freezing, room temp) as specified by the supplier. Lot numbers of reagents are recorded on sample preparation log sheets or in analysis log books to enable traceability (GQA037).

METHOD DETECTION LIMITS

Method Detection Limits (MDL) are normally determined by taking 7 or more aliquots of a sample containing the compounds of interest at a concentration 1 to 5 times the estimated detection limit and processing each sample through the entire analytical method (GQA014). The MDL is calculated from the standard deviation of the replicate measurements (MDL = 3. 143 x Standard Deviation for seven replicate measurements). MDL studies for each method are normally performed at least annually or when a major modification is made to the method or instrumentation used for analysis. Reference: 40 CFR, Ch 1, Part

136, Appendix B (7-1-86 Ed). Method Detection Limits are updated in the laboratory information management system (LIMS) and tracked by the quality manager.

QUALITY CONTROL PROCEDURES FOR MICROBIOLOGY

A complete record of the preparation of each batch of media is maintained in the media preparation log binder. For each batch of media the following information is recorded: date; type of media; lot number and expiration date of dried media; preparer's name; weight of dry media; volume of solution; pH measurements and adjustments, and results of control tests. A sterilization record of each batch of media is maintained in the autoclave log binder. The date, media or item identification, time in/out, maximum temperature, total time, sterilization time, controls and maintenance is recorded. Sterilization indicator tape is applied to each batch of prepared media. The pH of each batch of media is checked either before or after autoclaving and recorded in the media preparation log binder. Each batch of media is checked using an incubated positive control, negative control and sterility check sample. If any QC check sample fails, the batch is not used for sample analyses. A duplicate analysis is run on 5 percent of the samples of each type, or at least one per test run.

Quality Control on multiple tube dilution tests:

For routine analyses, the "Completed Test" is run on 10% of positive samples. If there are no positive results from potable water samples, at least 1 positive source water is run by the completed test quarterly. For public water supply samples with a history of heavy growth without gas in Presumptive-Phase tubes, all tubes are submitted to the Confirmed Phase to check for Coliform bacteria.

CONTROL OF ANALYTICAL PERFORMANCE

Method blanks, calibration blanks, quality control standards, matrix spikes and sample duplicates are utilized to ensure quality control. For chemical analysis, laboratory pure water, calibration blanks, are analyzed with each sample run. A method blank will be analyzed with each batch of samples or every twenty samples whichever is least. A second standard from a source different than that of the calibration standard (quality control standard) is run with each sample run. The percent recovery of this standard must be ± 10% for all methods where the standard must be ± 5% of true value. Every twentieth sample will be replicated and where feasible, spiked. The deviations from the replicated and spiked samples will

be entered into a LAB 2000 LIMS program as part of each sample batch worksheet. The program adds the new deviations to all previous deviations and calculates a new average range with a new warning limit and a new control limit. The warning limit is ±2 standard deviations from the mean replication deviation/mean spike recovery. The QA/QC section of the LIM system displays the new control limits along with the sum of the deviations squared in a graphical format. The analyst is then able to see if his data in "IN" or "OUT" of control. When the data is "OUT" of control the process is stopped. None of the data is recorded. The laboratory director or the QA officer will determine what corrective action will be taken, which will include; first, recalculation; second, re-run with new reagents; third, re-run with new standards; fourth, re-run with a different technician. All samples in the analysis will be retested until the data is found to be "IN" control (GQA 014). Final data will be reported only to the significant figure as determined by all the digits of true value and one last digit in doubt.

Drinking water coliform test – MPN QA/QC procedure: Samples are collected either by the client or by IEH-EEL personnel using sterile containers provided by IEH-EEL. These containers have sodium thiosulfate as a dechlorinator. Sterile containers for drinking water are purchased from IDEXX. All other systems will use glass containers with 2 drops of 10% sodium thiosulfate solution added before autoclaving. The bottles will then be autoclaved for 30 minutes at 17 psi and 121° C. The time between sample collection and the placement of sample in the incubator must not exceed 30 hours. Each batch or lot of sterile sample containers must be checked for the sterility by inoculating containers with tryptic soy broth and incubating at 35 C for 48 hours and checking for turbidity. With each sample batch a positive control (*E. coli*.) and a negative control (*E. aerogenes*) must be run with the samples in the 44.5ºC water bath.

Wastewater Coliform test – MPN QA/QC procedure: Samples are collected either by the client or by IEH-EEL personnel using sterile containers provided by IEH-EEL. Samples have 6 hours holding time before delivery to lab. Once the sample arrives at the laboratory there is 2 hours holding time to inoculate it into the appropriate culture media.

Drinking water Presence/Absence – MGG QA/QC procedure: Each lot of colilert reagent packets is to be tested for performance by inoculation with three control bacteria: *Escherichia coli* (fecal coliform),

Klebsiella pneumonia (total coliform) and *Staphylococcus aureus* (non-coliform), and incubate at 35°C ± 0.5°C for 24 hours. Three sterile colilert sample bottles are labeled as follows: *E. coli* (EC), *K. pneumonia* (KP), and *S. aureus* (SA). A 100 ml portion of reagent water is added to each labeled container. The contents of one reagent packet aseptically added to each bottle, and mixed. A culture of one of the listed bacteria transferred to the appropriately labeled vessel. Vessels incubated for 24 hours at 35°C ± 0.5°C. Results should be as follows: *E. coli* - Yellow and Fluorescent, *K. pneumonia* - Yellow, no fluorescence, *S. aureus* - No color, no fluorescence. If the above results are not obtained, QC procedures repeated with fresh packets and bottles from the same lot. Failure a second time warrants product rejection to manufacture. Each lot of sample containers should be checked for volume capacity and sterility by adding tryptic soy broth to several of the containers and incubating them at 35°C for 24 hours. The containers are acceptable if no turbidity is observed.

INTERNAL QUALITY CONTROL MEASURES AND ASSESSMENT

Quality assurance is a system of operating principles that, if strictly followed, through all points of sample collection, storage and handling, data reduction and reporting will result in data of known quality and defensibility. Included in quality assurance is quality control and quality assessment. Quality control is an *internal measure.* Internal quality control is managed at IEH-EEL laboratory through the application of six elements. The first element of internal quality control is the *certification of analyst competence*. Before an analyst is permitted to do reportable work, single operator precision and bias must be determined. A minimum of four replicate analyses of a check standard, prepared and recorded by the laboratory director(s) only, between 5 and 50 times the method detection limit (MDL) must be run. Results must agree to within 10% of the true value as determined by the laboratory director(s). The relative standard deviation of the results should not exceed 10%. Then the analyst is considered competent to perform the analysis. The second element is *performance of duplicate analyses and recovery of known additions/spikes.* Performance of duplicates and spikes by analysts are a regular and ongoing part of the analytical protocol. Duplicates assess precision and spikes verify the absence of matrix effects and are employed routinely on representative samples in the laboratory. When a new matrix is encountered, it must be duplicated and spiked. In the event a duplicate is not available due to absence of the analyte of interest, duplicate spikes must be employed. At least 10 % of the samples must be duplicated and spiked preferably at the pace of every tenth sample (for chemistry). Make the spikes in the range of 5 to 50 times the MDL or 1 to 10 times the ambient level whichever is greater. Do not spike a sample above the demonstrated linear range of the method nor dilute the sample beyond a negligible

amount. Analyses of *externally supplied standards* is the third element. These should be conducted on each analytical run at 5 to 50 times the MDL. Whenever possible use certified reference materials (e.g. NIST certified) or if internal reference materials are used, they must be prepared from a stock standard of different manufacture or lot number from the standards used for calibration. Element number four is the analysis of reagent blanks. Reagent blanks must be prepared for each analytical run at the rate of 5% of the total analytical run. In machine analyses, run a reagent blank whenever there is concern of "carryover" from a sample of high analyte concentration.

The next element in the group of seven is the *employment of calibration standards or "calibrants".*

A minimum of three, but preferably, five or more dilutions of the stock standard on every analytical run. Correlation coefficients, r or r^2, must be 0.9950 and 0.9900, respectively, for the calibration curves. On some automated tests analysis of a single calibrant within the linear calibration range is sufficient.

Do not report results above the range of calibrants used unless a greater linear range has been demonstrated prior to the run, no instrument settings have been changed and the sample value is not greater than 50% of the highest calibrant. The lowest reportable value is the reporting limit (RL) provided the lowest calibrant is 10 times lower than the RL. The last element is the control chart. IEH-EEL laboratory employs as part of the Laboratory Information Management System (LIMS) statistical tracking of external control, control duplicate, duplicate, matrix spike, and/or matrix spike duplicate, and reagent blank results obtained in the course of analytical runs. The results are entered and the LIMS computes a cumulative mean (x) and standard deviation (s). From the standard deviation, upper and lower ***warning*** limits (+/- 2s) and upper and lower ***control*** limits (+/- 3s) are set. From these criteria, the following analytical corrective actions are taken: a.) if one measurement exceeds the control limit repeat the analysis immediately; if the repeat is within the control limit continue analyses; if it exceeds the control limit stop analyses and correct the problem. b.) if two out of three successive points exceed a warning limit analyze another sample; if the next point is within the warning limit continue analyses; if not discontinue the analyses and correct the problem. Trends of 5 or more successive measures upward or descending between the warning limits and control limits but within the control limits requires corrective action before analyses resume. All analytical runs by an analyst will be subject to check by an alternate analyst certified to perform the test who will repeat a sample analysis at random. Entry of data in the LIMS will be reviewed by another analyst and the QA/QC results reviewed by the laboratory director(s) before posting of findings without exception.

EXTERNAL PERFORMANCE ASSESSMENT

Quality assessment at IEH-EEL laboratory is a combination of the quality control measures employed in the laboratory daily, in addition to the use of regular internal and external performance assessments. These assessments include such items as the performance evaluation tests (PET) with inter-laboratory comparisons monthly unknowns prepared and recorded by the laboratory director(s) and internal audits as well as external audits conducted by the applicable regulatory agencies concerned with laboratory accreditations. Final review of the results will be conducted by the quality assurance officer as well as the laboratory director for internal consistency. All outgoing reports must be signed by the director(s) and countersigned by quality assurance officer. On an annual basis IEH-EEL laboratory participates PET provided commercially for all fields of testing (FOT) for which it is certified. These PETs are inter-laboratory comparison programs as well as independent checks on laboratory performance. Any test results deemed unacceptable by the commercial provider who is solely responsible for the result evaluations must be repeated within the next available scheduled study. Consultation with the commercial supplier's technical support can be helpful with any difficulties. All analytical study results will be made available by the commercial provider to the environmental laboratory accreditation program (ELAP) as well as a copy to IEH-EEL laboratory.

To compare its bias, accuracy, precision to other laboratories and, sometimes, to known or expected values, in order to demonstrate the ability of IEH-EEL to successfully perform specific analyses, IEH-EEL subscribes to proficiency test sample providers to participate in any programs that are available and applicable to IEH-EEL's scope of testing. If available, the providers used will be accredited to ISO Guide 43-1 or be using a scheme that follows those requirements. The quality assurance officer or the laboratory director(s) will maintain an electronic spreadsheet of proficiency programs in which the laboratory participates. The spreadsheet must contain, at least, the following information about the proficiency sample program: the name of the organization conducting the program, the laboratory's identification code, the tests being compared, and matrix or matrix type involved. At least two sets of proficiency samples are analyzed per year for each analysis in IEH-EEL's ISO17025 scope of accreditation (SOP number GQA010). All proficiency samples are tested in-house by the analysts that normally perform the respective analyses. Upon receipt, all reports are reviewed with the analysts. If unsatisfactory results are obtained, an investigation of the root cause of the error is immediately started and, upon completion, corrective actions are identified and implemented. Finally, IEH-EEL will analyze the next available set of samples from the same provider. IEH-EEL is participating in FDA Shellfish Laboratory Evaluation program annually.

QUALITY ASSURANCE PLAN FOR THE SHELLFISH TESTING

Quality assurance procedures suggested by the National Shellfish Sanitation Program (NSSP), and the California ELAP are followed and documented in all areas involved in microbiological testing, especially for the shellfish. The following paragraphs cover some of the more noteworthy quality assurance efforts in the laboratory in regard to shellfish sanitation program.

Sample collection, preservation, and holding time

A representative sample of shellstock is collected, (10-12 animals or more, sufficient to yield 200-250 g). Shellstock is collected in clean, waterproof, puncture resistant containers. Shellstock are labeled with the collector's name, type of shellstock, the source, the harvest area, time, date and place (if market sample) of collection. Shellstock samples are maintained in dry storage between 0 °C and 10°C until they can be examined. Examination of the sample is initiated as soon as possible after collection. Shells that are broken or those that are dead as shown by permanently gaping shells will be discarded. However, shellfish samples are not examined if the time interval between collection and examination exceeds 24 hours.

Media preparation

The distilled water produced in house is used for the preparation of reagents and media. This distilled water is analyzed monthly for conductivity, pH, and total chlorine residual, total dissolved solids (TDS), and heterotrophic plate count (HPC) in our laboratory network and we receive a monthly suitability report. The water is analyzed for total heavy metal content annually, and the report is reviewed to ensure water suitability standards are met. Each bottle of dry media is dated on the label when it is received and when it is first opened. These two dates, the date the bottle is discarded, lot number, expiration date, and accession number are recorded in a log which is kept in the media preparation area. Freshly prepared media are stored in a cool, dry, dark area. Prepared media are labeled with identification and preparation or expiration date. Each batch of prepared media is tested for pH before or after autoclaving. Each new batch of laboratory prepared media is checked for sterility, and with positive and negative controls. The results are recorded. Medium is discarded if evaporation exceeds 10% of original volume. Fermentation media, if refrigerated, is incubated at room temperature overnight before use. Media with growth or gas bubbles are discarded. Broth media in loose-cap tubes are stored in the dark at <30°C and held no longer than 1 week. Broth media in screw-cap tubes stored in the dark at <30°C and held no longer than 3

months. Prepared plated media (agar) is stored in plastic bags or tightly sealed containers in the dark, refrigerated, and held no longer than 2 weeks.

Phosphate dilution buffer sterility check

Each batch of dilution water is checked for sterility by adding 100 ml of a double strength non-selective broth (e.g., tryptic soy, trypticase soy, or tryptose broth). It is incubated at 35°±0.5°C, and checked for growth after 48 hours. Results are recorded. If growth is detected, the batch will be discarded.

Environmental monitoring

Microbiology work surfaces, appliance handles, and water-bath water are monitored weekly for bacterial contaminants by aerobic plate counts and presence/absence methods, respectively.

Airborne bacterial contamination

Monitoring for airborne bacterial contaminants is performed whenever standard plate counts are conducted.

Stock cultures

Stock cultures are ordered from Microbiologics and /or ATCC. The working cultures are inoculated every week.

Calibration and maintenance of laboratory equipment

Records are kept of the operation, calibration, and maintenance of all laboratory equipment. Maintenance contracts provide regular service for the microscope, autoclaves, and balances. Balances are checked prior to each day's media preparation against certified weight standards. Autoclave performance is verified with spore testing once per week. Autoclaved loads are identified with temperature sensitive tape on the items in the batch. Autoclave run logs are kept showing the type of material run, length of sterilization run, 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.

Labware

Disposable petri dishes, pipettes, and culture tubes are used whenever possible to reduce the possibility of contamination from unclean glassware. Glassware used in media preparation and sample collection is tested for soap residue by using 0.04% bromthymol blue pH indicator solution and the results are entered into the appropriate log. The sterility of sample containers is verified by adding tryptic soy broth (approximately 50:1) to a portion of the containers which are then incubated at the appropriate temperature and humidity for 48 hours. After the incubation period, they are checked for bacterial growth and the results are entered into the appropriate log. If growth occurs, the entire batch is re-autoclaved.

Inhibitory residue test

A "Test for Inhibitory Residues" is run annually to determine detergent and cleansing efficacy. Standard methods 20th edition.

Multiple tube fermentation

With each sample batch a positive control (*E. aerogenes*) and a negative control (*Staphylococcus aureus*) are run with the samples in the 35±0.5 ^{o}C incubator. A positive control (*E. coli*) and a negative control (*E. aerogenes*) are run with the samples in the 44.5± 0.2 ^{o}C water bath. One media tube is run as a blank for each temperature.

Standard plate count

All bacterial plates are examined and recounted by a second analyst to assure the accuracy of the recorded information. Discrepancies in original versus recount numbers of greater than 10% are discussed and resolved with the microbiology supervisor. Calculations of bacterial density are checked and initialed by a member of the microbiology lab who was not involved with the original counts or calculations. Control plates are used to check the sterility of the air, agar and the diluent.

CORRECTIVE ACTION REPORTS AND DEPARTURES FROM DOCUMENTED POLICIES

A feedback, complaint, departure, preventive & corrective action form (FCDPCA) may be required when certain quality control criteria are exceeded in a sample analysis batch. The FCDPCA form is initiated by the analyst in the event of a sample exceeding holding time, quality control sample results outside control limits or other known non-conformance to the analytical method or client requirements (SOP number

GQA018). The FCDPCA form may also be initiated by the supervisor or director in the event client requirements are not met or other analytical problems are discovered. After the FCDPCA Form is initiated, the corrective action, if any, must be agreed upon by the supervisor or director and the quality assurance officer. This is documented and signed by the supervisor in the second part of the FCDPCA Form. The form is then forwarded to the QA officer. The QA officer then completes and signs the final part of the form. If necessary, verification of the corrective action is documented in this section. The original is filed in the Corrective Actions File which is maintained by the QA officer. When there are deviations from the requirements by the specific method, such as insufficient sample volume, improper preservation, the client should be notified as soon as possible. If the client agrees to the deviation, then an explanation of the deviation or non-compliance is required to be attached to the data package and final report.

REVIEW OF NEW PROJECTS

New projects and contracts are reviewed by laboratory management to ensure that the laboratory has the technical capability and resources to meet the requirements. Any potential conflict of interest or other problem noted in the review is discussed with the client prior to acceptance of the contract or samples (SOP numbers GQA022 and GQA024).

PROTECTION OF CLIENT CONFIDENTIALITY

IEH-EEL Laboratories recognizes the importance of client confidentiality (SOP number GQA020). Each Lab report contains the following statement: “The results in this report relate only to the portion of the samples tested. This report shall not be reproduced, except in full, without written approval of the laboratory. Trade secret-contains confidential commercial information".

LABORATORY LIMS SYSTEM

The laboratory information management system (LIMS) is a client-server network of computers used to login samples, track samples during and after analysis, and report the final results to the client. In addition the LIMS software which is database driven is able to generate historical reports and trends and generate other types of reports such as electronic deliverables which are increasingly used by clients to transfer data into their own computer systems without having to do manual data entry. The LIMS system is also

used to track laboratory data such as detection limits (MDL) and reporting limits for analytes. Security consists of a password login system. All reports are reviewed and signed by quality assurance officer and director(s) before release to the client. Tracking reports are generated daily from the LIMS system to insure timely analysis and reporting of all client samples. Electronic Delivery Capabilities - laboratory data can be delivered to the client in electronic data deliverable (EDD) formats such as: spreadsheet (Lotus, Excel); standard database file formats (dB, Paradox, etc); delimited or fixed field formatted ASCII; or word processing formatted. The data files can be transmitted to the client either by diskette or directly using e-mail (PDF file) or FTP protocols.

DOCUMENT CONTROL AND RECORD KEEPING

All documents relating to laboratory analyses and reporting are kept for 26 months (SOP number GQA016). After that time the records will be destroyed by shredding, unless special arrangements are made with the headquarters. The laboratory maintains a tracking system for SOP, MDL determinations, training documentation and corrective actions (SOP number GQA002). A Lab request is created by the laboratory LIMS system for each group of samples received from a client to enable organization and tracking of the analyses and final reporting. All analytical results are reported in the LIMS database system, including date of analysis and analyst initials. All documentation other than bound laboratory notebooks relating to the analyses of a client's samples including a copy of the final report, chain of custody, all sample preparation worksheets and analytical raw data will be kept in the laboratory. All acceptable data is approved by the authorized personnel only and the administrative assistant then prints all reports and invoices using the LIMs system. Copy of all signed reports will be kept for 26 months (SOP number GQA016). Other relevant analysis data may be written in bound laboratory notebooks which are maintained in each laboratory department. All calibration data and other relevant data such as calibration checks, which may apply to multiple Lab Requests are filed and retained in the individual departments.

Corrections

All generated data is recorded in permanent ink. Entries in records shall not be obliterated by methods such as erasures, overwritten files or markings. All corrections to record-keeping errors shall be made by one line marked through the error, followed by recording of the corrected data. The individual making the correction shall sign (or initial) and date the correction.

SAMPLE ACCEPTANCE POLICY

Sample acceptance policy determines if the sample is identified correctly, with proper documentation, packaging, adequate volume for the analyses requested, and correct preservatives (SOP number GQA032). For accurate analysis, the sample and sample source must be identified correctly. If there is an obvious discrepancy between the sample and documentation, this is normally investigated first by the sample receiving personnel. If the problem cannot be resolved, then the appropriate lab supervisor is notified. Sufficient documentation should be supplied with the sample to fill in the chain of custody completely. If there are any doubts as to the sample identification or analyses requested, the client should be called immediately. All communications and decisions regarding the client samples should be documented and signed in writing and attached to the original lab sheet (and all copies if necessary). The employee receiving the sample must note on the chain-of-custody form or an attached sample receiving form the following information for each sample and fraction:

1- Sample type.
2- Provider sample ID(s).
3- Number of sample(s)
4- Sample condition (Temperature, labeling, packaging).

Perishable samples for microbiological testing should be below 10°C (50°F). Environmental samples should be between 0°C and 7.2°C (32°F and 45°F). Samples coming directly from a warm environment may exceed the 50°F maximum. These samples should be processed within 15 minutes of receipt or refrigerated. If the samples are outside the proper temperature range record the temperature on the request form and contact the client before proceeding with any testing. Preservatives added must be listed on the sample container and/or the chain of custody form. The sample must be within the specified holding times for the analyses requested. Any irregularities noted in the samples (leaking, air bubble in volatile organics analysis vial, improper packaging, etc) must be brought to the attention of supervisory personnel. Generally all information and decisions involving sample integrity must be documented in writing with a date and signature.

SAMPLE RECEIVING AND CUSTODY

The receipt of a sample is laboratory's first chance to assure that the quality of our data is the highest it can be. At IEH-EEL the act of receiving a sample has several steps and is crucial to starting the analysis cycle correctly. The general steps to sample receipt are separating and opening the shipments, determining the general condition of the samples, noting the presence or absence of any specific

preservation conditions, logging the samples into a laboratory information management system (LIMS), sample preparation, and delivery to the laboratory area for analysis. Samples for IEH-EEL can arrive by many different means, e.g.: delivery services such as; FedEx, UPS, USPS, private courier, delivery by the customer or pickup by laboratory personnel. If some or all analyses are to be subcontracted, notify the functional area supervisor or lab director, to prepare sub-samples and provide a second-level review of shipping method, timing (especially for weekend shipments) and destination. A record of this review, in the form of initials or signature on the analysis request form and external log book, shall be created.

All samples are assigned a laboratory identification number during the login process. This number is a unique identifier assigned by the laboratory LIMS system. All samples received from a client on the same day on the same chain of custody are normally grouped together in a unique laboratory request number. The laboratory request number is also assigned by the laboratory LIMS system. A laboratory request summary is prepared which includes: date, client name, client sample ID, corresponding laboratory sample number, all analyses to be performed, laboratory area designations and other special instructions. The purpose of the chain of custody form is to legally document the transfer of the sample(s) from the customer to the laboratory. Since any sample may potentially be used as evidence in legal proceedings, it is important that the chain of custody form be filled in completely and accurately.

The chain of custody form should furnish an accurate record of the samples received, analyses requested, and any important information from the client regarding the samples. The information entered on the form should be as complete as possible. All samples, immediately upon arrival in the laboratory, are logged into LIMS. It is assigned a unique log number based upon the year and computer-generated sequential five digit work-order number and count in the work-order series. For example, 0734692-001 for the first sample of the 34,692th work 'order' of 2007. The date and time sampled and the date and time received are recorded in the LIMS system as well as a backup written in a logbook with the client's name, sample location or identification and the analyses required as per the request in the chain of custody form completed by the client. In the case of bacteria samples the time of analysis is recorded along with the technician's initials. When an idiosyncratic chain of custody documentation is required by the client, they normally provide the forms and their instructions are followed. A copy of the completed form is provided to the client. The chain of custody forms are retained in a file. When the client does not have a form, we provide them with one. No sample is analyzed without being properly logged into the laboratory data system, even if the sample is not to be billed.

Sample storage during login process

Possible samples are written up as soon as received. A designated sample storage refrigerator is used for storage of samples which need to be refrigerated during the login process (samples for volatile organics analysis are stored in a separate refrigerator). Most samples are stored in refrigerators until analyses are completed (SOP number GQA033).

Handling of Samples Received by Client Delivery:

When a client delivers a sample for analysis, it is important that information about the sample be as complete as possible. This is best done with a properly completed and signed chain of custody form, following information must be obtained before the sample can be accepted:

- Client's name and address
- Person to contact regarding the sample(s) and phone and/or Fax number
- Method of payment
- Both the client and lab employee receiving the sample must both sign the completed chain of custody form. The chain of custody will normally contain detailed information on the samples.
- The client receives a copy of the chain of custody upon request.

Sample pick up by our personnel:

All samples received from our drivers should be accompanied by a completed chain-of-custody form, signed by the client and by the driver. All coolers received must have a temperature reading immediately upon opening. This reading will be taken by placing the metal probe of the thermometer either into a temperature blank (if provided) or between the respective samples and the cooling media (ice, dry ice, or blue ice). The thermometer should remain in place for 60 seconds to ensure a proper reading. The exact temperature will then be read from the thermometer. The temperature should be in the range of 2 - 6 °C. The temperature will be noted on the sample receipt form. The chain of custody and samples must be checked to make sure that all information is in agreement. The pH of all chemically preserved aqueous samples, except volatile samples, must be checked and documented upon receipt at the laboratory. If discrepancies are noted, the laboratory must contact the client immediately. Any problems with improper preservation, sample container type, volumes, etc are to be noted on the sample receipt form. This is to document problems which may interfere with a proper analysis of the sample. The director(s) should be notified so that the client can be contacted as soon as possible.

Samples received by mail, UPS, Federal Express, etc.

Samples received by mail, UPS and Federal Express are handled in the same manner as samples received from our drivers with the exception that samples are not relinquished by the client. All coolers received must have a temperature reading and all samples must be verified against the chain of custody or paperwork as noted above.

Special Handling of Samples for Microbiological Testing

Due to the short holding times for microbiological samples, these must be handled on a first- priority basis. Drinking water samples (potable water) should be analyzed as soon as possible after sampling (30 hours maximum time from sampling to analysis). Samples must be maintained at 4 -10 °C during transport and storage. Potable water samples cannot be analyzed after 30 hours, these samples should be refused. Waste water and surface water samples must be analyzed within 6 hours after collection (6 hours maximum holding time). Samples must be maintained at 4 - 10 °C during transport and storage. Water/waste water samples older than six hours should be refused. For heterotrophic plate count the holding time for any water sample is 8 hours. Upon receipt in sample receiving area, samples need to be checked immediately for proper temperature and holding time. Samples should be transported in a cooler with blue ice or regular ice. Check chain-of custody form to be sure samples are within holding times. If samples are outside holding time or not held at proper temperature, notify the microbiology department supervisor immediately. The chain-of-custody shall also state the conditions of the samples as received (cooled, frozen, room temp, etc). Check condition of samples received for microbiological testing for potential contamination of samples. Containers must be sealed with no evidence of leakage. Containers must be protected from melted ice or other potential contamination. Notify the microbiology supervisor if problems are noted. If there is evidence of contamination the client should be notified that the samples are potentially contaminated. Samples should be refrigerated or placed in a cooler with blue ice upon receipt and logged in immediately.

Safety Precautions

The lab does accept radioactive materials for analysis. A radiation monitor device is available in the Sample Receiving Department for screening samples if radiation is suspected in any sample. If any sample tests higher than background 25 cpm level radiation, the safety officer must be notified immediately. All sample shipments received from hazardous waste sites or labeled as highly toxic must be initially opened in a fume hood or in a well-ventilated area.

Gloves are available in the sample receiving area for handling potentially hazardous samples or samples which are leaking. When in doubt about the safe handling of any sample, the Lab safety officer or appropriate Lab supervisor must be consulted before the sample is logged in.

HANDLING OF COMPROMISED SAMPLES

If, during receiving and login, or sample preparation and analysis any conditions are noted that would compromise sample integrity the laboratory director or their designee(s) will be informed immediately. The laboratory director or their designee(s) will contact the client by telephone (and fax or email, if applicable) to notify them of the problem with the sample(s) and determine the appropriate course of action. . If the client agrees that the sample should not be tested, a sample rejection form is initiated (SOP number GQA034).The nature of the problem will be recorded, signed and dated on the sample rejection form. No sample is to be discarded until the client has agreed that it is acceptable to do so. If contact cannot be made during normal business hours on the sample receipt date, then the sample will be analyzed according to the client's original instructions. All conversations with the client concerning the compromised sample(s) must be recorded and initialed on the sample rejection form. Include the name of the contact person, the date and time of contact and the disposition of the sample.

HANDLING OF SAMPLES TO BE SENT OUT TO OTHER LABS

Arrangements to send samples out for analysis are handled by the director(s) and must have the Client's consent. Samples to be transferred to another lab are logged into the LIMS for "Send Out" and the information is posted on the "Out Board" similar to posting to an in-house department. Samples to be sent out are sub-sampled and shipped by the Sample Custodian. A portion of each sample to be sent out is retained in the original container.

ANALYTICAL RESULTS REPORTING

Final Reports issued to clients contain at a minimum the following information:

Title, client name, address, client ID number and contact or project manager, dates of sample receipt at the laboratory, date of sampling and date of analysis, cross reference of lab identification and client sample identification, results of analysis for each sample, including the test method, analyte, result, detection limit, date and time analyzed and analyst initials, signature of quality assurance officer and laboratory director(s). A statement is included in the final report: “The results in this report relate only to

the portion of the samples tested. This report shall not be reproduced except in full, without written approval of the laboratory. Trade secret contains confidential commercial information".

PROCEDURE FOR HANDLING CUSTOMER'S COMPLAINTS

IEH-EEL Laboratories encourages feedback from customers. Complaints such as improper billing or incorrect sample identifications are normally handled by quality assurance officer, who make every effort to resolve the problem as quickly as possible. Where the complaint involves problems which cannot be readily corrected, then the customer's complaints are recorded on a Customer Complaint Form (FCDPCA) which contains the following information:

Date of complaint

Name of company

Name of person submitting the complaint

How the complaint was submitted

Name of person receiving complaint by phone

Nature of complaint

Department(s) involved

The customer's complaint form is submitted to the department(s) involved for investigation and resolution of the complaint. The results of the investigation and resolution of the complaint are recorded on the complaint form, signed and dated by the individual handling the complaint and submitted to the Lab director to be reviewed and approved. The customer is notified of the results of the investigation and resolution of the complaint by the Lab director or by a person authorized by the Lab director, either verbally, by phone, or in the form of a letter. The Complaint Form and all other documents pertinent to the complaint are filed in the Complaint File maintained by the quality assurance officer (SOP number GQA019).

LABORATORY SAFETY

LABORTATORY SAFETY EQUIIPMENT

All laboratory personnel are shown the location and use of the following safety equipment.

Fire extinguisher: five located at different locations through out the laboratory

Fire Blankets: one portable, one permanently affixed by safety shower

Safety shower

Eyewash

Fume hoods, Glass container, and Sharp container

Chemical storage area: located in the storage room

Material safety data sheets

Spill Kits: Acids, Bases, and Organic solvents

PERSONAL PROTECTIVE EQUIPMENT

Lab coats

Gloves

Safety glasses

Acid/organic respirators

Dust masks or particle masks

First-aid kit

Blood borne Pathogen Control Kit

LABORATORY HANDLING OF REAGENTS AND SOLUTIONS

Acids and Bases should be handled in the fume hood using protective clothing, gloves, and acid respirator as needed. Any handling of more than 500 ml of acid requires the use of SAFETY GLASSES. Acids are stored in labeled plastic bins to keep them separated. Bases are stored in the chemical storage room. Organic reagents (solvents such as Chloroform, Methylene Chloride, etc.) must be handled in the fume hood using protective clothing, gloves, safety glasses and an organic respirator. An organic respirator must be worn at all times when reading organic extracts in the spectrometer area. Biological reagents: Particle masks should be worn when preparing culture media. All used microbiological pipettes must be placed in a disinfectant solution for decontamination before washing. All work areas must be decontaminated using Amphyll 2% solution. Gloves should be worn when handling used media before and after autoclaving. Sewage samples or samples suspected of containing pathogenic microorganisms should be handled with gloves. Good personal hygiene practices are important to control contact exposures. All laboratory injuries and accidents must be reported to the laboratory director(s). All prepared solutions should be labeled by the technicians with the solution name, concentration, date, and preparer's initials. MSDS are located in the safety cabinet file. General Employee Health and safety information is posted in the lunch room. If an accident occurs in the lab the employee should report it immediately to the safety officer.

FUME HOODS:

Fume hoods: The fume hoods shall be turned on when deemed appropriate until the lab is closed. When working at a fume hood station, all surrounding hood windows shall be closed to maintain proper airflow around work area.

LABORATORY INSTRUMENTATION AND EQUIPMENT

Analytical Balance and top loader or pan Balance

The Scientific Products Model 180 readable to 0.05 mg and precise to ± 0.03 mg is mounted on a seismic mass of approximately 2000 pounds of concrete and reinforcing steel. The balance will be recalibrated and serviced each year by a commercial service technician (EPOI number MB815). In addition, each day it will be checked for accuracy against a standard weight set by the laboratory technician(s). In addition, the balance will be checked annually against a second weight set reserved for this purpose. The weight sets will be calibrated every five years by a commercial facility to an ISO 17025 standard. The balances will be maintained in a level attitude, kept clean and free from objects, covered and arrested when not in use. The laboratory also has two electronic top loader balances readable to 0.01 gms and precise to 0.10 gms (EPOI number MB807 & MB844. These balances will also be checked for accuracy against two separate standard weights, kept clean, covered and arrested when not in use.

Balances provide a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less. Balances calibrated monthly using ASTM Class 1, 2, or 3 weights (minimum of three traceable weights, with a readability of 0.1 g.) (ASTM, 1916 Race St., Philadelphia, PA 19103). Non-reference weights calibrated every six months with reference weights. Calibrations recorded in a logbook with the initials of the individual performing the calibration. Correction values should be on file and used. A reference weight will be re-certified every five years. Damaged or corroded weights will be replaced. Service contracts or internal maintenance protocols and maintenance records should be available. Maintenance, calibration, and cleaning conducted at least annually by a qualified independent technician.

Autoclaves

The two autoclaves are Market Forge Sterilmatics with automatic temperature regulation by means of a pressure-activated switch (EPOI number MB811). Time is set manually. Pressure and temperature are shown on two separate gauges. Temperature during the sterilization run is checked with a calibrated ‘max’ thermometer. Heating and cooling rates are sufficiently rapid so a complete fifteen-minute

sterilization cycle requires less than forty-five minutes. The autoclave has an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve. The autoclave maintain a sterilization temperature during the sterilizing cycle and complete an entire cycle (i.e., time between starting autoclave and removing items from autoclave) within 45 minutes when a 12-15 minute sterilization period is used. The autoclave depressurizes slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes. The date, contents, sterilization time and temperature, total time in autoclave, and analyst's initials recorded each time the autoclave is used. Copies of the service contract or internal maintenance protocol and maintenance records are kept. Maintenance conducted at least annually. A record of the most recent service performed is on file, available for inspection. Overcrowding is avoided. Spore ampoules are used weekly as bioindicators to confirm sterilization. Automatic timing mechanisms checked quarterly with a stopwatch or other accurate timepiece or time signal, and the results recorded and initialed. Autoclave door seals are clean and free of caramelized media. Also, autoclave drain screens are cleaned frequently and debris removed.

Blender

The laboratory uses a blender Osterizer deluxe model 403 with two speeds. There are eight glass jars for this blender (EPOI numbers MB825, and MB826).

Centrifuges

The laboratory uses a Clay Adams-Dynac bench top centrifuge with speed to 3,300 rpm, 4 x 50 ml and has a timer for 30 minutes. There are two mini centrifuges in the lab. One is manufactured by Fisher Scientific, model number 05-090-100. This centrifuge has a maximum speed of 6,600 rpm (2,000 x g) for 6 x 1.5/2.0 ml with an angle rotor or 6,300 rpm (2,200 x g) with a rotor for 8-strip PCR tubes (EPOI number MB833). The other mini centrifuge is manufactured by ISC Bio Express; model C1301-ISC-P (EPOI number MB834). The maximum speed for this centrifuge is 6,000 rpm (2,000 x g). Additionally the laboratory uses a Beckman high speed centrifuge, model J2-21 with a JA20 rotor (8 x 50 ml) (EPOI number MB831). The maximum speed with this rotor is 20,000 rpm (48,400 x g).

Chlorine Test Kits

Two Hach Pocket Colorimeter Chlorine Test Kits (both of lot number L5196) employing the DPD method are utilized for laboratory and field testing for total and free chlorine. Both instruments are tested against secondary gel standards available from Hach prior to use.

Chlorine Dioxide Test Kit

The Hach Chlorine Dioxide Test Kit is used laboratory analysis in drinking water by modified DPD method. The Pocket Colorimeter lot number is L1235.

Colony Counters

Bacterial plates are counted on two Quebec Darkfield Colony Counters with 1.5X magnification by American Optical (EPOI number MB827) and Reichart (EPOI number MB828).

Computers

All laboratory and accounting data is maintained on a central server workstation with Microsoft Small Business Server software. All computer workstations are password protected have MS Windows professional as the operating system. Laboratory information and QA/QC data is entered in the LIMs system by Genesis Microsystems LAB 2000 LIMS. All major equipment is operated by computer based programs and is password protected.

Conductivity Meter

Conductivity measurements in the laboratory will be made by YSI Scientific Corp. Conductivity Meter 35 (EPOI number MB805). A temperature measurement will be made on each sample and conductivity readings will be mathematically corrected to 25° C. The cell constant will be checked each day of analysis. The cell will be rinsed several times with deionized water prior to each measurement and kept moist with deionized water when not in use. Calibrate the meter for each day's use, following the manufacturer's recommendations and using an appropriate certified and traceable low-level standard. If the meter cannot be calibrated with a commercial standard, the cell constant should be determined for each day's use, using a method in Section 2510, "Conductivity," in *Standard Methods*.
If an in-line unit cannot be calibrated, it will not be used to check reagent-grade water.

Furnace

A Thermolyne Type 1300 resistively heated muffle furnace is used in volatile residue analyses. Temperatures are monitored only during analysis.

Gross Alpha/ Beta analyses

Gross Alpha/ Beta analyses will be performed on a Gamma Products GH542M-Quad Alpha/Beta Counting System. The measurement section of the instrument is a massively lead shielded 'cave' housing four flow proportion counters. All measurements will be performed with materials licensed for IEH-EEL use by the California Department of Health Services Radiological Division consistent with EPA methods and manufacturer's specifications.

Gieger Counter

Laboratory and personnel surveys are performed with a Ludlum 3 Survey Meter with pancake style 44-9 probe. Calibrations will be performed on the instrument annually by a certified commercial laboratory.

Hot plate/Magnetic Stirrer

The laboratory is equipped with six hotplate/magnetic stirrers (EPOI number MB846). All have variable speed, temperature control, and all stir bars are TFE coated.

Incubators

There are ten incubators in the laboratory: a Lab Line Inc. Environette Controlled Environmental Room maintained at 42 ± 2°C (EPOI number MB813), a Forma Scientific maintained at 35 ± 0.5°C (EPOI number MB812), a VWR incubator maintained at 37 ± 0.5°C (EPOI number MB843), four water baths (EPOI number MB847), and a Fisher Scientific Isotemp Incubator maintained at 30 $\pm$ 0.5°C (EPOI number MB837), Boekel incubator at 41 ± 1°C (EPOI number MB841), and a Lab Line incubator maintained at 22 ± 3°C (EPOI number MB843). Temperatures are monitored twice daily (AM/PM) by traceable thermometers and recorded in the temperature log.

The water bath temperatures are measured by means of a thermometer immersed in the bath. Water bath also has an immersed pump for circulating water. Incubator units have an internal temperature monitoring device and maintain the temperature specified by the method used. For non-portable incubators, thermometers placed on the top, middle, and bottom shelves of the use area and immersed in liquid as directed by the manufacturer.

Inoculating Equipment

Sterile metal or disposable plastic loops, sterile swabs, or sterile plastic disposable pipette tips are used. The metal inoculating loops and/or needles are made of nickel alloy or platinum. (When performing an oxidase test, we do not use nickel alloy loops because they may interfere with the test).

Ion chromatography

The laboratory uses a Dionex DX-120 Ion Chromatography for all anion analyses. The instrument is equipped with an external water suppressor supply for chemical suppression to maintain a "quiet" bateline for low level inorganic disinfection by-product analyses. This instrument was purchased new in 2002.

Microscopes

The laboratory uses three microscopes: two Swift Stereomicroscopes with 10 X and 20 X magnification and an Olympus Phase Contrast Microscope. The binocular Olympus Phase-Contrast Microscope has dual 10 X (and 15 X) wide field eyepieces, and four achromatic objective lenses; 4 X, 20 X, 40 X, and 100 X. The light source is a high intensity incandescent lamp with variable power transformer and filters. The microscope is also capable of photography (EPOI number MB830).

Ovens

Three gravity convection ovens are utilized in the laboratory. One is a Blue M Model OV18A is maintained at 103° ± 2.0° C for suspended solids analysis and reagent drying.180° ± 2.0° C. The other is a Fisher Scientific Isotemp oven model 630G maintained at 180° ± 2.0° C for dry heat sterilization and total dissolved solid residue analysis 103° ± 2.0° C. Both ovens have their temperature monitored twice daily (AM/PM) by a calibrated mercury thermometer placed in sand and recorded in the temperature log.

pH Meter

The laboratory is equipped with two Orion Three Star Series Meters each of which has an accuracy of ± 0.01 units and precision of ± 0.01 units (EPOI number MB813). One is dedicated to food analysis and the other for water. The meters will be recalibrated each day using two standard pH buffer solutions. The Orion SA720 will be calibrated between pH 7.0 and 10.0 while the Food pH meter will be calibrated between pH 4.0 and 7.0. The electrodes for the pH meters will be immersed in a buffer solution of 200

ml pH 7.00 buffer + 1 gm KCl when not in use The electrodes will be rinsed with distilled water between measurements and rinsed with a small portion of the sample to be measured. The meter will be placed on standby when not in use. If the slope is below 95% or above 105%, the electrode or meter may need maintenance. Follow manufacturer's instructions for electrode maintenance and general cleaning. Commercial buffer solution containers are dated upon receipt and when opened. Buffers discarded by the expiration date. The pH buffers used for calibration must not be reused.

Refrigerator/Freezer

Sixteen refrigerators located throughout the laboratory (EPOI number MB816). Temperatures are recorded twice daily (AM/PM) using standardized thermometers calibrated to ISO 17025 standards. Refrigerators maintain a temperature of 1°-4°C. Calibrated thermometers are graduated in at least 1°C increments and the thermometer bulb immersed in liquid.

Spectrophotometers

All spectrophotometric measurements are obtained by the use of a Bausch and Lomb Spectronic 100 colorimeter using 1 cm standard cells, or a Bausch and Lomb Spectronic 20 with 1.0 cm or 2.25 cm cells. The Spectrophotometer will be checked for alignment by using a diluted solution of Potassium Permanganate at 526 and 546 nm or a 0.1 ppm Diazotized Nitrite standard at the maximum absorbance wavelength of 543 nm. These checks will be performed quarterly. All cells will be kept clean, free from scratches, fingerprints, smudges, and evaporated film residues. Each spectrophotometric analysis will be performed with a standard curve derived concurrently with the analyses.

Thermocycler, Electrophoresis tank, power supply, and Gel documentation systems

The laboratory is equipped with Two 96 wells TECHNE TC-412 thermocyclers (EPOI number MB806). Two GeneMate electrophoresis tanks with different combs and One Power supply labnet 300. For visualizing gel(s) and taking picture (s) we have one gel documentation system UVP BioDoc-It.

Table top hood

The laboratory is equipped with one table top hood, flow sciences Inc., 3FT VBSE. This hood has UV light with 254 nm and a normal florescent light.

Turbidity Meters

The Hach Model 2100 P Turbidimeter (lot number A3013) is used for turbidity measurements. It is calibrated monthly as per manufacturer's instructions and checked against a laboratory prepared formazin standard (see Standard Methods 20th Edition) prior to each use. Measurements will be made on well suspended samples.

Titration Table

All burets are illuminated from behind with six fluorescent lights covered with white translucent plastic. This uniform lighting allows accurate reading of all burets.

Vacuum pump and manifold

For molecular microbiology and virology tasks, laboratory equipped with a Welch vacuum pump model 2522B-01 as well as Vac-Man vacuum manifold with 20 sample capacity (EPOI number MB832).

Vortexer

The laboratory uses four Thermolyne Maxi Mix Plus vortexers with variable speeds.

Equipment Manuals and Maintenance

Equipment manuals will be maintained in a folder for easy access to all laboratory technicians. All instruments in the laboratory will be maintained to factory specifications and all repairs and instrument changes will be documented in a log book.

LABORATORY SUPPLIES AND REAGENTS

Glassware

All volumetric pipettes, flasks and burettes are of borosilicate glass Type 1, Class A Grade and free of chips and cracks. Caustic solutions will not be stored in volumetric glassware and they will not be heated above 120° C. Measuring pipettes for water chemistry and bacteriological determination are Serological Type 1, Class B borosilicate glass. Borosilicate blowout pipettes with large tip openings are used when pipeting sample suspensions for sanitary chemistry. Flasks, beakers, funnels separatory funnels, distillation and reflux apparatus, graduated cylinders are borosilicate Type 1 glass. The cylinders are

either A or B Grade and are designated either ‘to deliver’ or ‘to contain’. All glassware will be washed with Alconox, rinsed with tap water and then rinsed three or more times with high purity deionized water by the laboratory assistant. Special cleaning procedures will be applied where necessary. Reagent storage containers are of borosilicate, lime glass or plastic (polyethylene or Teflon). The proper container for each reagent will be used. Graduated cylinders for measurement of sample volumes are accurate to within a 2.5% tolerance. Culture tubes and containers containing fermentation medium are of sufficient size to contain medium plus sample without being more than three quarters full. Tube closures are stainless steel, plastic, aluminum, or screw caps with non-toxic liners.

Culture Dishes

Laboratory uses sterile 100x15 mm Petri dishes from Fisher Scientific Co. Opened packs of disposable culture dishes always resealed between use periods.

Pipettes

To sterilize and maintain sterility of glass pipettes, stainless steel or aluminum canisters are used, or individual pipettes are wrapped in char-resistant paper or aluminum foil. Pipettes have legible markings and are not chipped or etched. Opened packs of disposable sterile pipettes are resealed between use periods. Pipettes delivering volumes of 10 ml or less are accurate to within a 2.5% tolerance. Calibrated micropipetters may be used if tips are sterile. Micropipetters are calibrated semiannually and adjusted or replaced if the precision or accuracy is greater than 2.5%.

Sample Containers

Sample containers are wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with non-toxic liners that withstand repeated sterilization, or sterile plastic bottles containing sodium thiosulfate. The capacity of sample containers are at least 120 mL (4 oz.) to allow at least a 1-inch head space. Glass and plastic bottles that have not been presterilized are sterilized by autoclaving. Empty containers are moistened with several drops of water before autoclaving to prevent an "air lock" sterilization failure. If chlorinated water is to be analyzed, sufficient sodium thiosulfate (Na2S2O3) is added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample. Dechlorination is addressed in Section 9060A of *Standard Methods*. At least one sample container selected at random from each batch of sterile sample bottles or other containers (or lot of commercially available sample containers), and the sterility confirmed by adding approximately 25 mL of

a sterile non-selective broth (e.g., tryptic soy, trypticase soy, or tryptone broth). The broth incubated at 35° ± 0.5°C, and checked after 48 hours for growth. Results recorded. If growth is detected, entire batch will be sterilized again.

Temperature Monitoring Device

Glass, dial, infrared, or electronic thermometers are graduated in 0.5°C increments (0.2°C increments for tests which are incubated at 44.5°C) or less, except as noted for hot air ovens and refrigerators. The fluid column in glass thermometers are not separated. Dial thermometers that cannot be calibrated are not be used. The calibration of glass and electronic thermometers checked annually, and dial thermometers quarterly, at the temperature used, against a NIST-traceable reference thermometer or ISO 17025 standards. The calibration factor and date of calibration indicated on the thermometer. In addition, the laboratory records in a QC record book the following information:

- serial number of laboratory thermometer
- serial number of NIST-traceable thermometer (or other reference thermometer)
- temperature of laboratory thermometer
- temperature of NIST-traceable thermometer (or other reference thermometer)
- correction (or calibration) factor
- date of check
- analyst's initials

If a thermometer differs by more than 1°C from the reference thermometer, it should be discarded. Reference thermometers are recalibrated at least once every year. Reference thermometer calibration documentation is maintained. Continuous recording devices that are used to monitor incubator temperature are recalibrated at least annually. A reference thermometer that meets the specifications is used for calibration. The digital thermometers are traceable and biannually checked against standards.

Desiccators

The laboratory utilizes five Pyrex desiccators and one polyacrylic desiccators filled with indicating desiccant. Desiccants will be regenerated and maintained when not in use at 103°C. The plastic desiccators are dedicated for radiological standards.

High Purity Water

High purity water for all glassware cleaning and most reagent preparation is prepared by passing tap

water through a cation exchange resin then an anion exchange resin. followed by two polishing mixed bed resins. The product water is passed through a macro filter and a carbon filter. Water purity is monitored continuously by two fail safe, in series, conductivity meters designed to fail below 1 megohm quality water. The deionized water is distributed to four sinks located in the laboratory through PVC pipes.
In addition, the pH and conductivity of the water is checked by a technician daily.

Media

All media will be prepared fresh weekly, or as needed, dated and autoclaved with autoclave tape. The pH will be taken and recorded in a permanently bound log book. Dehydrated media stored in a cool, dry location, and discarded by manufacturer's expiration date. Caked or discolored dehydrated media also discarded. For media prepared in the laboratory, the date of preparation, type of medium, lot number, sterilization time and temperature, final pH (after sterilization), and the technician's initials are recorded. For media prepared commercially, the date received, type of medium, lot number, and (if identified by the manufacturer or method) pH verification for each lot is recorded. Each new lot of dehydrated or prepared commercial medium and each batch of laboratory prepared medium checked before use for sterility and with positive and negative culture controls. Control organisms (total coliforms, fecal coliforms, and/or *E. coli*, as appropriate) are stock cultures (periodically checked for purity) or commercially available disks impregnated with the organism. Results are recorded. The following Table 1, identifies a few positive and negative culture controls that laboratory consider.

Table 1. Control Cultures for Microbiological Tests

Group	Positive Culture Control[9]	Negative Culture Control[9]
Total coliforms	*Escherichia coli* *Enterobacter aerogenes*	*Staphylococcus aureus*[1] *Proteus vulgaris*[2] *Pseudomonas aeruginosa*[1]
Fecal coliforms	*Escherichia coli* *Klebsiella pneumoniae* (thermotolerant)	*Enterobacter aerogenes*[3]
E. coli	*Escherichia coli* (MUG-positive strain)	*Enterobacter aerogenes* *Klebsiella pneumoniae*[4] (thermotolerant)
Enterococci[5]	[5] *Enterococcus faecalis* *Enterococcus faecium*	*Staphylococcus aureus*[6] *E. coli*[7] *Serratia marcesens*[8]

[1] *S. aureus, P. aeruginosa* - not lactose fermenter
[2] *P. vulgaris* - not lactose fermenter; uses hydrolyzed lactose, indicating "overcooked" medium
[3] *E. aerogenes* - ferments lactose, but is not typically thermotolerant
[4] *K. pneumoniae* - ferments lactose, but does not hydrolyze MUG
[5] Do not use closely related strains from genus *Streptococcus* as a positive control
[6] *S. aureus* - sensitive to nalidixic acid in medium
[7] *E. coli* - sensitive to sodium azide in medium
[8] *S. marcescens* - will not hydrolyze fluorogenic compound in medium
[9] Examples of appropriate ATCC strains include the following:
Enterococcus faecalis ATCC 11700
Enterobacter aerogenes ATCC 13048
Klebsiella pneumoniae (thermotolerant) ATCC 13883
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 6538
Enterococcus faecium ATCC 6057
Escherichia coli ATCC 8739 or 25922
Proteus vulgaris ATCC 13315
Serratia marcesens ATCC 14756

If prepared medium is stored after sterilization, it maintained in the dark, avoiding moisture loss, per the following Table 2. Prepared plates stored in sealed plastic bags or containers. For either broth or agar media, each bag or container include the date prepared or an expiration date. If the medium is stored in a refrigerator, it will warm to room temperature before use; tubes or plates that show growth and/or bubbles will be discarded. Liquid media discarded if evaporation exceeds 10% of the original volume.

Table 2. Maximum Holding Times and Temperatures for Prepared Media

Container	Max storage temp.	Max. storage time
Poured agar plates	1-5°C	2 weeks
Broth in tubes, bottles, or flasks with loose-fitting closures	1-30°C	1 weeks
Broth in tightly closed screw-cap tubes, bottles, or flasks	1-30°C	3 months

Chemical Supplies

All Chemicals and media will be dated when received in the laboratory and will receive a unique log number. In addition all chemicals and media will be dated when opened.

Reagents Solutions

All reagent solutions will be prepared with reagent grade chemicals or better and will receive a unique log number.

Standard Solutions

All purchased standard solutions will be dated when received and a log entry will be kept of a unique log number, the date received along with the supplier and lot number. If available, manufactures' certification of the standard will be on file while the standard is in use. The date opened will be recorded on the bottle. When stock standards are prepared in the laboratory all preparation data will be recorded in a standards solution log and will include a unique log number, date of preparation, initials of preparer, supplier, lot number of reagents and weight of solute and volume of solvent used.

STERILIZATION PROCEDURES

Autoclaving times at 121°C are listed below (table 3). Except for membrane filters and pads and carbohydrate-containing media, indicated times are minimum times and may necessitate adjustment depending upon volumes, containers, and loads. Carbohydrate-based media should not be over-sterilized.

Table 3.

Item	Time (min)
Membrane filters & pads	10
Carbohydrate containing media	12-15[1]
Contaminated test materials	30[2]
Membrane filter assemblies	15
Sample collection bottles	15
Individual glassware	15
Dilution water blank	15
Rinse water (0.5 - 1 L)	15-30[2]

[1] except when otherwise specified by the manufacturer
[2] time depends upon water volume per container and autoclave load

REAGENT-GRADE WATER

Only satisfactorily tested reagent water from distilled or deionization units will be used to prepare media, reagents, and dilution/rinse water for performing microbial analyses.

The quality of the reagent water tested and should meet the following criteria in table 4.

Table 4.

Test	Maximum Acceptable Limit	Monitoring Frequency
Conductivity	>0.5 megohms resistance or <2 micromhos/cm (microsiemens/cm) at 25 C	Monthly[3]
Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively, no greater than 0.1 mg/L	Annually
Total Chlorine Residual[1]	<0.1 mg/L	Monthly
Heterotrophic Plate Count[2]	< 500 CFU/mL[4]	Monthly

[1] DPD Method should be used. Not required if source water is not chlorinated.
[2] Pour Plate Method. See *Standard Methods* 9215B.
[3] Monthly, if meter is in-line or has a resistivity indicator light; otherwise, with each new batch of reagent water.
[4] CFU means colony-forming units (same as colonies, but is a more precise term).

DILUTION/RINSE WATER

Stock buffer solution or peptone water will be prepared, as specified in *Standard Methods*, Section 9050C or will prepared from commercially available concentrated pillows. Stock buffers autoclaved or filter-sterilized, and containers labeled and dated. Stored stock buffers are free from turbidity. Each batch of dilution/rinse water is checked for sterility by adding 100 mL of a double strength non-selective broth (e.g., tryptic soy, trypticase soy or tryptose broth). Incubate at 35°±0.5°C, and check for growth after 24 and 48 hours. Results recorded. If growth is detected batch will be discarded.

GLASSWARE WASHING

Distilled or deionized water is used for final rinse. Laboratory glassware washed with a detergent designed for laboratory use. A glassware inhibitory residue test (*Standard Methods*, Section 9020B, under *Laboratory Supplies*) will be performed before the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure, is used. This test will ensure that glassware is free of toxic residue. Each batch of dry glassware used for microbial analysis will be checked for pH reaction, especially if glassware is soaked in alkali or acid (*Standard Methods*, Section 9020B, under *Laboratory Supplies*). By using 0.04% bromthymol blue (or equivalent pH indicator) and observe color reaction. Clean glassware without an alkali or acid residual should have a neutral color reaction (blue-green for bromthymol blue). This test will ensure that glassware is at a neutral pH.

DISPOSAL OF LABORATORY SAMPLES

Biological wastes (culture media, petri dishes, culture discards etc.,) are sterilized by autoclaving before disposal. Samples will be checked and discarded, if necessary, at a minimum of once per week, according to the requirements of each functional area as described below:

Chemistry: Due to City of San Diego restrictions, no waste with a pH outside of the range of 5.0 to 10.0 can be disposed by way of the city sewer system. IEH-EEL Laboratory neutralizes all waste to a pH between 6.0 and 8.0 before disposal. Hazardous Acids and Organic Wastes are accumulated for disposal on a semi-annual basis. They are collected by a certified company for disposal. Perishable samples are kept in the refrigerator and shelf stable samples are kept in dry storage. All samples are retained at least two weeks after the final report is issued, unless the sample held per client request and it is documented on the chain of custody.

Residue: Perishable samples are kept in the refrigerator and shelf stable samples are kept in dry storage. All samples are retained at least two weeks after the final report is issued, unless the sample has been marked differently.

Microbiology: After sampling for analysis, perishable samples are stored refrigerated or frozen and retained at least two weeks after the final report is issued, unless held per client request. Shelf stable samples are kept in dry storage for a minimum of two weeks.

All samples are disposed of appropriately. Food samples are disposed of in the regular trash.

Under no circumstances are samples to be consumed in the laboratory or removed from the laboratory for personal use or consumption. Doing so constitutes grounds for immediate dismissal.

BIOHAZARD DISPOSAL

Bio-Hazards are defined as all materials in the microbiology room that have used to perform microbiological analyses. This includes, but is not limited to, media and test tubes used in tests, petri dishes with agar, and samples which are colilert positive. These materials can only be disposed of after they have been steam autoclaved. The procedure for this autoclaving is as follows:

Place all test tubes into baskets. Remove all tape from test-tube caps and remove all old autoclave tape from the baskets. Affix new strip of autoclave tape to each basket. Place all used petri dishes in metal pan. Place sample containers which are positive for colilert in pan. Loosen the caps of colilert positive bottles so that steam is allowed to enter. Affix a piece of autoclave tape to the pan itself.

Place all items in the autoclave and set the autoclave to run for 45 minutes.

Record in Media log book the following data:

1. Initials
2. Date
3. Time started
4. Time completed
5. Maximum pressure
6. Maximum temperature
7. Total elapsed time.

Run spore test once each month to monitor effectiveness of autoclave. Use the autoclave data logger as second temperature check for autoclave each week. Calibrate this thermometer at least once each year.

SAMPLE PRESERVATION LIST

PHOSPHORIC ACID & CuSO4 2 ml/L

Phenols

HYDROCHLORIC ACID (HCI) 2 ml/L

Grease and oils

Volatile Organic compounds from chlorinated sources

NITRIC ACID (HNO_3) 2 ml/L

All metals except Hexavalent Chromium

Alpha, Beta Radioactivity

SUFURIC ACID(H2SO4),CONCENTRATED 2 ml/L

Chemical Oxygen Demand (COD)

Nitrogen: Total Kjeldahl

Ammonia

Nitrate

Total Phosphorous

PHOSPHORIC ACID (H3PO4), COCENTRATED 2ml/L

Total Organic Carbon (TOC)

SODIUM THIOSULFATE (Na_2SO_4) 2ml/L

Trihalomethanes (only)

Organics as per SW846 table 2-16

ZINC ACETATE 2 ml/L

Total Sulfide only (do not add zinc acetate to dissolved sulfide)

SODIUM HYDROXIDE

Cyanide, Sulfate

ASCORBIC ACID Hc

Volatile Organic Compounds (non-chlorinated sources)

SPECIAL CONSIDERATIONS

Samples that are received unpreserved for metals analysis and which are subsequently acidified must be held for **24 HOURS** after acidification before any analysis can be performed. Sample login personnel are responsible for recording this in sample log. The turbidity of acid preserved drinking water samples must be measured and recorded in the metals digestion log. Samples with a turbidity of < 1.0 NTU do not need to be digested.

Ethylene Diamine (EDA) 2 DROPS/ 40 ml

Inorganic Disinfection by-products: Chlorite, Chlorate, and Bromate.

Ammonium Chloride

Haloacetic Acids