City of San Buenaventura
Wastewater Laboratory

Analytical Quality Assurance Program 2010

Date Revised: 3/24/2010

Florence B. Jay
Laboratory Supervisor

ANALYTICAL QUALITY ASSURANCE PROGRAM 2010

I. LABORATORY DUTIES AND OBJECTIVES

The City of San Buenaventura Wastewater Laboratory is responsible for all sampling and analysis for purposes of NPDES compliance monitoring related to the City operated wastewater treatment plant, industrial waste and for the City domestic water supply and water distribution system monitoring for SDWA compliance. It is aim to provide a product the does not cause harm to the public or the environment.

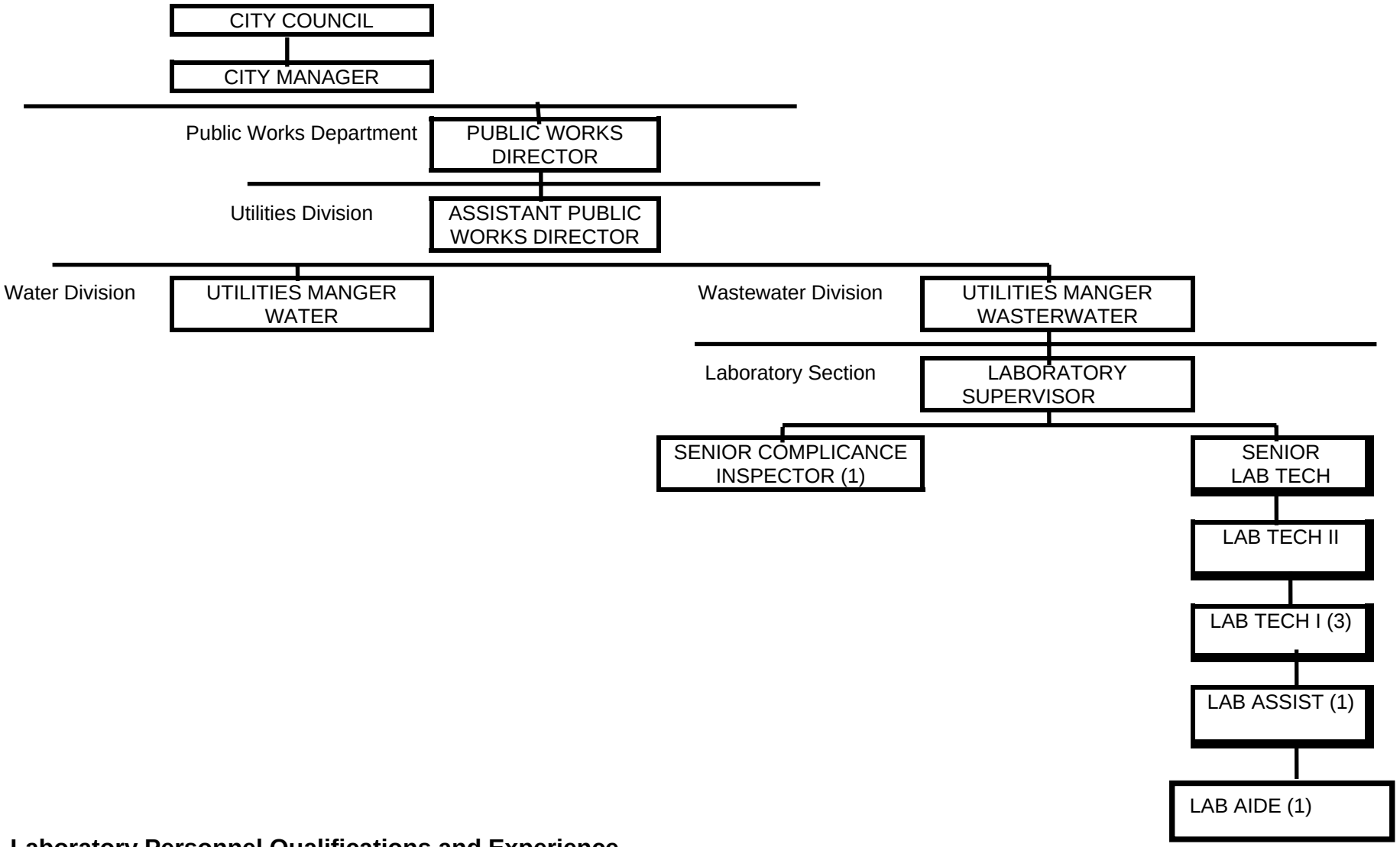
Current State of California Department of Health Services laboratory certification is attached.

All analyses for purposes of NPDES and SDWA reporting or for industrial waste monitoring conforms to the current requirements of 40 CFR Part 136, "Guidelines Establishing Test Procedures for the Analysis of Pollutants" or of 40 CFR Part 141, "National Interim Primary Drinking Water Regulations."

The purpose of this document is to outline the laboratory quality assurance procedures as they relate to compliance monitoring and to evaluate performance where statistically valid numbers of control results are available.

II. Laboratory Overview

A. Organization



B. Laboratory Personnel Qualifications and Experience

Laboratory Supervisor: Florence Jay - May 1998 to Present

Education:

Bachelor of Sciences Fort Valley State - Biology

Master of Sciences Iowa State University - Fisheries Biology

Experience:

Lab Tech I, City of San Buenaventura Water Division

Lab Tech II, City of San Buenaventura Wastewater Division

Senior Lab Tech, City of San Buenaventura Wastewater Division
Present Duties:

Responsibilities include the supervision of the laboratory and industrial waste staff, which involves the preparing of employees' evaluations and overseeing the day to day activities. Prepare water and wastewater monitoring reports of analyses including electronic transfer of water data. Prepare the laboratory budget and the ordering of supplies and chemicals. Other administrative duties include attending various meetings, coordination of sample schedule with other departments and assist with the chemical, physical and biological analysis of water, wastewater and industrial waste samples.

Senior Technician: Michael L. Torres - August 1999 to Present

Education:

Bachelor of Science Microbiology – California State University @ Northridge (Pending)

Experience:

Microbiologist – Montgomery Watson Laboratories

Present Duties:

Responsibilities include the inorganic and organic analyses of water, wastewater and industrial waste samples using the Gas Chromatogram and the Ion Chromatograph. As the bench supervisor direst, train and assist the staff with physical, chemical and biological analyses. Operate the laboratory's quality control program, maintain records, data entry of results and oversee the laboratory safety program. Supervise the laboratory in the absence of the supervisor.

Lab Tech II: Craig Jones – September 2000 to Present

Education:

Bachelor of Science in Biology - University of North Carolina @ Chapel Hill

Experience:

Laboratory Technician I City of San Buenaventura Wastewater, Ventura California

Laboratory Technician, Ventura County Waterworks, Moorpark, California

Present Duties:

Analyze water, wastewater and industrial waste sample using the Atomic Absorption Spectrometer for mineral and metals. Perform other physical, chemical and biological analyses of water, wastewater and industrial waste samples Daily input and recording of laboratory data. Maintenance and upkeep of laboratory equipment.

Lab Tech I: Mary Champion - July2005 to Present

Education:

Bachelor of Science in Biology, minor in Chemistry, University of May Harding – Baylor

Experience:

Laboratory Technician, Los Angeles County Sanitation District Via Fastek

Laboratory Technician, San Manuel Bottle Water Group

Present Duties:

Perform routine chemical, physical and biological analysis of water, wastewater and industrial waste samples. Effluent chronic toxicity, collect water, wastewater, and receiving water samples for laboratory analyses. Daily input and recording of laboratory data. General laboratory housekeeping.

Lab Tech I: Felicitas Ramirez – September 2005 to Present

Education:

Bachelor of Science in Commerce Management - Saint Louis University Philippines

Bachelor of Science - Biology Ventura College

Experience:

Laboratory Technician I Aquatic Bioassay Consulting Laboratories, INC

Aquatic Biologist Aquatic Bioassay Consulting Laboratories, INC

Laboratory Supervisor Aquatic Bioassay Consulting Laboratories, INC

Present Duties:

Perform routine chemical, physical and biological analysis of water, wastewater and industrial waste samples. Chronic bioassay testing of effluent, collect water, wastewater, and receiving water samples for laboratory analyses. Daily input and recording of laboratory data. General laboratory housekeeping.

Lab Tech I: Manuel Zapien – July 2006 – Present

Education:

Associate of Science in General Science – Ventura College

Bachelor of Science in Geology – University California at Santa Barbara

CWEA Grade 1 Laboratory Analyst Certificate

Experience:

Laboratory Technician – Ventura County Water Works

Laboratory Technician – NUSII Technology, INC

Present Duties:

Perform routine chemical, physical and biological analysis of water, wastewater and industrial waste samples. Collect water, wastewater, and receiving water samples for laboratory analyses. Daily input and recording of laboratory data. General housekeeping and equipment maintenance.

Laboratory Assistant: Jason Wong – August 2006 – Present

Education:

High School Diploma - Adolf Camarillo High School

Experience:

Laboratory Aide – City of Ventura Wastewater Laboratory

Present Duties:

Collect water, wastewater, industrial waste and receiving water samples for laboratory analyses. Perform simple chemical, physical and bacteriological analyses on the various water samples. Input laboratory data and perform maintenance of laboratory equipment.

C. Instrumentation and Equipment

The division laboratory owns and maintains the following equipment and instrumentation.

UNIT	MANUFACTURER/MODEL	MAINTENANCE
Water Still	Corning 3 Liter Megapure	Division
	Barnstead Nanopure Diamond	Division
D. I. Water Supply	Culligan Commercial Units	Culligan
Forced Convection Oven	VWR S/P Model 1370FM	Division
Oven	VWR Model 1670 HAFO Series	Division
Muffle Furnace	Barnstead/Thermolyne Furnace Model F304203C	Division
Incubator (Air)	Precision Model 30M	Division
Incubator (Air)	Fisher Scientific Counter Top Model 6500	Division
Incubator (BOD)	Fisher Scientific Model FFU20FC4CWO	Division
Speed Vac	Horizon Speed Vac 9000	Division
Autoclave	Getinge/Castle Model 133LS	Getinge/Castle
	Market Forge Sterilmatic	Thermo - Scientific
pH Meters	Orion Model 701	Divison
	Orion Model 701	Division
	Cole-Parmer 5938-00 Portable	Division
Specific Ion System	Orion Model EA 940 Meter/Electrodes	Division
Sealer	Idexx Quant- Tray Sealer Model 2X	Idexx
Conductivity Meter	Orion Model 162A	Division
Centrifuge	Sorvall Legend	Division
D.O. Meters	Orion SL 9 Portable Probe	Division
	Orion Model 9708 Electrode	Division
	Thermo Orion Model 826A	Division
Nepthelometers	Hach Model 2100A	Hach
Water Bath	Blue M Magniwhirl Model 1110A	Division
	Thermo-Scientific Lindberg/Blue M circulating bath	Division
	Precision Circulating Bathe Model 270	Division
Analytical Balances	Mettler Model AT 201 Mettler Model AE 163	Mettler
Top-Loading Balances	Mettler Model PM2000	Mettler
	Mettler Model PM2000	Mettler
Microscopes	American Optical 40-1000X Phastar	Division
	American Optical .7-3X Stereo	Division
	Nikon Eclipse E600	Division
Refrigerators	3 - Thermo Scientific Isotemp	Thermo Fisher ,Winkler

Spectrophotometers	2 – Agilent 8453 UV-Visible Spectrophotometer	Division
	Bausch & Lomb Spectronic 20	Division
	Hach DR/4000	HACH
Spectrophotometer AA-AE	Varian Spectra220/Furnace Atomizer/ GTA110 Autosamplers	Varian
Ion Chromatograph	Dionex, ASRS-I Self Regenerating Supressor/Dionex, CD20 conductivity Detector	Dionex
Gas Chromatograph	HP 6890 GCSystem Series Autosampler; Micro EC Detector Flame Ionization Detector	SC Chromatography
Purge/Trap system	HP 7695	SC Chromatography
Dispenser/Diluter	Gilson 222	Division
Samplers	5 ISCO Model 6712Fr	Division/ MRC Technologies, Inc
	2 - American Sigma 900	Division/America Sigma
	1 ISCO Model 3700	Division/ MRC
	5 American Sigma 800SL	Division/America Sigma
Dishwashers	2 - Miele Professional Washers G-7783	Miele
SC Chromatography	-- SC Chromatography Torrance, CA	
Hach	-- HACH Company , Loveland, CO	
Getinge/ Castle	-- Getinge/Castle Rochester, New York	
Idexx	--Idexx Westbrook, Maine	
Dionex	--Dionex Sunnyvale, CA	
Varian	-- Varian Sugarland, Texas	
MRC Technologies, Inc	--MRC Technologies, Newbury Park, CA	
Mettler Toledo	--Mettler Toledo Columbus, OH	
Miele	--Miele, Inc, La Verne, CA	
American Sigma	-- Ponton Industries, Inc, Santa Fe, Spring CA	
Division	-- Ventura Sanitation Division Personnel	

III. PROCEDURES, RECORDS AND REPORTS

A. Sampling

Procedures for sampling, sample preservation, handling, storage, disposal and transportation conform to the requirements of 40 CFR Part 136 and/or to 40 CFR Part 141 and amendments.

1. Collection

Samples are collected and delivered to the Wastewater Laboratory for analysis by wastewater personnel (laboratory staff and plant operators), industrial waste inspector, water division and other City's staff.

Samples collected maybe a grab or a 24-hour composite. All composite samples are collected using ISCO Models 6712 FR, 3700. Samplers operate in flow proportion by utilizing the non-uniform time option of the control electronics.

Sample containers are of a material that does not produce positive or negative errors or cause contamination to the sample. Sample containers used for composite samples are pre–clean ICHEM plastic cubtainers or stainless steel container for organic analysis. Grab samples are collected in pre-cleaned plastic ICHEM cubtainers, pre-cleaned glass amber bottles and pre-cleaned 40 vials depending upon the analysis.

Potable water distribution and groundwater samples are collected from designate sample box. Bacteriological samples are collected in sterile Idexx sample bottles containing sodium thiosulfate. Non-routine or grab sample sites for verifying disinfection are flamed using a propane torch. Samples are stored into ice chest with ice packs for transporting to the laboratory.

Receiving water and beach samples are collected in sterile 125ml samples bottle with sodium thiosulfate using a adjustable pole with a clamp to hold the container.

Field samples are transported to the lab in ice chest with ice packs to keep the samples cooled.

All samples are collected using aseptic techniques to prevent contamination. Sample is collected daily, weekly, monthly, quarterly, semi-annually in accordance with the NPDES permit or as a special request, one time basis.

2. Sample Preservation

Sample preservations are done in accordance with the analysis to be performed in 40CFR. At sample collection the sampler does field measurements for pH, chlorine residuals and temperatures.

The laboratory preserves all samples not analyzed immediately that are collected and delivered to the laboratory by wastewater personnel (laboratory staff and plant operators). The technician performing the analysis preserves samples delivered by the Water Division or outside agencies.

The industrial waste inspector or the lab assistant preserves industrial samples collected for metals cyanide and total sulfide analyses. The lab technician performing the analysis preserves all other industrial waste samples.

3. Handling and Storage

The person preserving the sample is responsible for storage of the sample. When possible the samples are stored in their original containers in the containers.

All samples not requiring immediate analysis are preserved and refrigerated at 4^{o} C or less. They remain in storage until all the analyses have been completed and data approved. The technicians responsible for performing the required analysis remove and replace the samples in storage.

Samples placed into storage must be labeled with the sample name, date, and time sampled, the analysis required and the initial of the sampler. As part of the chain of custody the technician fills out the sign in and out label on the sample container or custody sheet when the sample is taken out of storage.

The amount of time a sample is held in storage varies from .5 hours to 6 months depending upon the analysis to be performed. The holding time for each sample is checked before it is placed into storage to ensure that the analysis is done within that time frame.

4. Disposal

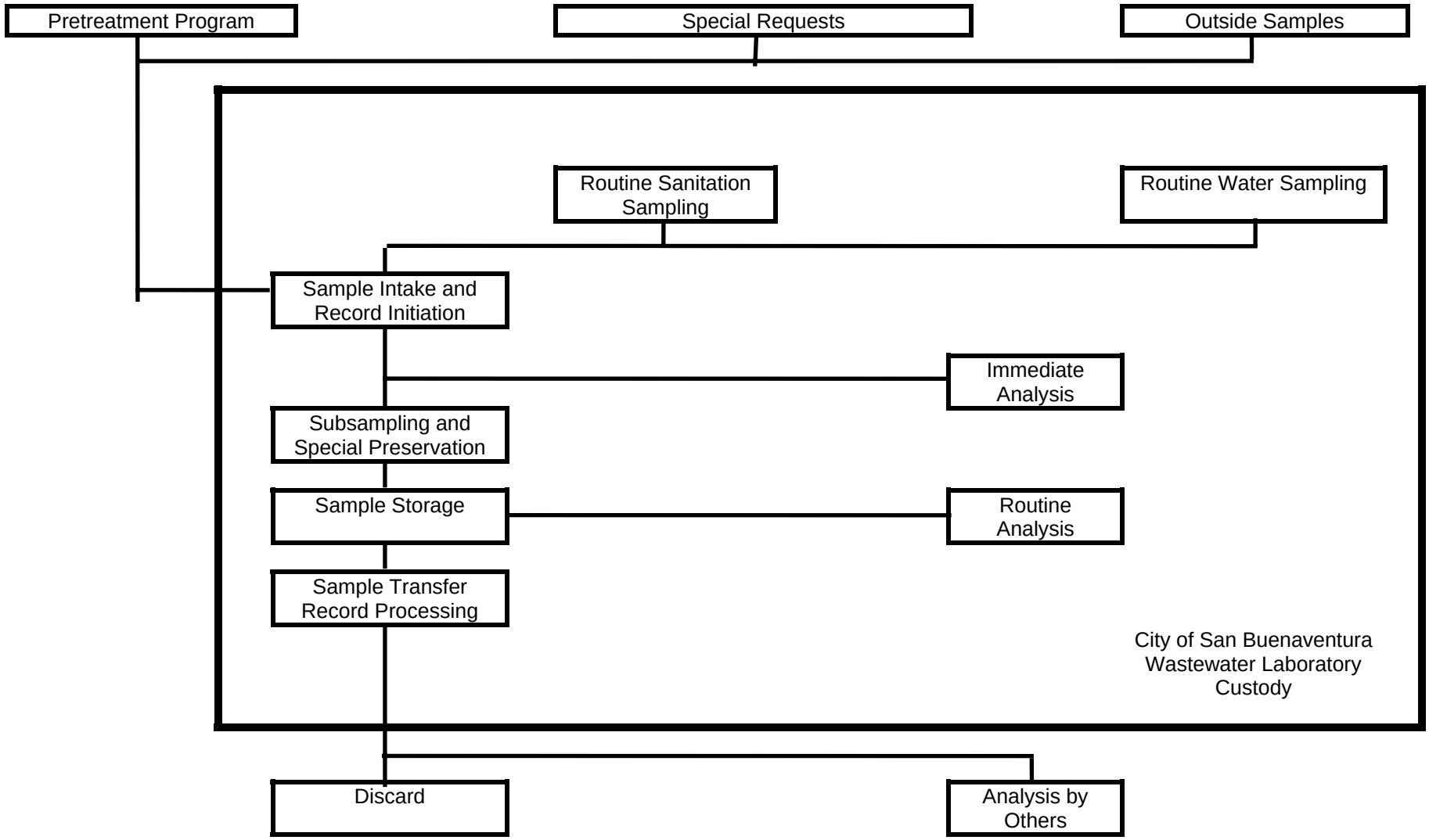
A sample can be disposed after the analysis is completed and the data has been reviewed by the laboratory supervisor, senior lab tech and industrial waste inspector. Samples should be disposed of in a safe manner that will not harm employees or the environment. Special care must be taken with samples that have been stored for long periods of times.

Wastewater and some industrial waste samples that have been stored for several weeks can create hazardous odor such as sulfides at the time of disposal. Proper safety attire and precaution must be taken when disposing these samples.

Samples analyzed for minerals and some metals maybe dumped down the drain and flushed with large of water for disposal.
Others must be neutralized before they can be disposed as in the case of COD vials. Samples that have been analyzed for pesticides or phenols can be evaporated under the fume hood. Large volumes of waste solvents and other hazardous materials are disposed of at hazardous waste disposal sites.

Review the Material Safety Data Sheet (MSDS) or check with the laboratory supervisor or the senior lab tech if you have questions on proper disposal of laboratory chemical or reagents.

The laboratory sample path is shown in the chart below.



B. Sample Identification

Sampling sites for routine wastewater and drinking water, which are, monitored daily, weekly, quarterly or semi-annually have fixed identity by name, number or acronym. This identification is used on location maps, in sample logs, on bench worksheets, on permanent records and on analysis reports.

The Laboratory Computer Data System assigns a Laboratory Identification Number (LID) to other water, wastewater, industrial waste samples or any non-routine sample received. The LID is in numerical order and is automatically assigned by the computer. This number is used in sample logs, bench worksheets, permanent records and on analysis reports. A copy of the Computer Data System is in the laboratory's Standard Operation Procedures (SOP).

C. Custody

A Chain of custody is initiated when a sample enters or leaves the laboratory unit. All samples done on a regular bases have printed worksheet which sample collectors log in custody information. All other samples enter the laboratory are log in the incoming sample book and given a laboratory Identification number.

Custody documents vary with the sampling purpose, but all custody transfers identify the sample by name and/or LID number, the sample collector and documents date, time, location, analysis required and circumstances of sample collection along with the history of sample transfers by person and/or organization. An example of the chain of custody form is in the laboratory's SOP.

C. Analysis Procedures

Analytical Procedures, which the laboratory is certified to perform, are according to the Standard Methods for the Examination of Water and Wastewater 18^{th} – 20^{th}, EPA Chemical Method for Analysis of Water and Wastes and EPA 40 CFR 136 & 141.

Bench procedures for analytical methods performed by the laboratory are maintained in a loose-leaf notebook in the laboratory work area. These are derived from approved standard procedures; which include reagents, standard preparation and concentration, test procedures, equipment and instrumentation with the analytical options for interference correction; samples and sample volumes defined for the samples analyzed. These procedures are reviewed periodically and revised to accommodate method and sample changes.

For unfamiliar and non-routine samples, the primary analytical procedures are followed to determine dilution, interference correction and all other method variables.

D. Records

Systematic procedures for record keeping and retention have been established in conformance with the requirements of compliance monitoring and good practice.

The following summarizes the purposes and retention criteria for each general type of written laboratory record.

Record	Function	Retention
Field Logs	Record of Field Measurements and Circumstances of Sampling	7 Years
Receiving Log	Record of Samples Received from Others	7 Years
Sample/Custody Form	Pretreatment Program Sampling	7 Years
Chromatographs	Analysis from Gas Chromatograph, Ion Chromatograph and Atomic Absorption	7 Years
Bench Logs	Worksheets for Data and Calculation	7 Years
Bound Record Books	Permanent Record of Analysis Results	7 Years
Reports	Transmittal of Information to Others	7 Years

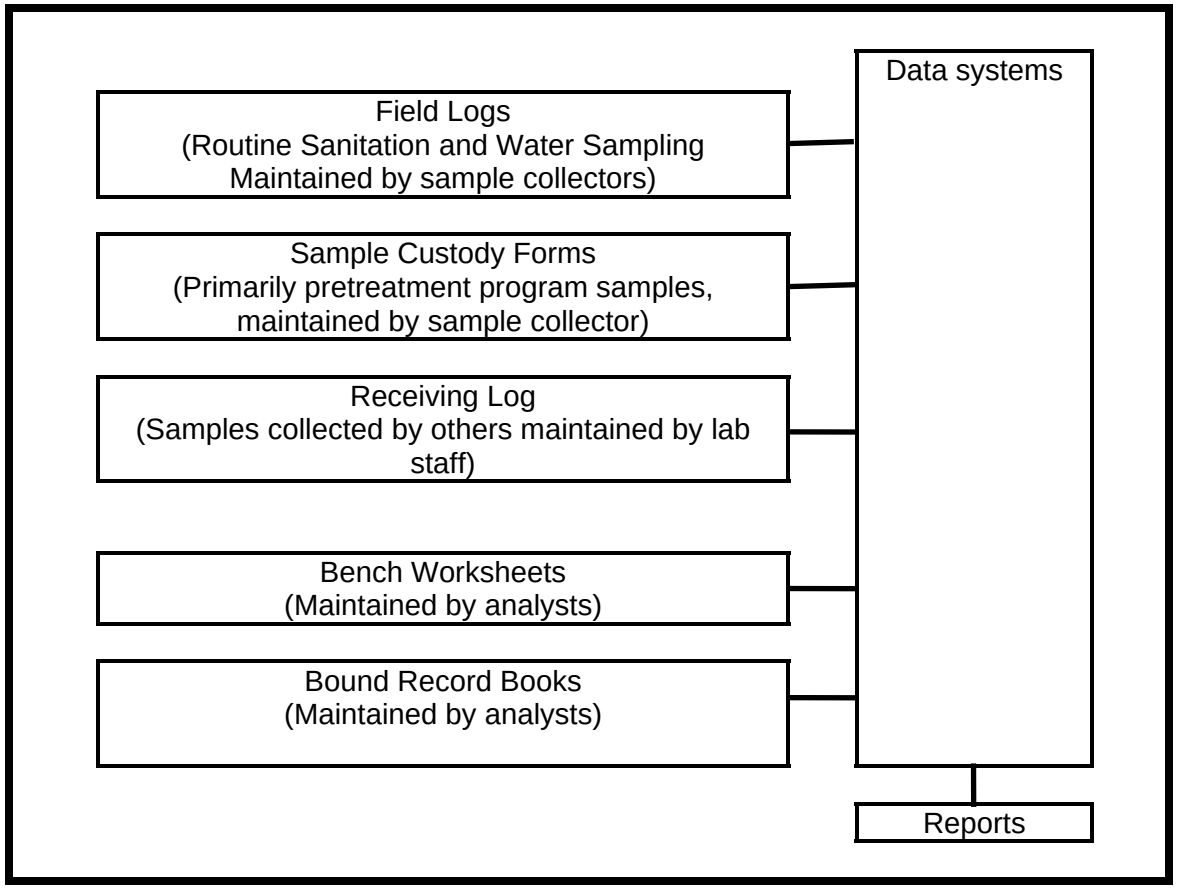
E. Reports

Report formats and contents are generally specified by the agency requesting the reports. Reports of routine monitoring are provided by computer methods designed to meet these specifications. Reports for water samples are generated from data entered into Run: Input1and Input 2 through the City Vax system before September 2009. OPS Strategic Data Management program is used to generate wastewater and water reports. Industrial Waste reports are generated from the Environmental Control Database. Reports generated from data with LID numbers are generated from the Vax using Run Tra Input3. A copy of the Laboratory Computer Data Systems is located in the SOP manual in the Laboratory.

Data from field logs, custody forms, receiving logs, bench worksheets, bound logbooks, chromatographs and spectrophotometer is used to compile information required for these report. A flow chart for data input information is below.

All current procedures, records and reports are available at the laboratory for review and inspection. Records of annual analytical results are available from 1971 to present. Reports are reviewed and signed by the Laboratory Supervisor.

Data flow from generation to reporting is shown below.



Sample Retention Requirements

Sample Source	Frequency	Subsample	Discard After:	Authorization by:
Drinking Water	Weekly	Turbidity, Iron Filters	Analysis Complete	Analysts
Drinking Water	Any	Bacti	Inoculation Complete	Analysts
Drinking Water	Monthly	Chemical & Physical	Report Reviewed	Lab Supervisor
Drinking Water	Quarterly	THM	Report Reviewed	Lab Supervisor
Drinking Water	Annual	Metals	Report Reviewed	Lab Supervisor
Submerged Wells	Any	All	Report Reviewed	Lab Supervisor
Surface and Ocean	Any	Bacti	Inoculation Complete	Analysts
Wastewater	Daily Grab	pH, Turbidity, Residual	Analysis Complete	Analysts
Wastewater	Daily Grab	Bacti	Inoculation Complete	Analysts
Wastewater	Daily Composite	pH, Solids, Oxygen Demands, Conductivity	Analysis Complete	Analysts
Wastewater	Weekly Composite	Nitrogen, Chloride, Sulfate	Analysis Complete	Analysts
Wastewater	Monthly Composite	MBAS, Total P	Report Reviewed	Lab Supervisor
Wastewater	Monthly Composite	PO4, Alkalinity, B, F	Analysis Complete	Analysts/Supervisor
Wastewater	Weekly Grabs	Oil & Grease	Analysis Complete	Analysts
Wastewater	Quarterly Grabs	Cyanide	Analysis Complete	Analysts
Wastewater	Monthly Composite	Bioassay	Test Complete	Analysts/Supervisor
Wastewater	Quarterly Composite	Metals,	Report Reviewed	Lab Supervisor
Wastewater	Quarterly Composite	Pesticides, Phenol	Report Reviewed	Lab Supervisor
Receiving Waters	Weekly	Bacti, Chemical & Physical	Analysis Completed	Analysts
Receiving Waters	Monthly	Nitrogen, PO_4, Total P	Analysis Completed	Analysts/Supervisor
Receiving Waters	Quarterly	Priority Pollutants	Report Reviewed	Lab Supervisor
Source Control	Any	All	Report Reviewed	IW Inspector
Special	Any	All	Report Reviewed	Lab Supervisor

IV. QUALITY ASSURANCE PROCEDURES AND DOCUMENTATION

A. General

The quality assurance procedures employed by the laboratory are intended to accomplish the following objectives:

1. Provide primary control over the accuracy reagents, standards and other related materials employed in analysis.
2. Provide day-to-day control over the accuracy of measurements.
3. To ensure that the technicians understand the analyses.

Specific actions designed to accomplish these goals in each area of laboratory measurement are discussed below.

B. Laboratory Equipment

Equipment effected by the environmental, mechanical or electronic reasons is checked periodically for alignment. Other units, such as ovens or incubators are monitored for accuracy and consistency. Readings are taken or calibration procedures are performed and recorded at the frequency indicated below.

Unit	Calibration Procedure	Frequency
Ovens	Verify Temperature and Adjust as needed	Daily
Incubators	Verify Temperature and Adjust as needed	Daily
Furnace	Verify Temperature and Adjust as needed	Daily
Conductivity Meter	Calibrate with 1413 calibration standard	Daily
pH Meters	Calibrate with Buffer Solutions	Prior to Use
D.O. Meters	Air Calibrate	Prior to Use
D.O. Meters	Check Against Winkler Titration	Weekly
Light Merer		Annually
Turbidimeters	Calibrate with Secondary Turbidity Standards	Daily
Turbidimeters	Calibrate with Certified Standards	Daily
Turbidimeters	Calibrated by Hach	Annually
Spectrophotometer	Verify Wavelength Accuracy with Holmium Oxide Filter	Quarterly
Autoclave	Verify Accuracy of Integral Recorder with Lag Thermometer	Weekly
Weights	Calibrated by Troemner Precision Weights	Annually
Balances	Verify Accuracy with External Calibration Weights	Weekly
Balances	Calibrated Mettler Toledo	Annually

C. Primary Quality Control (QC)

Stock standard and reagents used in the analysis are logged with: the quantity used, dilution, finally volume, initials of the preparer, date prepared and discards date. This information is on each sample container. The method procedure is checked for the stability and storage of the stock solution or the reagent.

Titration reagents used on a daily basis is standardized weekly. The results from that standardization such as the multiplication factor adjustments; the normality of the reagent; and the initials of the person doing the standardization is label on the buret and the logged into the prep book. Other titration reagents are standardized prior to use and labeled with the same information listed above.

D. Chemical Analysis

Analysis reagents and standards are prepared from Primary standard materials, calibrated against Primary Standard materials, or purchased as certified purity and/or certified concentration standards from an approved agency such as NSI.

These procedures are used to assure conformance to narrow concentration or purity limits when procedures require it. It helps in determining when a reagent must be discarded and calculation factors to avoid errors in analysis results.

E. Day-to-day Control for Accuracy of Results

Testing for chemical and physical composition is conducted on a batch basis. Each sample batch is run with controls and spikes. An acceptance of sample results as valid is based on the results of the control analyzed and spike recovery. Weekly QC is performed on all daily analyses. QC is performed on all monthly, quarterly and annual analyses following the same format as for weekly QC.

Routine control samples are prepared in house for frequently performed analyses. For other procedures the Division Laboratory analyzes NSI and Accustandard traceable commercial reference samples.

In addition to these primary checks on the accuracy and precision of measurement, blank, sample replicates and matrix spikes are carried through all procedures.

F. Corrective Actions

Some laboratory data reduction is automated in many cases including instrument data generation. For automated applications, when a control, spike or sample duplicate evaluation fails to meet standard criteria for method performance, the analysis process is halted and/or sample results are withheld by the software system. Analysis cannot continue until the cause of the failure is identified and acceptable results from the control materials are produced.

In procedures where automation is not employed, the analyst performs the same function: data is not reportable unless results from analysis of control, spike and sample duplicates analyzed with the analysis batch are within acceptance standards.

All controls, spikes and duplicates must be within the acceptance limits before the results from the analysis can be recorded. After reviewing the analysis procedures, calculations and repeat of the analysis it cannot be determine the reason for the failure you must check with the QA person and the laboratory supervisor before recording the data. If it is determined that the QA material failed and the sampled material was accurate an explanation must be recorded for the failure in the "QIR" Qualitative Investigate Report book.

G. Special considerations for Trace Inorganic and Organic Analyses

The Quality Assurance requirements for trace inorganic and organic analyses are narrowly defined by the approved analytical procedures. These requirements are adhered to.

Materials used for preparing standards, spikes and control for Trace Inorganic analysis are obtained from SCP Science, Champlain, NY, AccuStandard, Inc, New Haven, CT and VHG, Manchester, NH.

Materials used to prepare standards; spikes and control for trace organic analysis are normally obtained from Supelco/Sigma Aldrich, Milwaukee, WI. If appropriate materials are not available from this source, they are obtained from NSI Solution, Raleigh, NC or from normal chemical supply sources.

As with all other measurements, acceptability of sample results is dependent on controls, spikes and duplicates analysis results being within acceptance limits. No QA analysis data can be recorded if the control, spike or duplicate fail without a valid reason.

H. Special Considerations for Toxicity Analysis

Instrument Calibration

Continuous temperature recorders for monitoring test solution temperatures are Taylor Instrument drum recorders with remote sensor probes. Recorders are calibrated against ASTM certified reference –1 ^{0} to 101 ^{0} glass thermometer by adjustment of the pen arm.

ASTM 30 ^{0} C thermometers are calibrated annual against the reference thermometer any corrections are labeled on the thermometer.

pH measurement is made with Thermo Orion meter which is calibrated prior to use.

D.O. measurement is made with a Thermo Orion meter which is calibrated daily.

Reference materials are analyzed as noted below.

Analysis	Reference Material	Frequency of Reference Analysis
Algae Growth Chronic Toxicity	Zinc	With Every Test Sample
Ceriodaphnia Survival and Reproduction	Copper	With Every Test Sample
Larval Fathead Minnow Survival and Growth	Copper	With Every Test Sample

Other test acceptance criteria:

Analysis	Criterion
Algae Growth Chronic Toxicity	Control cell counts >/= 1,000,000,000/ml
Algae Growth Chronic Toxicity	Control Replicate Counts << 20% Different
Ceriodaphnia Survival	Survival in Controls >> 80%
Ceriodaphnia Reproduction	Number of young must be 15 or greater
Ceriodaphnia Survival and Reproduction	60% of Surviving Adults produce 3 broods
Larval Fathead Minnow Survival and Growth	Survival in Controls >> 80%
Larval Fathead Minnow Survival and Growth	Control Average Dry Weight >> 0.250 mg

Moderate hard synthetic dilution water is used as the control for all chronic bioassay analyses. The control is exposed to the sample conditions as the sample. A summary of the test acceptance for each species is below and a table for each is at the end of the QC report.

For Green Alga, *Selenastrum Capricornutum,* at the end of 96- hour the cell mass density in the control must be at least 1 x 10^6 cells/ml and variability (CV $^o/_o$) among control replicates less than or equal to 20 $^o/_o$ required. Test organisms must be 4 to 7 days old and have a cell density of 10,000 cells/ml.

For Daphnid, *Ceriodaphnia Dubia,* $60^o/_o$ or more of the control females must have three brood within an 8 day time period and $80^o/_o$ or greater survival of all control organisms. Each surviving control female must produce an average of 15 or more babies. Test organisms must be less than 24 hours old and hatched within an 8 hours period.

For, Fathead Minnow,*Pimephales Promelas,* at the end of 7 days the control must have $80^o/_o$ or greater survival. Each survivor must have a dry weight or 0.25 mg or greater. Test organisms must be newly hatched larvae less than 24 hours old. If shipped not more than 48 hours old.

I. Bacteriological Analysis

Bacteriological analysis required by NPDES and SDWA monitoring is routinely performed by the multiple-tube fermentation procedure. Drinking water samples are analyzed using Colilert and recreational water by Idexx Quanti Tray method.

The Laboratory is equipped to perform Multiple Tube Fermentation (MTF) for total coliform, fecal coliform and fecal streptococci; membrane filter tests for total and fecal coliform analyses. Heterotrophic plate count (HPC) is performed monthly on water samples.

Quality assurance is for MTF, Colilert, and idexx methods consist of analyses of a blank, positive and negative control using the appropriate bacteria strand. Each new batch of MTF media and bacti supplies is tested before use. Controls are analyzed with each colilert an idexx tests.

Total coliform testing is performed following the procedures of Section 9221B of "Standard Methods for the Examination of Water and Wastewater," 18^{th} – 20^{th} Edition. All Samples are carried through the Brilliant Green Bile confirmation step.

At least 5% of all samples testing positive in the confirmed coliform procedure are carried through the completed procedure.

Fecal Coliform testing is performed following the procedures of Section 9221E of "Standard Methods for the Examination of Water and Wastewater," 18^{th} - 20^{th} Edition.

Fecal streptococcus testing is routinely performed following the procedures of Section 9230B of "Standard Methods for the Examination of Water and Wastewater," 18^{th} – 20^{th} Edition.

Control tests for water suitability and for inhibitory residues are performed annually following the procedures of Section 9020B(3)(a)(2) and 9020A(3)(c)(1) of "Standard Methods for the Examination of Water and Wastewater," 18th – 20th Edition.
Commercial dehydrated media is used for all analysis. Media is tested for accurate response by inoculati on of portions from each prepared batch with Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923) and Streptococcus faecalis (USEPA-EMSL Cincinnati 111054).

Coliform test materials failing to give a positive response to Echerichia coli, a negative response to Staphylococcus aureus and no response upon incubation of non-inoculated media are discarded.

Fecal strep test materials failing to give a positive response to Streptococcus faecalis, a negative response to Staphylococcus aureus and no response upon incubation of non-inoculated media are discarded.

Both media and equipment are prepared in weekly batches, and materials are tested for sterility using Tryptic Soy Broth before use and dated to assure they are used within acceptable holding periods or discarded.

Revised 3/24/10

Sampling and Testing Procedures

Ref: Standard Methods 18th Ed.
Revised 7/ 2002

MULTPLE-TUBE FERMENTATION BACTERIOLOGICAL PROCEDURES

The following describes specific sampling and test procedures for the MTF determination of total coliform, fecal coliform and fecal streptococcus.

GENERAL OUTLINE

The MTF test involves determination of bacterial density by the process of diluting the test sample and observing for growth at each dilution.

In a simplistic way, an estimate of bacterial population could be made by simply observing the dilution below which no growth at all occurs. It would be possible to say that the population (the count) was between the last dilution with growth and the first without.

Because of the normal variations which occur in the processes of mixing, diluting and pipeting the sample, a single test at each dilution is usually not very accurate and at least 5 inoculations at each dilution are are commonly used for calculating reportable results.

For potable water samples, where any significant presence of coliform organisms is unacceptable, it is usually adequate to set up only one set of 10 tubes each with 10ml of undiluted sample.

For other applications where higher counts of coliform or streptococcus organisms are normal and better data on the actual bacterial numbers is wanted it is necessary to inoculate 3 or more sets of 5 tubes, each succeeding row at a concentration 1/10 of the previous row. The starting inoculation volume and the number of dilutions used will depend on the Coliform numbers expected. Sample amount for the first set is usually 10ml but it can be from 100ml when very low counts are expected to 1/10, 1/100ml or even less when heavily contaminated waters are being examined.

For the total and fecal coliform tests, the initial innoculation is into Lauryl tryptose broth.

For the fecal streptococcus test the initial inoculation is into Azide dextrose broth.

For the total coliform test, confirmation is by transfer from all positive Lauryl tryptose tubes into Brilliant green bile broth.

For the total coliform test, completion is by streaking Levines EMB agar plates from all positive BGB tubes, transfer from typical/atypical EMB colonies to a nutrient agar slant from which a gram stain is prepared and to a secondary LTB tube to confirm gas production.

For the fecal coliform test, confirmation is by transfer from all positive Lauryl tryptose tubes into EC medium.

for the fecal streptococcus test, confirmation is by streaking Pfizer selective enterococcus agar plates from all positive Azide dextrose broth tubes.

MATERIALS

Sample containers are either 4 or 8 ounce (depending on the volume required

for the tests to be performed) Nalgene polypropylene bottles containing 0.1 ml of 10% Sodium thiosulfate solution. Bottles are capped (not tightly) labeled with sterile indicator tape, covered with an inverted drinking cup for protection of the screw top in the field and sterilized. Caps are tightened after cooling.

For each sample to be examined by the confirmed total coliform procedure, assuming you will begin inoculation with 10 ml of sample and use 3 dilutions total, you will require:

5 culture tubes with inner Durham tubes each with 10ml of double-strength (twice the label concentration) Lauryl tryptose broth. Cap with BLUE caps.

10 culture tubes with inner Durham tubes each with 10 ml of normal strength Lauryl tryptose broth. Cap with BLUE caps.

Several culture tubes with Durham tubes each with 10 ml of Brilliant green bile broth. Cap most with WHITE and a few with RED caps.

Broth is prepared using manufacturer's weight per volume specifications by dissolving dehydrated media in distilled water, dispensing into culture tubes, caping and sterilizing.

One Milk dilution bottle containing 90 ml of dilution buffer (2 bottles if you will be inoculating a total of 4 five tube rows)

Buffer is prepared by diluting stock buffer ingredients (refer to Standard Methods for formula specifications) in distilled water, dispensing into bottles, capping (not tightly) and sterilizing. Caps are tightened after cooling.

One 10 ml and one 5 ml Serological Pipet (two 10 ml and one 5 ml pipets if you will be inoculating a total of 4 five tube rows)

Pipets are placed in stainless steel cans labeled with sterile indicator tape for sterilization. If all pipets in a can are not expected to be used in one inoculation sitting, they should be sealed in use groups in glassine envelopes to protect them from contamination after the can is opened. If not in envelopes, once opened a can of pipets may not be reclosed and stored for later use.

Wooden applicator sticks for culture transfers

Applicator sticks are also sealed in use batches in glassine envelopes and in stainless steel cans labeled with sterile indicator tape.

If you will carry the test through the completed step you will also require:

Several petri dishes with Levine's eosin methylene blue agar

Agar is prepared using manufacturer's weight per volume specifications dissolved by boiling for 1 minute in an Erlenmeyer flask covered with an inverted beaker, then sterilized. After sterilization, agar is dispensed into sterile dishes, allowed to harden and then inverted.

Several slant tubes with Nutrient agar

All of the above must be sterilized and protected from subsequent contamination.

Media are sterilized for 15 minutes in the autoclave on liquid cycle. Store in the closed cabinet at room temperature.

All other materials are sterilized for 30 minutes in the autoclave on dry cycle. Cap everything tightly after cooling. Put everything away in cabinets or drawers. Protect from water which can penetrate loose fitting covers and contaminate sterile materials. Do NOT store on bench tops.

QUALITY ASSURANCE PROCEDURES

Media preparation should me monitored for correctness and proper organism response. The following information should be recorded for each separate batch of media prepared: date, manufacturer, batch control number, weight of dehydrated media, volume of water, post sterilization ph, incubated response of the
media to inoculation with an expected positive standard culture, inoculation with an
expected negative culture, and incubated response of uninoculatedmedia.

Autoclave charts are labeled with the date and the materials sterilized for each temperature tracing. Charts are retained for 6 months from the run date. At least once each 7 days the accuracy of the autoclave temperature chart recorder is checked against a lag thermometer included with a batch of materials being sterilized. If the difference exceeds 2oC, the autoclave recorder reading will be adjusted to correspond to the lag thermometer reading. The result of the check will be logged with media preparation information.

Media racks are tagged with the preparation date (and strength, if appropriate). Media prepared on different dates should not be mixed in test racks. Media are used oldest first. Media older than 14 days will be discarded.

Air incubator temperature will be checked daily, logged and adjusted if temperature is above 35.5oC or below 34.5oC.

Water bath incubator temperatures will be checked daily, logged and adjusted if temperature is above 44.7oC or below 44.3oC.

The conductivity of the distilled water supply used for media preparation will be checked and logged monthly.

Before released for test use, each weekly batch of dilution water, sample containers, transfer sticks and pipets will be checked for sterility before use. Pour 100 ml double strength TSB into one dilution bottle, pour 100 ml single strength TSB on one sample bottle,and pipet 10 ml TSB in a tube and pipet back. The TSB broths should show no turbidity after 48 hours of incubation.
If turbidity is observed, tested batch should be re-sterilized and re-tested before
use.

Agar is dissolved by boiling for 1 minute in an Erlenmeyer flask covered with an inverted beaker, then dispensed into screw top culture tubes, capped, (not tightly) and sterilized. After sterilization, tubes are slanted and the agar allowed to harden. Caps are tightened after cooling.

Crystal Violet stain, Gram's iodine solution, Acetone/Ethyl alcohol mixture and Safranin stain for the Gram's stain procedure. Refer to Standard Methods for the formulation of each of these.

For each sample also to be examined by the fecal coliform procedure you will need, in addition to the materials required for the total coliform procedure:

15 culture tubes with inner Durham tubes each with 10 ml of EC medium. Media is prepared like LTB and BGB using manufacturer's weight per volume specifications. Cap each tube with BLACK caps.

For each sample to be examined for fecal streptococcus, assuming you will begin inoculation with 10 ml of sample and use 3 dilutions total, you will require:

5 culture tubes each with 10ml of double-strength (twice the label concentration) Azide dextrose broth. Media is prepared like LTB and BGB using manufacturer's weight per volume specifications. Cap each tube with GREEN caps.

10 culture tubes each with 10 ml of normal strength Azide dextrose broth. Cap each tube with GREEN caps.

One Milk dilution bottle containing 90 ml of dilution buffer (if the fecal strep test is to be performed in conjunction with the total coliform test, an additional dilution bottle will not be required)

One 10ml and one 5ml Serological Pipet (if the fecal strep test is to be performed in conjunction with the total coliform test, additional pipets will not be required)

Several Petri dishes with Enterococcosel (PSE) agar

Agar is prepared like EMB agar using manufacturer's weight per volume specifications.

If inoculation volumes and dilutions are different from the above assumptions you must adjust the quantities of materials accordingly.

Media is commonly prepared from commercial dehydrated materials. these are:

Difco 0241-01-8 Lauryl tryptose broth
Difco 0007-01-2 Brilliant green bile, 2%
Difco 0314-01-0 EC medium
Difco 0387-01-2 Azide dextrose broth
Difco 0005-01-4 Levine's EMB agar
BBL 12205 Enterococcosel agar

If it is necessary to formulate these from component materials, or to check that the commercial formulas conform to the requirements of the methods, the preparation formulas and procedures for each of these are available in Standard Methods.

Once annually the water supply and the washing procedure will be evaluated following the standard methods procedures for water suitability and inhibitory residue.

PROCEDURE

ASEPTIC TECHNIQUE

Bear in mind that bacteria are to be found everywhere. Your hands, the bench top, books and virtually everything people have touched and which has not been sterilized and sealed is loaded with living coliform organisms which, if they wind up in your media tubes, will give you errors in your test.

A. Do not touch any part of any apparatus which will later contact sample or media.

B. Do not lay anything down which will later contact sample or media.

C. Do not use or re-use for dilute sample any piece of apparatus which has contacted a more concentrated sample. The other way around is OK.

SAFETY

We are growing bacteria here. Although we select for certain kinds by the conditions of culture, we do not know what kinds might be contained in a sample or what kinds might propagate under the conditions of the tests we perform, so caution is warranted. It is generally not desirable to injest bacteria or permit them entry to eyes, ears, nose open wounds or burns.

Wear suitable protective clothing and equipment.

Never pipet anything by mouth.

Always terminally sterilize every culture before discarding.

Clean the work areas frequently with disinfectant.

Attend to injuries promptly: Wash, apply antiseptic and protect with a bandage.

Wash your hands frequently.

SAMPLING

Before opening the sample bottle, identify the sample by marking the sterile indicator tape label. Remove the cap using the paper cup protector and fill the bottle to the shoulder ONCE from the water source taking precautions to avoid touching the inside of the cap or bottle and avoid contact with any part of any structure, fixture or stream. Re-cap the bottle and put it into the ice chest or return immediately to the lab with it.

Standard sampling locations are established for the following routine samples:

Water distribution system samples numbered 1 through 35 and the Ventura River at Foster Park, Avenue Plant Flume

Receiving water stations R1 through R4 and L5

For these locations it is only necessary to mark the label with the location designation and log the time and conditions of sampling, if appropriate, in the field sampling log book. If resamples are taken, record the sampling conditions as above and mark these labels with the designation, date and "Resample".

EWRF disinfected effluent sample points for Bay 1, Bay 2 and Bay 3 of the Chlorine Contact Tanks must be marked with the designation, date and time of sampling as well as logged in the plant sample log book.

If any other samples are taken or received from others, obtain a Laboratory Identification Number from the computer data system and log the sample information as appropriate both in the incoming sample log and in the computer record. The sample may then be designated on the label and in logbooks only by the LID number.

SAMPLE INOCULATION

1. Mix the sample by shaking vigorously 25 times, transfer the sample label to the cap of a tube in the first media row and:

For Environmental samples, transfer 10ml to each of 5 tubes containing double strength Lauryl tryptose broth (for total or fecal coliform) and/or 5 tubes containing double strength Azide dextrose broth (for fecal streptococcus). Do not put down this pipet!. Use it to transfer an additional 10ml of sample into the 90ml of dilution buffer. OK, now put it into the disinfectant jar.

If you will be inoculating a fourth row of tubes with 0.01 ml of sample, you should have prepared one additional 10 ml pipet and 90 ml dilution buffer bottle. Use the pipet now to transfer 10 ml from the first dilution bottle to a second. This pipet now goes into the disinfectant jar.

Mix each diluted sample by shaking vigorously 25 times. With the 5ml pipet, transfer 1ml of the most dilute sample into each of 5 single strength Lauryl tryptose broth and/or Azide dextrose broth tubes (the last row). Do not put down this pipet either!. Use it to transfer 1 ml of succeedingly more concentrated dilutions, if any, into the next row(s) forward and finally 1 ml of the original full strength sample to each of the second row (next to the double strength media) containing single strength Lauryl tryptose broth and/or Azide dextrose broth tubes. OK, now put it too into the disinfectant jar.

For water distribution system samples, transfer 10ml to each of 10 tubes containing double strength Lauryl tryptose broth.

2. Put the rack into the 35oC incubator.

3. Record the date and time of presumptive inoculation in the bacti logbook.

CONFIRMATION

For total coliform:

1. After 24 hours of incubation (no less than 22 and no more than 26 hours),

examine each Lauryl tryptose tube for evidence of Coliform growth: turbidity in the media and any gas trapped by the inverted Durham tube. From each positive presumptive Lauryl tryptose tube, transfer a loop full of culture with a flame sterilized inoculating loop or with a wooden applicator stick to a tube containing Brilliant green bile broth and having a WHITE cap. Remove the positive Lauryl tryptose tube from the rack and replace it with the just inoculated BGB tube. Put everything back into the incubator. Record the number of positive transfers made from each row.

2. After a total incubation time of 48 hours (no less than 45and no more than 51hours), examine the presumptive tubes again and inoculate additional Bile tubes, this time having RED caps, from any additional Lauryl tryptose tubes you find positive. Put everything back in the incubator and record the transfers again.

3. After a total of 72 hours (again +/- 3 hours) from the time of the initial inoculation, examine the first set of Bile tubes (WHITE caps) for positives. Record the number of positive tubes in each row. Put them back in the incubator.

4. After a total of 96 hours examine the second set of Bile tubes (RED caps) for positives and record the numbers. The confirmed test results will be based on the sum of these and the white capped BGB tubes from 3 above for each row.

For fecal coliform

1. After 24 hours of incubation (no less than 22 and no more than 26 hours), examine each Lauryl tryptose tube for evidence of Coliform growth: turbidity in the media and any gas trapped by the inverted Durham tube. From each positive presumptive Lauryl tryptose tube, transfer a loop full of culture with a flame sterilized inoculating loop or with a wooden applicator stick to a tube containing EC medium. If you are transfering to BGB for the total coliform test at the same time use a different applicator for the EC transfer. You will need to prepare a second tape label and mark it with the same information as the first label. Place the EC tube in a second rack in the same position as the positive LTB tube from which it was inoculated. Put the LTB rack back into the incubator at 35oC and the EC rack in the 44.5oC water bath. Record the number of positive transfers made from each row.

2. 24 hours after inoculation (no less than 22 and no more than 26 hours) of EC media tubes, examine for turbidity and gas and record the results. These tubes are now discarded.

3. After a total LTB incubation time of 48 hours (no less than 45and no more than 51 hours), examine the presumptive tubes again and inoculate additional EC tubes from any additional Lauryl tryptose tubes you find positive. Put everything back in the incubators as above and record the transfers again.

4. 24 hours after inoculation of the second set (no less than 22 and no more than 26 hours) of EC media tubes, examine for turbidity and gas and record the results. These tubes are also discarded. The fecal coliform test results will be based on the sum of the first and second EC media positives for each row.

For fecal streptococcus

1. After 24 hours of incubation (no less than 22 and no more than 26 hours), examine each Azide dextrose broth tube for turbidity.

From each positive (turbid) AD tube, prepare a streak plate on Enterococcel (PSE) agar. Identify the plates with the sample label tape information and the row and position of the positive AD tube from which it was inoculated. Invert the plates and incubate at 35oC for an additional 24 hours (no less than 22 and no more than 26 hours) and examine for characteristic brown/black colonies with brown halos extending away from the colony in the plated media.

2. After 48 hours of incubation (no less than 45 and no more than 51 hours), examine each remaining Azide dextrose broth tube for turbidity.

Streak additional PSE plates as above and check for characteristic colonies after 24 +/- 2 hours. The fecal streptococcus test results will be based on the sum of the first and second set of positive PSE agar plate results.

Total coliform completion

1. From each positive Bile tube from the last row with any positive tubes, prepare a streak plate on EMB agar. Identify the plates with the sample ID and which row it came from then incubate these at 35oC for 24 hours.

2. At 24 hours, examine the growth on the plate and determine if the bacterial colonies are:

Typical - Metallic green surface sheen and with a colony nucleus.

Atypical - Pink and mucoid (moist and runny appearance).

Negative - Anything else.

3. From any plates which are Typical or Atypical, transfer a portion of culture from a single colony to both a Nutrient agar slant and to a single strength Lauryl tryptose tube. Identify and incubate these also.

4. After another 48 hours, examine the secondary Lauryl tryptose tube for positive growth.

5. At the same time, prepare a smear from growth on the Nutrient agar slant, carry out the Gram's stain and examine the stained cells under the microscope. The Gram's stain procedure should include control smears of known Gram positive (S. aureus) and Gram negative (E. coli) cultures.

1. The total coliform MPN is determined from the numbers of tubes which:

Were positive as Lauryl tryptose tubes

and

Produced positive Bile tubes

and

Produced typical or atypical colonies on EMB agar if attempted

and

Produced positive secondary Lauryl tryptose tubes

and

Showed Gram negative rods with no evidence of spores when examined microscopically.

1. The fecal coliform MPN is determined from the numbers of tubes which:

Were positive as Lauryl tryptose tubes

and

Produced positive EC media tubes

1. The fecal streptococcus MPN is determined from the numbers of tubes which:

Were positive as Azide dextrose tubes

and

Produced characteristic Enterococcsel colonies

OBJECTIVE OF THE PROJECT

Bacteria indicator samples (total coliform, fecal coliform, and enterococcus) were collected by the City of Ventura in the Ventura Keys, and additionally in the waterbodies connected to the Ventura Keys: Ventura Harbor, Arundell Barranca, and Peninsula Beach (Harbor Cove). Samples were collected for the purpose of characterizing the water quality in these waterbodies.

The Ventura County Department of Environmental Health also collected bacteria indicator samples at Peninsula Beach (Harbor Cove Beach) under the AB411 monitoring program. Because theses samples were collected at a location also sampled by the City of Ventura, the data are submitted together.


K1
K2
K3
K4
K5
M1
M2
Ventura Keys
Ventura Harbor
Arundell Barranca
Harbor Cove Beach
Peninsula Street
Sailor Avenue
Seahorse Avenue
Seaview Avenue
Bayshore Avenue
Weymouth Lane
Hanover Lane
Camden Lane
Nathan Lane
Greenock Lane
Beachmont Street
Seafarer Street
Navigator Drive
Harbor Boulevard
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