PROCEDURE MANUAL APPROVAL

DRAFT

WATER MICROBIOLOGY QUALITY ASSURANCE PROCEDURE MANUAL

	LAB DIRECTOR SIGNATURE	DATE	REMARKS
APPROVED			
REVISED	**SIGNATURE**	**DATE**	**REMARKS**
DISCONTINUED	**LAB DIRECTOR SIGNATURE**	**DATE**	**REMARKS**

San Diego County Public Health Laboratory
3851 Rosecrans St. Suite 716
San Diego, CA 92110
Patricia McVay, MD, Chief

WATER MICROBIOLOGY

QUALITY ASSURANCE

MANUAL

TABLE OF CONTENTS

ORGANIZATION AND PERSONNEL RESPONSIBILITIES

Organization

1. In general, trained laboratory assistants and public health microbiologists perform all water testing procedures.

2. Results are checked before reports are issued by the senior or Supervising Public Health Microbiologist. For drinking water analyses performed by laboratory assistants, the actual results will be visibly verified by a microbiologist.

3. Reports are then given to the Chief of the Public Health Laboratory for final review before mailing or faxing by clerical staff. If the Chief is not available, this step is omitted.

CLIA-Regulated Personnel

Testing Personnel: Public Health Microbiologists

1. Review the procedure and quality assurance manuals for the duties assigned (a) within 2 weeks after a change in assignments and (b) every 12 months if the assignment is > 6 months.

2. Review the laboratory safety manual (a) within 2 weeks after beginning employment and (b) every 12 months thereafter.

3. Under the direction of the Supervising or Senior Public Health Microbiologist, become proficient in performing all microbiology and serology tests which you are assigned to perform.

4. Perform any microbiological or serological test, as assigned, following training and orientation under the supervision of the Supervising Public Health Microbiologist.

5. Process specimens, perform tests, and prepare and initial reports and forward to Senior or Supervising Public Health Microbiologist for review.

6. In each section to which you are assigned, follow the written procedures for specimen collection, criteria for specimen acceptance or rejection, testing, quality control and assurance, and reporting as documented in procedure manuals and the safety manual.

7. Document all quality control activities, instrument and procedure calibrations, and maintenance performed.

8. Follow established policies and procedures whenever test systems are not within the established acceptable level of performance. Record remedial action taken when tests are out of control.

9. Handle and test proficiency testing (PT) samples in the same manner as routine patient samples and document this.

10. Use written procedures and quality control to identify problems that may adversely affect performance or reporting of test results. Notify Senior or Supervising Public Health Microbiologist immediately when problems are identified.

11. If licensed as a Clinical Laboratory Technologist, carry out responsibilities of that job class, as requested.

12. Complete the weekend coverage checklist and hand in to your supervisor on Monday morning before the weekend you are scheduled to work.

General Supervisor: Senior Public Health Microbiologist

1. Perform onsite general supervision and oversight of laboratory operations and personnel performing testing in one unit of the laboratory, as assigned. Be present in the laboratory when testing is being performed. Assignments can be in any one of the following units: Mycobacteriology, General Bacteriology, Virus Serology, and Virus Isolation.

2. Review the procedure and quality assurance manuals for the particular unit to which you are assigned (a) within 2 months after a change in assignments and (b) every 12 months if the assignment is >6 months. Prepare and revise manuals as directed by your technical supervisor.

3. Review the laboratory safety manual (a) within 2 weeks after beginning employment and (b) every 12 months thereafter.

4. Under the direction of the Supervising Public Health Microbiologist, become proficient in performing all microbiology and serology tests performed in the unit you are supervising.

5. Perform any microbiological or serological test, as needed to assist other staff in completing daily workload, following training and orientation under the supervision of the Supervising Public Health Microbiologist.

6. In each unit which you are assigned to supervise, monitor test analyses and specimen examinations to ensure that acceptable levels of analytic performance are maintained, as defined in the procedure and quality assurance manuals. This includes making sure that testing personnel do the following:

    a. Follow the written procedures for specimen collection, criteria for specimen acceptance or rejection, testing, quality control, and reporting as documented in procedure manuals and the safety manual.

    b. Document all quality control activities, instrument and procedure calibrations, and maintenance performed.

    c. Forward reports to Senior or Supervising Public Health Microbiologist for review prior to sending out.

    d. Follow established policies and procedures whenever test systems are not within the established acceptable level of performance. Record remedial action taken when tests are out of control.

e. Test results are not reported until any necessary corrective actions have been taken and the test system is properly functioning.

f. Handle and test proficiency testing (PT) samples in the same manner as routine patient samples and document this.

7. Provide orientation and training to new testing personnel and determine when they are capable of performing accurate and reliable testing.

8. Annually evaluate performance of all Public Health Microbiologists (testing personnel) assigned to the unit you are supervising. Assist the Technical Supervisor in evaluating competency of testing personnel.

9. If licensed as a Clinical Laboratory Technologist, carry out responsibilities of that job class or Senior Clinical Laboratory Technologist, as assigned.

10. Complete the weekend coverage checklist (form Lab 57) and hand in to your supervisor on Monday morning before the weekend you are scheduled to work.

Technical Supervisor: Supervising Public Health Microbiologist

1. Provide onsite technical and scientific oversight of the laboratory during normal operating hours, Monday through Friday.

2. When the General Supervisor is not available, monitor work performed by testing personnel, checking quality control, and accuracy of test reports before sending out.

3. Review procedure and quality assurance manuals in the areas assigned to supervise within 2 months after hiring and annually thereafter. Prepare and revise manuals or oversee the preparation and revision of manuals by general supervisors as needed. Submit new and revised procedures to Chief, Public Health Laboratory for review before implementation.

4. Make sure that a copy of each procedure is maintained with dates of initial use and discontinuance. Retain copies of discontinued procedures for 2 years.

5. Provide onsite or telephone consultation for testing personnel, physicians, nurses, and public health workers as needed.

6. Select and implement new or improved test methodologies as appropriate for their clinical use.

7. Verify test procedures performed and establish test performance characteristics including (where applicable) accuracy and precision of each test and test system.

8. Enroll and participate in an HHS approved proficiency testing program commensurate with services offered.

9. Establish a quality control program and prepare quality assurance manuals appropriate for the testing performed and establish standards for acceptable levels of analytical performance. Ensure these standards are maintained through the entire specimen receiving, testing and reporting process.

10. Resolve technical problems. Ensure remedial action is taken and documented whenever test systems deviate from established performance standards.

11. Ensure patient test results are not reported until necessary corrective actions have been taken and the test system is functioning properly.

12. Identify training needs and ensure that Testing Personnel and General Supervisors under your supervision receive regularly scheduled in-service training and education appropriate for the type and complexity of testing services they perform.

13. Evaluate competency of Clinical Laboratory Technologists and Public Health Microbiologists (Testing Personnel) under your supervision, assuring that they maintain their competency to perform and report tests accurately and efficiently. This shall be done on an ongoing basis using all of the following methods:

    a. Direct observations of patient test performance including patient preparation (if applicable), specimen handling, processing, and testing.

    b. Monitoring the recording and reporting of test results.

    c. Review of intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records.

    d. Direct observation of performance of instrument maintenance and function checks.

    e. Assessment of test performance through testing previously analyzed specimens, internal blind testing samples, or external proficiency testing samples.

    f. Assessment of problem solving skills.

14. Performance evaluation of testing personnel using the above methods must be completed at least semiannually during the first year the individual tests specimens. Thereafter, evaluation must be conducted at least annually, unless methodology or instrumentation changes, in which case evaluation must be done within 6 months following the changes.

Laboratory Director: Chief, Public Health Laboratory

1. Assume responsibility for overall laboratory operation and administration, including hiring personnel competent to perform test procedures, record and report test results promptly, and assuring compliance with applicable regulations.

2. Assume the responsibilities and duties of Technical or General Supervisor or Testing Personnel when needed due to staff vacancies or absences.

3. Be accessible to the laboratory to provide onsite, telephone, or electronic consultation as needed.

4. Must direct no more than 5 laboratories.

5. Ensure that methods used to perform lab tests provide quality lab services for all aspects of test performance including preanalytic, analytic, and post-analytic phases of testing.

6. Ensure that physical and environmental lab conditions are appropriate for tests being performed and that employees are protected from physical, chemical, and biological hazards.

7. Ensure that test methods used provide the quality of results required for patient care.

8. Ensure that adequate verification procedures are used to determine accuracy, precision, and other pertinent performance characteristics of the method.

9. Ensure that Testing Personnel are performing tests as required for accurate and reliable results.

10. Ensure that the lab is enrolled in a HHS-approved proficiency testing program for the testing being performed.

11. Ensure that proficiency testing samples are handled as follows:
    a. Samples are tested with the regular workload using routine methods by personnel who routinely perform the tests.
    b. Samples are tested the same number of times as routine specimens.
    c. There is no communication with other labs, sharing of results, or referral of samples for proficiency testing to other labs.
    d. Pre-analytical, analytical, and reporting steps are documented. Maintain copies of records for at least 2 years.
    e. Results are returned to the proficiency testing service within the specified time limits.
    f. Appropriate staff review proficiency testing report received to evaluate the lab's performance and identify and correct any problems.

    g. When corrective action is necessary, appropriate staff review all steps in analysis, repeat testing if applicable, determine source of problem, and implement corrected procedure.

12. Ensure that quality control and assurance programs are established and maintained to assure high quality of services and identify failures in quality as they occur.

13. Ensure establishment and maintenance of acceptable levels of analytical performance for each test system.

14. Ensure that necessary remedial actions are taken and documented when performance standards are not met and that patient results are only reported when test systems are functioning properly.

15. Ensure that reports of test results include information required for interpretation.

16. Ensure that consultation regarding quality of test results and their interpretation in relation to patient conditions is available to those submitting specimens for testing.

17. Employ sufficient staff with appropriate education, training, and experience to provide necessary consultation and supervision of the performance and reporting of test results, as defined in personnel responsibilities.

18. Ensure that prior to testing specimens, personnel have necessary education, training, and experience and have demonstrated they can perform and report the tests accurately.

19. Ensure that policies and procedures are developed to monitor all phases of collection, testing, and reporting by testing personnel make sure that results are reported promptly and accurately. Identify needs for remedial training and continuing education to improve skills.

20. Ensure that approved procedure manuals are available to Testing Personnel covering each aspect of the testing process.

21. Specify responsibilities and duties of Clinical Consultant, Technical Supervisors, General Supervisors, and Testing Personnel engaged in any aspect of testing. Specify which procedures each individual is authorized to perform.

22. Ensure that microbiology and serology lab reports are checked by a Senior or Supervising Public Health Microbiologist and that clinical chemistry, hematology, and urinalysis lab reports are checked by a Senior or Supervising Clinical Lab Technologist prior to sending out reports.

23. Approve all new procedures or modifications of procedures (except typographical errors) by signing name and date at the time they are implemented. Document discontinuation of procedures by signing name and date at the time they are discontinued.

24. Review and amend if necessary the Quality Assurance Manual and Program for Water Microbiology annually or whenever there are changes in methods or lab equipment employed, in the laboratory structure or physical arrangements, or changes in the laboratory organization.

QA OBJECTIVES FOR MEASUREMENT DATA

Quality Control/Quality Assurance Procedures

Media and reagents must be checked and found satisfactory prior to routine use. For media, check for sterility and also determine that it supports the growth of desired organisms and gives correct indicator reactions. Specific information on QC procedures for the various types of media is given in the various procedures in which they are used in this manual. Information on shelf life is given throughout the procedures also. If shelf life is not listed, assume the medium is to be prepared fresh each time it is needed.

Generally all QC results are to be recorded in the QC log book of Media, Reagent and Stain Preparation Record Sheets. pH meter checks with standard buffers are recorded on reverse of Media preparation log sheets.

QC Procedure for Purified Laboratory Water

Specific procedures for the tests listed below are found in the following sections of this manual.

Test	Frequency	Limits
Conductivity of purified water	daily	<1.0 umhos/cm (State limit; EPA limit <2.0)

(NOTE: Resistivity of deionized water is checked daily. Resistivity must be greater than 0.5 megohms.)

SPC	Monthly	<500 CFU/mL
Total Chlorine	Daily	≤0.1 mg/L
pH	Daily	5.5-7.5
Heavy Metals	Annually	<1.0 mg/L
Bacteriologic quality of laboratory water test	Annually or when conditions change	ratio of 0.8-1.2
The Glassware Inhibitory Residues test	Annually or when conditions change	

Routine tests performed on laboratory reagent water

- SPC (standard plate count) is performed monthly with both 0.1 and 1.0 mL of sample as described in section 8. Acceptable results are <1,000 cfu/mL. When unacceptable counts occur the microbiologists are to define the problem.

- Resistivity (reciprocal of conductivity) is checked and recorded daily on the form in the Media Section of the laboratory using the in-line resistivity meter. Acceptable results are $\geq$ 0.5 megohm. Conductivity is also determined using a hand-held meter and the results are recorded on the same form used for recording pH meter and analytical balance calibration.

- pH is checked daily after calibrating the pH meter with pH 7.0 buffer. The meter is also calibrated each Monday with both pH 4.0 and 10.0 buffers. Immerse the pH electrode approximately one inch deep in the container of water, avoiding the sides and bottom. Acceptable results are 5.5-7.5.

- Total chlorine is checked daily using the HACH reagents and a meter. Acceptable results are $\leq$ 0.1 mg/L chlorine.

- Hardness of water is checked daily using the HACH Hardness Test Kit model 5B. Instructions are listed on the kit and under Calibration Procedures in this manual.

All QC is recorded in the Water System QC Notebook kept in the Water Room. If any QC parameters are out of range they should be rechecked. If still out of range, the Supervising PH Microbiologist should be notified. The results are to be checked by the Senior Laboratory Assistant and Supervisor.

Reference:

1. Standard Methods for Examination of Water and Wastewater, 18th Edition, APHA.

Sterility Check Procedure for Water Specimen Collection Containers

All containers used for bacteriological sampling:

One sample cup or vial from each new lot is checked for sterility by adding 100 mL of tryptic soy broth (TSB) and incubating at 35°C for 48 hours. If growth is observed, containers are unsuitable and should not be used. The accuracy of the 100 mL mark on the Colilert containers is verified at the same time by checking the level of TSB. Record results on the water media QC sheet located in the Water Section QC notebook. Notify the Senior or Supervising Microbiologist if results are unacceptable.

Sterility testing of buffered dilution water:

In addition to recording the pH of each batch of dilution water, each batch needs to be tested for sterility. This is done by adding 20 mL of dilution buffer to 100 ml TSB and incubating for 48 hours at 35ºC. If growth is present the batch should be rejected and re-sterilized.

Tryptic soy broth QC:

Tryptic soy broth is prepared by the media section and placed into a 100 mL bottle and kept in the media refrigerator. Additional TSB is dispensed into four 16x125mL tubes. The tubes are inoculated with the following organisms: *E. coli*, *E. aerogenes*, and *P. aeruginosa*. One tube is uninoculated as a sterility check. The tubes are incubated for 48 hours at 35ºC. A positive reading is growth or turbidity. A negative reading is no growth or clear.

90 mL DI water bottles:

Check for sterility by adding 10-20 mL of the DI water from a bottle to 100 mL of tryptic soy broth. Incubate or 48 hours at 35ºC. A positive reading is growth or turbidity. A negative reading is no growth or clear.

IDEXX:

Sterility check for sample containers from IDEXX are checked per each lot and recorded on the Water Media QC Form. At least one bottle from each lot of sample containers is tested for sterility by adding 25 mL of a sterile Tryptic Soy Broth (TSB) to a bottle, incubating it at 35 $\pm$ 0.5ºC for 24 hours and checking for growth.

All forms are reviewed monthly by the Supervising Microbiologist. Any corrective actions performed because of QC failures or values "out-of-range" should be recorded on these forms and the Senior or Supervising microbiologist informed.

SAMPLING PROCEDURES

Water Sample Collection Procedure

GENERAL INFORMATION

Drinking water samples may be submitted to and received by the Public Health Laboratory for testing on Monday through Wednesday. Other water samples should be submitted Monday and Tuesday. When samples need to be tested on other days, please consult with the Laboratory before submitting samples. Normal lab hours are Monday through Friday from 8:00 AM to 5:00 PM.

When samples other than potable water or sea water are submitted consult with the lab in advance to specify what upper endpoints are anticipated and needed. Routine sea water testing has an upper endpoint of $\geq$ 16,000 organisms/100 mL while potable water is tested by the Presence/Absence method and no MPN is given.

Two types of containers are provided for collecting water samples. Choose the correct type for collecting the sample:

1. Rigid CLEAR Plastic screw-cap bottles with 100 mL mark for potable water samples only. These bottles are sterile and contain sodium thiosulfate powder which is sufficient to dechlorinate 125 mL of water containing 10 mg/liter (or 10 PPM) of chlorine. Each bottle is pre-numbered on the lid. Bottles MUST contain at least 100 mL or the specimen will be unsatisfactory for testing.

2. Rigid, sterile, OPAQUE plastic screw cap bottles for samples such as salt water, streams, lakes, sewage, seepage, and other non-potable waters. These bottles contain 30 mg of sodium thiosulfate which is sufficient to dechlorinate 125 mL of water containing 45 mg/liter (or 45 PPM) of chlorine. Each bottle is pre-numbered with a number on the side.

Equipment and supplies needed for water sample collection:

1. Sample collection containers (see above for appropriate type)
2. Water-Bacteriology lab forms (DHS Lab 10)
3. Pen with waterproof ink
4. Cardboard or metal screw cap mailing containers for potable samples.
5. Ice chest with ice pack(s)
6. Rubber bands

COLLECTION AND LABELING

1. PROCEDURE FOR POTABLE WATER COLLECTION:

   a. Allow water to run from tap or pump from well at least two minutes before sampling. Do not collect samples from leaking faucets, swing faucets or faucets with aerators or screens. Do not rinse or empty the chlorine neutralizing thiosulfate powder out of the container.

   b. While waiting for the water to run for two minutes:
      Completely fill out Water Bacteriology form with waterproof pen. Be sure to write the bottle number in the space provided on the form.

      Include the following information:
      - Date of collection
      - Time of collection
      - Sample identification.
      - Client name(s)
      - Type of sample
      - Name of sample collector

   c. Reduce flow and fill container from running faucet to at least the 100 mL line. Please do not overfill; overfilled bottles cannot be properly mixed prior to testing. Leave a small airspace of approximately 1/4 inch at the top of the bottle.

      NOTE: At least 100 mL is needed for testing. If less than 100 mL is submitted, the specimen will be rejected as "Unsatisfactory, sample less than 100 mL.".

   d. Screw cap firmly on bottle.

2. PROCEDURE FOR NON-POTABLE WATER COLLECTION:

   a. Complete lab form (DHS Lab 10). Write the bottle number in the space provided.

   b. Remove cap from opaque screw cap bottle. Taking care not to place fingers into bottle, grasp bottom portion of bottle and plunge the bottle mouth down into the water to avoid introducing surface scum. Position the mouth of the bottle into the current, if any, and away from the hand of the collector. Tip the bottle slightly upward to allow air to exit and the bottle to fill.

   c. Beach and bay marine waters being tested in conjunction with AB411 regulations are to be collected in ankle- to knee-deep water, approximately 4-24 inches below the water surface. If wave conditions are unsafe, sampling should be as close to the 4-24 inch depth and to the sampling station as can be safely accomplished.

   d. Do not fill the bottle to the brim. Leave a small airspace of approximately 1/4 to 1/2 inch at the top of the bottle to allow mixing of the sample in the laboratory.

   e. Screw cap firmly on bottle.

PACKAGING AND DELIVERY

1. For potable samples, place the bottle in a mailer. Mailers have space for one plastic container. Screw lid on mailer. Wrap lab form around mailer and secure with a rubber band.

2. For non-potable samples, keep lab forms in a clean dry place - not in ice chest.

3. Place all samples and mailers with samples into an ice chest with ice packs.

4. Send specimens to the lab via department courier or deliver directly to the lab office. Always keep specimens refrigerated. If you are using the courier to deliver your specimens, it is your responsibility to make sure the specimens will be received by the laboratory within the time limits listed below. Maximum allowable transit times for specimens (time of collection to time received by the lab) are as follows:

    a. Potable water samples - less than 24 hours

    b. Surface and waste waters - less than 6 hours

Specimens received in the lab after these maximum times will be reported as "Unsatisfactory: Sample received >24 hours (or 6 hours) after collection. Please resample."

SAMPLE CUSTODY, HOLDING AND DISPOSAL

General Instructions for Handling of Water Samples for Microbiological Analysis

1. Review of sample and related paperwork:

   a. All log entries, worksheets and results must be recorded in ink. If corrections are made, the old entry should be lined out and the new entry (result) initialed.

   b. Check to make sure the lab form is completed and matches the specimen submitted with it. The following information should be logged into the computer:
      1. Dates of collection, receipt and analysis
      2. Time of collection, receipt and analysis
      3. Sample identification
      4. Client name(s)

   c. If anything appears to be out of order or improperly labeled, consult with the Supervising P.H. Microbiologist for directions on how to proceed. Potable samples >24 hours old are reported as "Unsatisfactory--sample received >24 hours after collection." Surface and waste water samples >6 hours old are reported as "Unsatisfactory - Sample received >6 hours after collection"

   d. Routine drinking water samples should be received no later than Thursday. If one is received after Thursday, consult with the supervisor. Water samples should be refrigerated upon receipt in the laboratory and processed within 2 hours.

   e. Check if the sample is marked "treated". Treated samples should have a chlorine reading indicated on the lab form. At least 10% of such treated specimens are to be checked in the lab for total residual chlorine, using the Hach DPD total chlorine test kit, to insure the chlorine in the sample has been inactivated. Results of the test are written in the Treated Drinking Water Log. Results are recorded in the "remarks" field on the slip and in the computer. Any samples showing presence of chlorine are to be reported out as "Unsatisfactory - Residual chlorine present. Please resample. Be sure sodium thiosulfate is in the sample container." Retest resamples of above to be sure chlorine has been inactivated in the resample.

2. Time- and date-stamp the lab form and initial by the stamp when the samples are received in the lab. Enter the sample into STARLIMS and place a barcode label on the sample and on the upper right corner of the form.

   Record the time actually set-up and the dates and times read and your initials along the left side of the slip.

4. Use back-up log form if unable to enter into STARLIMS. Record test results on front of form accompanying specimen or on reverse of file copy. For all drinking water samples, mark coliforms "Present" or coliforms "absent" and *E. coli* "present" or *E. coli* "absent". Also mark slips as "Pass" if no coliforms present, or "Fail" if coliforms detected. Mark non-potable water exams with the MPN value from the chart. Complete report forms when

testing is finished. Report plate counts using two significant figures. Discard culture tubes in baskets and plates in autoclave bags; both these items are autoclaved.

5. Record all results in the computer and submit reports to Supervising P.H. Microbiologist for review.

6. When tests indicate presence of coliforms in drinking water proceed as follows, then note on lab form the person notified, the date and time:

   a. Exams completed during normal working hours: phone or fax results to an environmental health specialist in the Environmental Health Office, or phone results to other appropriate responsible party. A log of all reports will be kept indicating the date, time and person or organization notified.

   b. At times other than normal Monday-Friday working hours (evenings, weekends, etc.): Phone only final positive drinking water reports on public water supplies when results show coliforms present. Contact Hazardous Materials Management Specialist on call (see weekend and emergency telephone number list) or other responsible party.

7. Positive drinking water tests are to be repeat tested with a freshly collected water sample. Collection is routinely done by the environmental health specialists.

8. When testing is completed, all samples and culture tubes are autoclaved before disposal.

   Recreational (bathing beach) water samples with results >10,000 MPN/100mL for total coliforms, >400 MPN/100mL for fecal coliforms, and >104 MPN for 1:10 dilution for enterococcus must be reported immediately by phone to the Department of Environmental Health (338-2386).

   Contact persons at Environmental Health for questions, discussions, or problems concerning water samples:

   Drinking waters: All positive public drinking waters should be phoned to:
   Peter Neubauer (858) 694-3113 or
   Wendy Martinez (858) 694-2242
   All positive private drinking waters should be phoned to:
   Duty Sanitarian - Ruffin Road Office
   (858) 694-2949
   (M.S. 0-564)
   Fax: 858-694-3105 or
   Lina (858) 565-5173 main number or
   (858) 694-3148 voice mail (Try this number first).
   This message must be given to a person and not left on voice mail.

   Beach and Bay waters:
   (858) 495-5579
   Fax 858-694-3670

PHONE NUMBERS FOR KEY STAFF AND AGENCIES ON EVENINGS AND WEEKENDS:

Refer to Weekend Worker's information binder in the laboratory office for contact information

CALIBRATION PROCEDURES AND FREQUENCY

ROUTINE TESTS FOR PURIFIED LABORATORY WATER

1. SPC (standard plate count) is performed monthly with both 0.1 and 1.0 mL of sample as described under Analytical Procedures in this manual. Acceptable results are <1,000 cfu/mL. When unacceptable counts occur the microbiologists are to define the problem.

2. Resistivity (reciprocal of conductivity) is checked and recorded daily in the Water Purification system room of the laboratory with an in-line resistivity meter. Acceptable results are $\geq$ 0.5 megohm. Conductivity is determined using a hand-held meter and results are recorded on the same form as used for recording pH meter and analytical balance calibration checks.

3. pH is checked monthly after calibrating the pH meter with pH 7.0 buffer. The meter is also calibrated each Monday with both pH 4.0 and 10.0 buffers. Immerse the pH electrode approximately one inch deep in the container of water, avoiding the sides and bottom. Acceptable results are 5.5-7.5.

4. Total chlorine is checked daily using the HACH reagents and comparator wheel. Acceptable results are $\leq$ 0.1 mg/L chlorine.

5. Hardness of water is checked daily using the HACH Hardness Test Kit model 5B. Instructions are listed on the kit and can be found in the Boiler Room Book. The HACH Kit and Boiler Room books are kept in the Water Room.

Except as noted, all QC is recorded in the Water System QC Notebook kept in the Water Purification System room. If any QC parameters are out of range, they should be rechecked. If still out of range, the Supervising PH Microbiologist should be notified.

See “Internal Quality Control Checks and Corrective Actions” section for procedures on performing conductivity and pH meter calibration.

Reference:

Standard Methods for Examination of Water and Wastewater, 18th Edition, APHA.

ANNUAL TESTS FOR PURIFIED LABORATORY WATER

These tests are performed once a year:

Trace Metals testing is performed on our purified water on a complimentary basis by the City of San Diego Water Production Laboratory (5530 Kiowa Dr, La Mesa, CA 91942). Call Dana Chapin, Chemistry section at (619) 668-3237 around May 1st and request permission to send a sample of our purified water for annual heavy metal testing. State regulations require that water be tested for the following metals: Cd, Cr, Cu, Ni, Pb, and Zn. Limits: Cd, Cr, Cu, Ni, Pb, Zn – each <0.05 mg/L – Total $\leq$ 0.1 mg/L. Obtain a container from the Water Utilities lab. Fill the container with our laboratory water and return it to them. For sample analysis requested, include Date of collection, Time of collection and sample Identification. Request a written report and leave our laboratory's card with name, address, and phone number.

Bacteriologic Quality of Laboratory Water – The source of reference water is The San Diego City Water Production Laboratory, 2392 McCain Road. The contact person is Laila Outman (619) 758-2312. Always provide clean containers to hold 4 liters of purified water. To perform the test prepare reagents and follow instructions on the following pages.

Other phone numbers are
General Plant (619) 668-2010
Rick Amador (619) 758-2311

TOTAL CHLORINE - HACH TEST KIT

This test is performed daily on the water used in the Public Health Laboratory to establish the Total chlorine content.

Materials:

1. DPD Total Chlorine Reagent Powder Pillows
2. Clippers
3. Color Comparator
4. Color Viewing Tube
5. DPD Chlorine Color Disc, 0-3.5 mg/L
6. Stopper for Color Viewing Tube

Procedure:

1. Fill a color viewing tube to the 5 mL mark with the water to be tested.

2. Fill the other viewing tube to the 5-mL mark with the water to be tested.

3. Use the clippers to open one DPD Total Chlorine Reagent Powder Pillow. Add the contents of the pillow to the sample to be tested. Swirl to mix. Let stand for three minutes, but not more than six minutes. Place the sample tube in the right top opening of the comparator.

4. Hold the comparator up to a light source and view through the opening in front. Rotate the disc until a color match is obtained. Read the mg/L total chlorine (Cl_2) through the scale window.

5. Record the results in the daily water Q.C. notebook. Notify Supervising or Senior Microbiologist if chlorine is detected.

NOTE: If chlorine is detected the Supervising or Senior Microbiologist will call for service from U.S. Filter. Reagents are also ordered from U.S. Filter.

WARNING: The chemicals in the kit may be hazardous to your health if used inappropriately. Please follow the procedure using appropriate safety precautions.

Reference:

HACH Water, Free and Total Chlorine Test Kit, Model CN66/66F/66T, Cat. #22431.

HACH WATER HARDNESS TEST PROCEDURE

This test is performed daily to establish the hardness level of the water used in the Public Health Laboratory. Below are the procedure and the materials required to perform this test:

Materials:

1. Titrant Solution, Hardness 3 (4 oz. dropper bottle)
2. UniVer III Hardness Reagent (1 oz.)
3. 1- 4 oz Mixing Bottle
4. Measuring Spoon 0.1 g
5. Plastic Measuring Tube

Procedure:

1. Fill the plastic measuring test tube to the top with the water to be tested and pour into mixing bottle.

2. Add one level measuring spoonful of UniVer III Hardness Reagent into the mixing bottle.

3. Using the dropper provided, add Titrant solution, Hardness 3 to the mixing bottle a drop at a time. Swirl the bottle and count the drops of Titrant added until the color changes from pink to blue.

4. The total hardness of the water in grains per gallon, as calcium carbonate is equal to the number of drops of titrant used to produce the color change. Rinse out mixing bottle well with DI water after use.

NOTE: If one drop produces a color change the actual hardness may be less than 1 gr./gal.

5. Record the reading obtained in the daily water system Q.C. book: The laboratories reagent water normally starts out blue, indicating <1 gr./gal. Hardness.
   Acceptable range = 0-2 gr./gal.
   If > 2 gr./gal. Is obtained, notify Supervising or Senior Microbiologist.

Note: The Supervising or Senior Microbiologist will call for service if any problem occurs with the test.

Warning: The chemicals in this kit may be hazardous to your health. Please follow the procedure using appropriate safety precautions.

References:

1. HACH Water Hardness Test Kit Procedure, Model 5B, Cat. #1453-00
2. HACH Chemical Company, Ames Iowa, USA. 4/94

TEST OF BACTERIOLOGIC QUALITY OF LABORATORY WATER

To determine the bacteriological quality of laboratory reagent water the growth of *Enterobacter aerogenes* in a defined minimal growth medium prepared with laboratory reagent water is compared to the growth in the same medium but prepared with high quality reference control water. The presence of a toxic agent or a growth-promoting substance in the lab reagent water will alter the 24 hour population by an increase or decrease of 20% or more when compared to a control water. This test is performed annually, when the source of laboratory reagent water changes and when an analytical problem occurs.

The test is complex and requires skill, patience, and 4 days to complete. The following procedure is a modification in the amounts of reagents prepared in the Standards Methods test for bacteriological quality of reagent water (17^{th} Edition, 1989).

Preparation:

All glassware used for reagents and testing in this test should be very clean. To clean glassware, chromic acid cleaner must not be used. If glassware needs thorough cleaning, use 10% nitric acid followed by 10 distilled water rinses. All clean glassware should be dry sterilized before use.

A. Sodium citrate solution: Dissolve 0.029 gm of $Na_3C_6H_5O_7 .2H_2O$ in 50 mL reference distilled water in a 100 mL bottle. Loosen cap and place in boiling bath 1-2 minutes.

B. Ammonium sulfate solution: Dissolve 0.060 gm $(NH_4)_2SO_4$ in 50 mL reference distilled water in a 100 mL bottle. Heat in bath as above for 1-2 minutes.

C. Salt mixture solution: Always make up day of test. Have media section add reagents only to a sterile 125 mL flask as follows:

   0.026 gm $MgSO_4.7H_2O$
   0.017 gm $CaCl_2.2H_2O$
   0.023 gm $FeSO_4.7H_2O$
   0.250 gm NaCl

   Microbiologist performing test will add 50 mL of reference water, mix and boil for 1-2 minutes. Discard after 1 day old. Ferric chloride which is toxic for test may precipitate out.

D. PHOSPHATE BUFFER SOLUTION
Note: Two buffer solutions are made from stock buffer below:

STOCK BUFFER: Stock buffer is prepared by dissolving 3.4 gm KH_2PO_4 in 50 mL reference distilled water, then adjusting to pH 7.2 + 0.5 with 1.0 N NaOH, diluting to 100 mL with reference distilled water, and autoclaving 15 minutes at 121ºC. Buffer is stored in the prep refrigerator.

D-1 Phosphate buffered dilution water: Add to a 250 mL flask or beaker with a magnetic stir bar 0.25 mL stock buffer, 1.0 mL of $MgCl_2$ (8.1 gm/100 mL) solution, and 198.8 mL of reference water. Stir on magnetic stirrer to mix thoroughly. Dispense 9.2 mL quantities in 18 16X125 mm screw cap tubes. Autoclave the tubes and bottles of buffered water 15 minutes at 121ºC. At the same time, autoclave 12 empty tubes also.

D-2 Phosphate buffer solution: Mix 2.0 mL of stock phosphate buffer (see bottom of previous page) with 48 mL of fresh boiled (1-2 minutes) reference water in a 100 mL bottle. **Discard if a precipitate forms and make a fresh dilution (1:25 dilution of stock buffer).**

E. Test Water: Our laboratory purified water. Note: Use fresh water and store in refrigerator if not ready for test. Older water may grow bacteria and be more toxic for testing. Boil water for 1-2 minutes

F. Reference Water: Note: Dry sterilize four 125 mL Erlenmeyer flasks (bottles) capped with aluminum foil (caps). Use fresh (refrigerated) double distilled water. Need about 4 liters. Reference and test water: On the day of testing, place 150 mL of each of these waters in separate 250 mL flasks. Cover with foil and boil 1-2 minutes.

G. Standard plate count agar: Order standard plate count agar - 600 mL per water tested. Add 1200 mL reference distilled water to a 2 liter flask with a magnetic stir bar. Next add 28.2 gm of powdered standard plate agar medium. Stir and heat on a hot plate mixer until medium is completely dissolved and well mixed. Dispense approximately 100 mL in each of 12 milk dilution bottles. Autoclave 15 minutes at 121ºC.

Day 1:

Use *E. aerogenes* EPA 10658. Inoculate a fresh 6.0 mL TSB tube using a water loop (3-4 mm) from a previous TSB culture. Incubate 35ºC ± 0.5 for 24 ± 2 hours.

Day 2:

A. Prepare fresh solutions A and B in sterile 125 mL flasks (bottles) according to Table 1 (Standard Methods, page 9-5, 18th edition). Be sure to boil test and reference water 1-2 minutes before using. Make 2 sets if desired. Add additional flasks (C, D, etc.) if additional batches of water need to be tested.

TABLE 1

Media - Reagents	Flask A (mL)	Flask B (mL)
A. Sodium citrate solution	2.5	2.5
B. Ammonium sulfate solution	2.5	2.5
C. Salt-mixture solution	2.5	2.5
D-2. Phosphate buffer (7.3 ± 0.1)	1.5	1.5
E. Test water (our lab)	---	21.0
F. Reference water	21.0	---
TOTAL VOLUME	30.0	30.0

B. Prepare dilutions of *E. aerogenes* (24 hour growth) as follows:

1. Match a 0.5 McFarland standard in a 5.0 mL buffered TSB broth using 4-5 drops of *E. aerogenes* 18 hour broth. Inoculum is equivalent to 10^8 organisms/mL. Standardize inoculum to between 80 and 85 %T on a spectrophotometer set at 530 nm.

2. Using a one mL pipette, transfer 0.1 mL to a 9.9 mL buffered water blank (first dilution tube). Mix well.

3. Transfer 0.1 mL of the first dilution tube to another 9.9 mL buffered water blank. Mix well.

4. Transfer 1.0 mL of the second dilution tube to a 9.0 mL buffered water blank (third dilution tube) to make a 10^{-5} dilution.

C. Using 3 sets of A and B flasks add the following inocula:

Flask A1 and B1: 0.75 mL of the third dilution from above.
Flask A2 and B2: 1.5 mL of the third dilution from above.
Flask A3 and B3: 3.0 mL of the third dilution from above.

D. Set up original plate counts:

1. Inoculate triplicate plates with 1 mL of flask A and B — 18 plates
2. 1 each of 1 mL of reagents A, B, C, D — 4 plates

3. 1 of 1 mL of water blank — 1 plate
4. 1 agar control plate — 1 plate
5. 1 each of 1 mL of reference water and lab water — 2 plates

26 TOTAL

E. Incubate flasks A and B and plate counts at 35ºC ± 0.5 for 24 ± 2 hours.

Day 3

A. Count colonies on all plates.

1. Select the set of flasks (A and B) which have counts between 30-80 colonies/mL for further testing.

2. All reagents and controls should have no colonies.

NOTE: If plate counts do not fit these numbers, re-evaluate and repeat test.

B. Using the flasks A and B that have between 30-80 colonies, make 10-fold serial dilutions* from 10^{-1} to 10^{-4} and plate as indicated below:

1. 1 plate each of 1 mL and 0.1 mL — 4 plates
2. 3 plates each of 0.01 mL, 0.001 mL, and 0.0001 mL — 18 plates
3. 1 plate each for blank and agar control — 2 plates

24 TOTAL

Incubate at 35ºC for 24 ± 2 hours.

*NOTE: It is important to have exactly 9.0 mL in each dilution tube.

Day 4

A. Select a dilution of flasks A and B that shows 30-300 colonies in each of the 3 plates. Average the triplicate counts for each flask and apply the proper dilution factor to obtain the count per mL. (It is better to repeat the test if the count is over 300 in the 0.0001 dilution; otherwise refer to Standard Methods for estimated plate count rules).

B. Calculate the ratio as follows:

$$\frac{\text{Colony count/mL flask B}}{\text{Colony count/mL flask A}}$$

A ratio of 0.8 to 1.2 (inclusive) shows no toxic substances.
A ratio of less than 0.8 shows growth-inhibiting substances in the laboratory water.

A ratio greater than 1.2 indicates growth promoting substances in the laboratory water, but growth promoting substances may be ignored if the ratio is less than 3.0.

References:

1. California State Department of Health
Sanitation and Radiation Laboratory
2151 Berkeley Way
Berkeley, California 94704

2. Standard Methods for the Examination of Water and Wastewater, 18th edition, APHA 1992.

TEST FOR INHIBITORY RESIDUES ON GLASSWARE AND PLASTICWARE

INTRODUCTION

Certain wetting agents or detergents used in washing glassware may contain bacteriostatic or inhibiting substances requiring 6 to 12 rinsings to remove all traces and insure freedom from residual bacteriostatic action. This test should be done annually.

Day 1

1. Wash and rinse six glass Petri dishes according to usual laboratory procedure and designate as group A.

2. Wash six glass Petri dishes as above, rinsing 12 times with successive portions of distilled and/or deionized water and designate as group B.

3. Wash six glass Petri dishes with the detergent water in normal use concentration, dry without rinsing, and designate as group C.

4. Dry sterilize the glass dishes in groups A, B, and C at 170ºC for two hours.

5. Use six pre-sterilized, plastic Petri dishes and designate as group D.

Day 2

1. Prepare and sterilize the Plate count agar. Cool to about 45ºC.

2. Prepare dilutions of *E. aerogenes* (24 hour growth) as follows:

    a. Match a 0.5 McFarland standard in a 5.0 mL buffered TSB broth using 4-5 drops of *E. aerogenes* 18 hour broth. Inoculum is equivalent to 10^8 organisms/mL. Standardize inoculum to between 80 and 85 %T on a spectrophotometer set at 530 nm.

    b. Using a Pasteur pipette, transfer 0.1 mL to a 9.9 mL buffered water blank. Mix well.

    c. Transfer 0.1 mL of the first tube to another 9.9 mL buffered water blank. Mix well.

    d. Transfer 1.0 mL of the second dilution to a 9.0 mL buffered water blank.

3. Add 1 mL from the dilution made in step d, above, into each of 3 dishes in groups A, B, C and D. Add 0.1 mL into each of 3 dishes in groups A, B, C and D.

4. Add 10-15 mL of agar into each dish and swirl gently in a figure 8 motion to evenly distribute the bacteria. Let the agar solidify on a flat surface. Invert the dishes and incubate them for 48 hours $\pm$ 3 hours at 35ºC. As a sterility check, pour a blank agar plate from each agar flask used.

Day 4

Count the plates of the significant dilutions (30-300 CFU/plate).

CALCULATIONS:

1. B-A
   ----- X 100 = Percent difference between routine rinsed dishes and well rinsed dishes.
   B

2. B-C
   ----- X 100 = Percent difference between unrinsed dishes and well rinsed dishes.
   B

3. B-D
   ----- X 100 = Percent difference between pre-sterilized plastic dishes and well rinsed
   B dishes.

4. A-C
   ----- X 100 = Percent difference between unrinsed dishes and routine rinsed dishes.
   B

INTERPRETATION OF RESULTS:

1. Difference of less than 15% in the average colony counts demonstrates no inhibitory residue left on dishes by routine washing procedure.

2. Difference of 15% in the average colony counts demonstrates inhibitory residue left on dishes by cleaning detergent.

3. Difference of 15% or more in the average colony counts demonstrates no inhibition by pre-sterilized plastic dishes.

4. Disagreement in average colony counts of less than 15% between groups A and B (1) and greater than 15% between groups A and C (4) demonstrates that the cleaning detergent has inhibitory properties that are eliminated during routine washing.

<u>References:</u>

1. California State Department of Health Sanitation and Radiation Laboratory 2151Berkeley Way, Berkeley, California 94704

2. Standard Methods for the Examination of Water and Wastewater, 18th edition. APHA 1992

ANALYTICAL PROCEDURES

MOST PROBABLE NUMBER (MPN) METHOD FOR TOTAL COLIFORM DETERMINATION

General Instructions

A. All lots of media are to be pretested to assure sterility. In addition, individual types of media are to be tested as follows:

LTB - should give a positive presumptive test with *E. coli* and *E. aerogenes*, negative with *P. aeruginosa*.

BGLB - should give a positive confirmed test with *E. coli* and *E. aerogenes*, negative with *P. aeruginosa*.

Levine's EMB agar - should give green sheen colonies with *E. coli* but not with *E. aerogenes*.

Nutrient agar - should support growth of *E. coli* and *E. aerogenes*.

Record all results in QC log book.

B. Shake all samples 25 times in a one-foot arc in approximately seven seconds to mix immediately prior to testing. Dilutions of samples are mixed in a similar fashion.

C. Transfer of growth from presumptive to confirmed is done using sterile 3 mm diameter inoculating loops or sterile applicator sticks. When using applicator sticks, immerse them as deeply as possible into the tube without putting your fingers in -- normally 1-2 inches from the top. Do fecal coliform on all presumptive positive public drinking waters and all resample specimens.

D. EPA requires performance of the completed test on 10% of confirmed positive drinking water samples and on at least one sample quarterly. According to Standard Methods, 18th edition, pages 9-47, the EC broth (for fecal coliforms) incubated at 44.5ºC is considered a completed test. Our lab policy is to inoculate an EC broth on all positive sea waters.

E. Record results on specimen form and initial it. Enter pertinent information in computer.

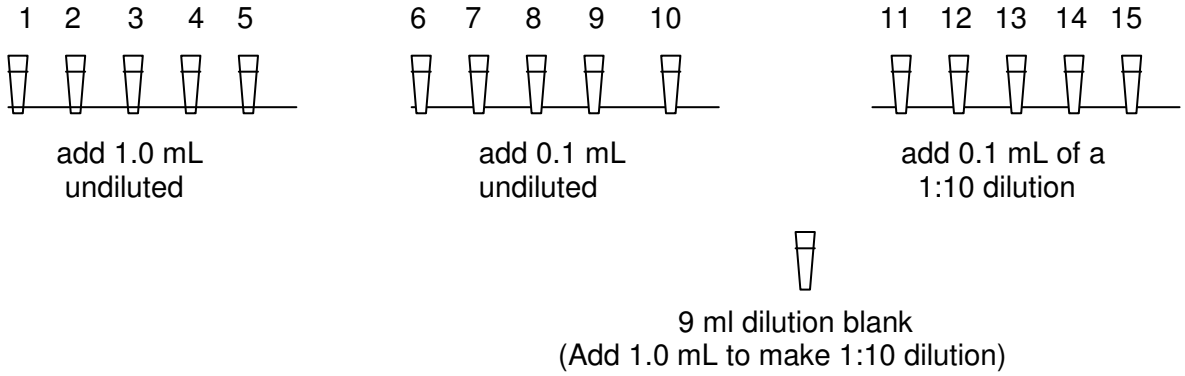
Sea Water Samples

A. 5-5-5 Test

1. This test is performed using 16 X 125 mm loose capped tubes containing 10mL of LTB and similar tubes containing 10 mL of BGLB. The presumptive test using LTB tubes is set up using 15 tubes arranged in three groups of five. Add 1.0 mL of sample to the first group, 0.1 mL to the second group, and 0.01 mL to the third group.

2. First place the specimen number label on the test tube rack adjacent to the row of LTB tubes to be used.

3. Using a 5.0 mL pipette, add 1.0 mL to the first 5 tubes. Switch to a 1.0 mL pipette and add 0.1 mL to the second set of 5 tubes. Using the same 1.0 mL pipette make a 1:10 dilution of the sample in a 9.0 mL water blank. Switch to a new 1.0 mL pipette and add 0.1 mL of this diluted sample to each of the last group of tubes. Add sample to tubes in the order shown (start with 1, 2, 3...) for maximum efficiency (Standard Methods for Examination of Water and Wastewater, 18th Ed. APHA. 1992).



4. Examine tubes for gas production after 24±2 and 48±3 hours. Transfer growth from positive (with gas) tubes to BGLB tubes.

5. Use the 5-5-5 test MPN chart to determine the MPN/100 mL to be reported based on the number of positive BGLB tubes at the various dilutions.

Reference:

1. Standard Methods for the Examination of Water and Waste Water, 17th Edition, 1989, pp 977 to 979. Chart follows.

Sewage Samples

A. 5-Tube 8-Dilution Test (EPA Standard Procedure)

1. This test is performed in a manner similar to the 5-5-5 test for seawater samples except five additional dilutions are used.

2. Add sample to tubes in the following manner:

   Add 1.0 mL of sample to the first 5 tubes. Make a series of 1:10 dilutions of the sample in phosphate buffered water to a 10^{-5} dilution (i.e. -1, -2, -3, -4, -5). Add 1.0 mL to each of 5 tubes corresponding to that dilution.

3. Incubate, transfer to BGLB, and incubate again as described in the 5-5-5 sea water test above.

Determine MPN using the 5-tube MPN test chart. See section 9221 C, page 9-50 of Standard Methods 18th edition for dilutions to use for determination of MPN.

MOST PROBABLE NUMBER METHOD FOR FECAL COLIFORM DETERMINATION

This test is routinely done in conjunction with the MPN total coliform test. The most frequent use in our lab is for monitoring bacterial level in sea water.

1. Perform the presumptive test the LTB as described in the preceding section (MPN Method for Total Coliform Determination) using the desired number of dilutions and replicates of tubes.

2. Any positive LTB tubes (gas in inverted Durham tubes) are subcultured with a sterile loop or applicator stick to individual appropriately labeled EC broth tubes (10 mL each).

3. Set up a positive control EC tube inoculated with *E. coli* and a negative control tube inoculated with *E. aerogenes*.

4. Incubate inoculated EC broth tubes within 30 minutes at 44.5±0.2ºC. for 24±2 hours. Make sure that the water level of the waterbath is as high as the top of the EC medium in the tubes.

5. Shake tubes gently and read for gas production in the inverted Durham tubes as evidence of presence of fecal coliforms. Make sure that the positive and negative controls show correct reactions before recording results of routine specimens. Record control results in the QC log book. Record specimen results on reverse side of first page of the lab form accompanying the specimen.

6. Use the MPN tables to determine the MPN of fecal coliforms for each sample and record results.

MOST PROBABLE NUMBER METHOD FOR MUD, SEDIMENTS AND SLUDGES

The MTF technique is applicable to the analysis of muds, sediments and sludges.

1. Prepare solid or semisolid samples by weighing 25 g of sample and adding 225 mL sterile buffer or 0.1% peptone water in a sterile blender jar. This will be your starting 10^{-1} dilution.

2. Blend at 1-2 minutes at low speed to mix.

3. Pipette 1 mL of the 1:10 dilution into each of 5 LSTB tubes and continue on to prepare further decimal dilution as desired. Prepare further dilutions as quickly as possible to minimize settling.

4. Continue to incubate and read the tubes as per the SOP.

COLILERT PRESENCE/ABSENCE METHOD FOR DRINKING WATER

PRINCIPLE

Colilert reagent contains salts, nitrogen and carbon sources that are specific to total coliforms. Specific indictor nutrients are ONPG (O-Nitrophenyl-B-d-Galactopyranoside) and MUG (4-Methylumbelliferyl-B-d-Glucuronide) for the target organisms: total coliforms and Escherichia coli. As these nutrients are metabolized, yellow color from ONPG and fluorescence from MUG are released, confirming the presence of total coliforms and E. coli. Non-coliform bacteria are suppressed.

PROCEDURE

1. Water samples are received in nonfluorescing, sealed plastic containers with 100 mL volume indication.

2. Number and date samples and lab slips. Any samples that are misidentified, not identified or have less than 100 mL of water are unsatisfactory. If a sample is unsatisfactory, so note in the "remarks" area of the report form and turn out. Check to see if any specimens had a residual chlorine level. If there are any, and there is at least 105 mL, draw off 5 mL for the Hach total chlorine test. Samples containing residual chlorine are unsatisfactory. At least 10% of the treated waters should be tested per month to insure that the chlorine is being inactivated.

3. If a sample volume is greater than 100 mL, draw off excess with the vacuum suction apparatus, using a fresh, sterile MLA tip for each sample.

4. Aseptically add one vial of Colilert reagent to each sample, cap the sample tightly and shake to dissolve the reagent.

5. Incubate the sample/reagent mixtures at 35$\pm$0.5 C for 24-28 hours, then read the tests.

6. If yellow color is present, check for fluorescence. Color should be uniform, if it is not, mix by inversion before reading.

7. Compare each sample to the color comparator, which has been dispensed into an empty, sterile sample container. **If the sample has a yellow color greater or equal to the comparator, the presence of total coliforms is confirmed.** If the sample is yellow, observe it for fluorescence by placing it 5 inches from the U.V. lamp in a darkened environment (the space under the workbench is adequately darkened). **If the fluorescence of the sample is greater or equal to the comparator the presence of E. coli is specifically confirmed.** Results are invalid if there is an indeterminate color or a color other than yellow.

NOTE 1: THE COMPARATOR IS THE LOWEST LEVEL OF YELLOW AND FLUORESCENCE WHICH CAN BE CONSIDERED POSITIVE.

8. Samples are negative for total coliforms if no color is observed at 24 hours.

NOTE 2: DO NOT INCUBATE MORE THAN 28 HOURS. IF SAMPLE IS INADVERTENTLY INCUBATED OVER 28 HOURS: NO YELLOW COLOR IS A VALID NEGATIVE

TEST; A YELLOW COLOR, WITH OR WITHOUT FLUORESCENCE IS NOT A VALID TEST AND MUST BE REPEATED.

9. Mark the lab forms in the results portion of the form. Mark the "Total coliforms present" box if sample is positive for total coliforms (yellow) or "Total coliforms absent" box if sample is negative. If sample fluoresces, mark the "E. coli" present box or the "E. coli absent" box if sample does not fluoresce. Mark the "Pass" box in the "Bacterial test results" section if coliforms are absent and "Fail" if coliforms are present. The Supervising or Senior P. H. Microbiologist must check the vials and slips before being reported.

10. Results are invalid if there is an indeterminate color present or there is an error made. Invalidation should be thoroughly documented and a new sample requested.

11. All positive drinking waters are to be phoned to the Duty Specialist at Ruffin Road 858-565-5173
    Results are not to be left on voice mail, but must be given to a person.

QUALITY CONTROL

Quality control procedure is to be done on each new Colilert lot received.

1. Aseptically empty a tube of Colilert reagent into a sterile vessel containing 100 mL sterile, distilled water (do not use buffered water); mix thoroughly.

2. Aseptically aliquot one-third of the sterile water/Colilert mixture into each of 3 sterile, transparent, non-fluorescent 20 x 200 mm borosilicate glass tubes (old drinking water test tubes).

3. Label the tubes "*E. coli*", "*E. aerogenes*" and "*P. aeruginosa*" respectively.

4. Inoculate each tube with 18-24 hour culture of corresponding organism.

5. Incubate inoculated tubes at 35 $\pm$ 0.5ºC for 24 hours.

6. Valid results:

E.coli	yellow and fluorescent
E. aerogenes	yellow, no fluorescence
Ps. aeruginosa	no color, no fluorescence

   Record QC results in the water QC notebook.

7. If valid results are not obtained, repeat test on additional reagents from the same lot. If, again, valid results are not obtained, notify supervising microbiologist. Idexx should be called: 1-800-321-0207. Drinking water may not be tested with Colilert reagent from a lot which does not pass quality control testing.

8. If there is no Colilert reagent from a valid lot available, the water may be tested by the presence-absence (P-A) test: see Standard Methods, 1989, 17th Edition, 9221 E, pp. 9-80.

Reference: Colilert Product Insert, 6/90

VERIFICATION OF TOTAL COLIFORMS AND Escherichia coli IN COLILERT CULTURES

PRINCIPLE

Five percent of all MUG positive and turbid MUG negative results must be verified by additional non-MUG testing. This verification procedure uses BBL Crystal to identify isolates.

MEDIA AND REAGENTS

1. EMB plates
2. BBL Crystal ID kit

PROCEDURE

1. Subculture one ONPG positive-MUG positive sample per month (approximately 5%) to EMB.

2. Incubate 24 hours at 35ºC.

3. Pick one to five isolated lactose positive colonies with or without a green sheen. Do Gram stain, oxidase, and indol. Inoculate a Crystal if necessary for identification.

4. Incubate for 24 hours at 35ºC. Read and record reactions.

5. Record results in the Colilert verification section of the Water QC notebook.

Reference: Colilert Product Insert, IDEXX Laboratories Inc.

HETEROTROPHIC PLATE COUNT

1. Sterile prepared SPC agar is stored in the refrigerator in 100 mL bottles and may be used for up to two months after date of preparation. Each lot is to be pretested for sterility and ability to support growth of *E. coli* and the results recorded in the QC log book. Melt a 100 mL bottle of SPC agar (tryptone glucose yeast extract agar) in a boiling water bath and place in a 45ºC water bath to allow temperature to stabilize (approximately ten minutes). Use the melted agar within three hours and discard any unused melted agar.

2. All plate counts should be done in duplicate. Label 15 X 100 mm sterile plates with date and identification of sample being tested. For testing distilled water, two dilutions are usually adequate -- one pair labeled 1.0 mL and another pair labeled 0.1 mL. (If necessary, further dilutions may be used. Prepare dilutions in 9.0 or 99 mL sterile dilution blanks.) Also label one plate "sterility". Into the plate labeled "sterility" aseptically pour 12-15 mL agar. Handle the same as the water sample plates. An air control plate must be performed with each run. Expose the air plate for 15 minutes and incubate at 35$\pm$ 0.5ºC for 48 hours. The control plate should have less than 15 colonies. If dilutions of the samples are to be made, also prepare and label a plate to check sterility of 1.0 mL of dilution water.

3. Shake water sample 25 times in a one-foot arc in approximately seven seconds to mix. Pipette 0.1 and 1.0 mL of water samples in appropriate plates. Add 12-15 mL of agar to each plate aseptically. Plates with water samples are swirled to mix 5 times clockwise, 5 times counterclockwise, and 5 times side-to-side to mix.

4. Allow plates to remain at room temperature unstacked for 15 minutes. Invert and incubate all plates at 35$\pm$0.5ºC for 48$\pm$3 hours.

   Plates from commercially bottled drinking water are incubated a total of 72$\pm$4 hours.

5. Count colonies using a Quebec Colony counter. To obtain accurate counts choose plates with 30-300 colonies if possible. Use two significant figures on the final report. Report all counts as CFU/mL, the time and temperature used and the method used. For example: CFU/mL, 35ºC/48 hours, plate count agar.

6. Interpretation: For tests to be valid the medium sterility control plate should show no growth. If used, the dilution water control must also show absence of growth. The water is acceptable for microbiology testing if <1,000 cfu/mL.

Reference:

1. 9215, 921B Heterotrophic Plate Count. In Greenberg, Arnold E. Ed. Standard Methods for the Examination of Water and Wastewater. 18th Ed., 1992 APHA.

TEST METHOD FOR ENTEROCOCCI IN WATER BY MEMBRANE FILTRATION PROCEDURE

The enterococci test measures the bacteriological quality of recreational waters. The significance of finding enterococci is the direct relationship between the density of enterococci in water and swimming-associated gastroenteritis. The MF method provides a direct count of bacteria in water based on the development of colonies on the surface of the membrane filter. A water sample is filtered through a membrane filter that retains the bacteria. The membrane is incubated on mE agar and then Esculin iron agar. Red colonies that have a black halo under them are counted as enterococci.

Media and Reagents

ME agar (BBL)
EIA (BBL)
Store plates at 2-10ºC. Plates have a shelf life 30 days
Buffered dilution water
0.45 μm membrane filters
Filter apparatus

Procedure

1. Mark the Petri dishes and worksheet(s) with sample identification number and sample volumes, usually 100 mL, 3 mL and 1 mL. This will give a countable range from 20 to 6000 enterococci per 100 mL.

2. Place a sterile membrane filter on each of 3 filter bases, grid-side up, and attach the funnels to the bases: the membrane filter is now held between the funnel and the base.

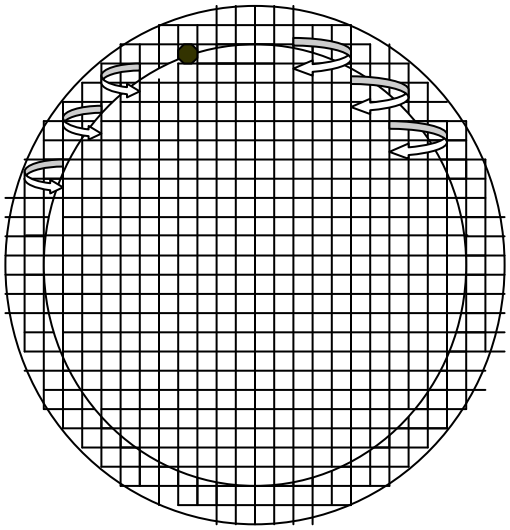
3. Shake the water sample bottle vigorously, about 25 times in seven seconds in a one foot arc, to mix the sample well. Add 10 mL sterile dilution water to the 3 mL and 1 mL funnels and measure the desired volume of sample into the funnels. Turn on the vacuum and filter sample.

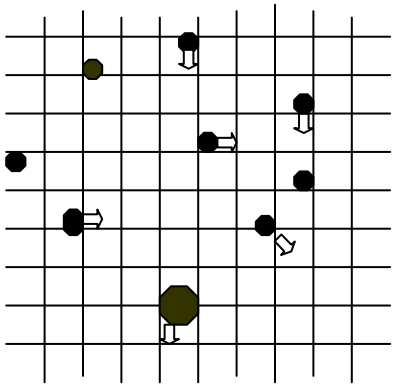
4. Rinse the sides of the funnels three times with 20-30 mL sterile buffered water. Turn off the vacuum and remove the funnels from the filter base.

5. Using sterile (alcohol -flamed) forceps to aseptically remove each membrane filter from the filter base and roll it onto mE agar, avoiding air entrapment. Incubate at 41ºC $\pm$ 0.5ºC for 48 hours in a Tupperware container with a tight-fitting lid.

6. Carefully transfer the membrane(s) with pink to red colonies to EIA medium. Incubate at 41ºC $\pm$ 0.5ºC for 20 minutes.

7. Count pink to red enterococci colonies that have a black or reddish-brown precipitate on the underside of the filter. Count colonies using a fluorescent lamp and a magnifying lens. Count the membranes as shown below:





Calculation of results

Select and count the membranes with ideally 20-60 pink to red colonies with black or reddish-brown precipitate on the underside. Calculate the final value using the formula:

$$\text{Enterococci/100 mL} = \frac{\text{No. of enterococci colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

1. If the counts are zero, calculate the result using the largest volume filtered. For a 100 mL sample report "<1/100 mL".
2. Counts greater than the upper limit of 60, but still countable, use the count from the smallest filtered volume and report as "estimated Count per 100 mL".
3. If all membranes are TNTC, use the upper limit for counts (60) and report ">6000/100 mL".
4. If several membranes have <20 colonies, add the number of colonies together and divide by the total volume filtered. For example, if the counts are 15, 5 and <1 on the 100, 3 and 1 mL membranes, respectively, the count would be 19 per 100 mL.

$$\frac{(15 + 5 + 0) \times 100}{104 \text{ mL}} = 19/100 \text{ mL}$$

Quality Control

1. Each new lot of media should be tested for sterility and positive (*E. faecalis*) and negative (*E. coli*) reactions.
2. After the filtration of 10 samples, or at the end of each series of samples, filter 50 mL sterile dilution water to control for carry-over and reagent/media sterility.
3, Verify one positive sample per month by picking 10 well isolated colonies to two SBA plates. Incubate overnight and test each isolate with a PYR/Esculin disk and by gram stain. Gram-positive cocci that are PYR and esculin positive are confirmed as enterococci.

References:

1. Eaton, A. D., L.S. Clesceri, and A.E. Greenberg, (eds.), 1995. Section 9230C Standard Methods for the Examination of Water and Wastewater. 19th Ed. APHA Washington D.C.
2. Bordner, Robert, J.A. Winter, and P.V. Scarpino (eds.), 1978. Microbiological Methods for Monitoring the Environment: Water and Wastes. USEPA Cincinnati, Ohio.
3. Method 1600: Membrane Filter Test Method for Enterococci in Water. 1997. EPA-821-R-97-004. USEPA.

Insert Enterolert package insert here

ENTEROLERT ENTEROCOCCUS MPN PROCEDURE

PRINCIPLE:

Enterolert reagent is used in this laboratory for the detection of enterococci in marine waters. The reagent uses a nutrient indicator that fluoresces when it is metabolized by enterococci. The test utilizes the 51-well Quanti-Tray and a 1:10 dilution of the sample, which allows for an MPN between 10 and 2,005 MPN/100 mL. For higher-counts, the water samples can be diluted 1:100 and 1:1000. Marine water samples to be tested must be < 6 hours old from time of collection.

MATERIALS:

Enterolert™ (Idexx) reagent snap-packs
90 mL sterile DI water blanks
Quanti-Tray 51-well trays
Quanti-Tray heat sealer

PROCEDURE:

1. Turn on the Quanti-Tray heat sealer. Allow about 10 minutes for it to warm-up. Sealer is ready when the green light is lit on the front of the sealer.

2. Pipette 10 mL of the marine water sample into a bottle containing 90 mL sterile DI water.

3. Separate one snap pack from its strip.

4. Tap the reagent pack to ensure that all of the Enterolert reagent is in the bottom part of the pack. Open the pack by snapping back the top at the scoreline. Do not touch the opening of the pack.

5. Add the reagent to the 1:10 dilution of the sample(s). Mix well by shaking until the reagent is dissolved.

6. Pull back the tab on the tray while squeezing the sides to form an opening. Avoid touching the insides of the foil below the tab. Pour the sample/reagent mixture into a Quanti-Tray.

7. Place the tray in the rubber insert and feed through the heat sealer to seal the tray.

8. Incubate the sample(s) for 24 + 4 hours at 41° $\pm$ 0.5°C. Note the time into the incubator.

9. Read the results at 24 hours by placing a 6-watt, 365nm wavelength UV light within five inches of the tray in a darkened environment. Blue fluorescence indicates the presence of enterococci.

10. Count the number of fluorescent wells and refer to the MPN table provided with the reagents to determine the Most Probable Number of enterococci in the sample. Remember to include the dilution factor (usually 1:10) in the calculation of the MPN. Record the date and time read, and results on the water slip.

Dilution of samples:
For counts >2005/100 mL, the re-sample may be diluted 1:100 and 1:1000.

1. Add 10 mL of the water sample into 90 mL sterile DI water blank for a 1:10 dilution.

2. Add 10 mL of the 1:10 dilution made above to a second 90 mL sterile DI dilution blank for a 1:100 dilution.

3. 1:100 SAMPLE DILUTION: Add 10 mL of the 1:10 dilution made in step 1 to 90 mL sterile DI water blank, add Enterolert reagent, mix to dissolve and pour into QuantiTray. Seal and incubate as per the SOP.

4. 1:1000 SAMPLE DILUTION: Add 10 mL of the 1:100 dilution made in step 2 to 90 mL sterile DI water blank, add Enterolert reagent, mix to dissolve and pour into QuantiTray. Seal and incubate as per the SOP.

PROCEDURE NOTES:

1. Be sure the UV light is facing away from your eyes and towards the tray.
2. If the sample is inadvertently incubated over 28 hours with out observation, the following guidelines apply: Lack of fluorescence after 28 hours is a valid negative. Fluorescence after 28 hours is an invalid test result.
3. Marine water samples must be diluted at least tenfold with sterile DI water.

REPORTING:

Results are entered in the computer and reported by group result sheet once per day. The results are then faxed to DEH.

All results greater than or equal to the following should be called to DEH immediately:
Total coliforms $\geq$ 10,000 MPN /100 mL
Fecal coliforms $\geq$ 400 MPN /100 mL
Enterococcus $\geq$ 104 MPN /100mL

Record the in Water notification log that it was reported.

QUALITY CONTROL/QUALITY ASSURANCE:

The following QC should be performed on each lot of Enterolert reagent.

Organisms used for Enterolert QC:
Enterococcus faecalis ATCC 29212
Enterococcus faecium ATCC 35667
Serratia marcescens ATCC 8100
Aerococcus viridans ATCC 11563

Procedure:

1. Prepare light suspensions of the organisms above by touching one colony with a 1 µl loop and inoculating a tube containing 5 mL sterile DI water.
2. Close the cap and vortex tube.
3. For each bacterial strain, take a 1 µl loop from the test tube suspension and inoculate a vessel containing 100 mL sterile DI water. These are your controls.
4. Follow the Enterolert Enterococcus MPN procedure for inoculation and incubation.
5. Expected results:

Enterococcus faecalis ATCC 29212	Fluorescence
Enterococcus faecium ATCC 35667	Fluorescence
Serratia marcescens ATTCC 8100	No fluorescence
Aerococcus viridans ATCC 11563	No fluorescence

Record results in the Water section QC notebook. Notify the supervisor of QC failures.

Verification of Positive Wells:

Verify at least one positive sample per week by picking up to 10 positive wells and subculturing to SBA. From each culture 5 to 10 colonies should be picked. Perform Gram stain, catalase, BEA, 6.5% NaCl, growth @ 45ºC from SBA, Pyroglutamate aminopeptidase (PYR), and Leucine aminopeptidase (LAP).

Enterococci are catalase negative; BEA, 6.5% NaCl, growth at 45ºC are positive, PYR positive and LAP almost always positive. Record results in QC book.

References:

1. Enterolert product insert. IDEXX Laboratories Inc. 1999.
2. Standard Methods for the Examination of Water and Wastewater. 18th Ed. APHA. Washington D.C. 1992.

Table 51-Well Quanti-Tray MPN table for 1:10 Dilution

Note: Multiply by 10 for 1:100 dilution or 100 for 1:1000 dilution

[illegible]	[illegible]
0	<10
1	10
2	20
3	31
4	42
5	53
6	64
7	75
8	87
9	99
10	111
11	124
12	137
13	150
14	164
15	178
16	192
17	207
18	222
19	238
20	254
21	271
22	288
23	306
24	324
25	344
26	364
27	384
28	406
29	429
30	453
31	478
32	504
33	531
34	560
35	591
36	624
37	659
38	697
39	738
40	782
41	831
42	885
43	945
44	1013
45	1091
46	1184
47	1298
48	1445
49	1652
50	2005
51	>2005

G:\WP51\WATER\Ent_MPN.doc

COLILERT–18 QUANTI-TRAY NUMERATION PROCEDURE FOR SEAWATERS

PRINCIPLE:

Colilert reagent contains salts, nitrogen and carbon sources that are specific to total coliforms. Specific indicator nutrients are ONPG (O-Nitrophenyl-B-d-Galactopyranoside) and MUG (4-Methylumbelliferyl-B-d-Glucuronide) for the target organisms: total coliforms and *Escherichia coli*. As these nutrients are metabolized, yellow color from ONPG and fluorescence from MUG are released, confirming the presence of total coliforms and *E. coli*. Non-coliform bacteria are suppressed. The test utilizes the Quanti-Tray/2000 and a 1:10 dilution of the sample that allows for an MPN between <10 and >24,192 MPN/100 mL. Marine water samples to be tested must be <6 hours old from time of collection. Repeat water samples from a same site must be done by the Multiple Tube Fermentation method.

MATERIALS:

Colilert-18 (IDEXX) reagent snap-packs
90 mL sterile DI water blanks
Quanti-Tray/2000
Quanti-Tray heat sealer

PROCEDURE:

1. Turn on the Quanti-Tray heat sealer. Allow 15 minutes for it to warm-up. Sealer is ready when the green light is lit on the front of the sealer.

2. Pipette 10 mL of the marine water sample into a bottle containing 90 mL sterile DI water.

3. Separate one snap pack from its strip.

4. Tap the reagent pack to ensure that all of the Colilert reagent is in the bottom part of the pack. Open the pack by snapping back the top at the scored line. Do not touch the opening of the pack.

5. Add the reagent pack to the 1:10 dilution of the sample(s). Cap the vessel and shake until dissolved.

6. Pull back the tab on the tray while squeezing the sides to form an opening. Avoid touching the insides of the foil below the tab. Pour the sample/reagent mixture into a Quanti-Tray/2000.

7. Place the tray in the rubber insert and feed through the heat sealer to seal the tray.

8. Place the sealed tray in a 35±0.5ºC incubator for 18 hours (prewarming to 35ºC is not required).

9. Read results according to the Results Interpretation below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain the Most Probable Number.

Results Interpretation:

Appearance	Result
Less yellow than the comparator	Negative for total coliforms and *E. coli*
Yellow ≥ the comparator	Positive for total coliforms
Yellow and fluorescence ≥ the comparator	Positive for *E. coli*

Look for fluorescence with a 6 watt, 365 nm, UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.

Colilert-18 results are definitive at 18-22 hours. In addition, positives for both total coliforms and *E. coli* observed before 18 hours and negatives observed after 22 hours are also valid.

PROCEDURAL NOTES:

1. A sight tinge of yellow may be observed when Colilert-18 is added to the sample.

2. If excess foam caused problems while using Quanti-Tray, you may choose to use IDEXX Antifoam Solution.

3. Colilert-18 can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater* MPN tables should be used to find most Probable Numbers.

4. If a water sample has some background color, compare inoculated Colilert-18 sample with a control blank of the same water sample.

5. Colilert-18 can be used for *E. coli* (but not coliforms) in marine water. Samples must be diluted at least tenfold. Multiply the MPN value by the dilution factor to obtain the proper quantitative result.

6. Use only sterile, non-buffered, oxidant-free water for dilutions.

7. Colilert-18 is a primary water test. Coliliert-18 performance characteristics do not apply to samples altered by any pre-enrichment or concentration.

8. In samples with excess chlorine, a blue flash may be seen when adding Colilert-18. If this is seen, consider sample invalid and discontinue testing.

9. Aseptic technique should always be followed when using Colilert-18. Dispose of in accordance with Good Laboratory Practices.

QUALITY CONTROL PROCEDURES

The following QC should be performed on each lot of Colilert-18 for seawaters:

Organisms used for Colilert-18
Escherichia coli ATCC 25922
Klebsiella pneumoniae ATCC 31488
Pseudomonas aeruginosa ATCC 10145

Procedure:

1. Prepare light suspensions of the organisms above by touching one colony with 1 µL loop and inoculating a tube containing 5 mL sterile DI water.
2. Close the cap and vortex tube.
3. For each bacterial strain, take 1 µL loop from the test tube suspension and inoculate a vessel containing 100 mL sterile DI water. These are your controls.
4. Follow the Colilert-18 procedure for inoculating and incubation.
5. Expected results:

QC organisms	Appearance	Result
Escherichia coli ATCC 25922	Yellow and fluorescence equal or greater than the comparator	Positive for *E.coli*
Klebsiella pneumoniae ATCC 31488	Yellow equal to or greater than the comparator. No fluorescence	Positive for total coliforms
Pseudomonas aeruginosa ATCC 10145	Less yellow than the comparator	Negative for total coliforms and *E.coli*

Record results in the Water Section QC notebook. Notify the supervisor of QC failures.

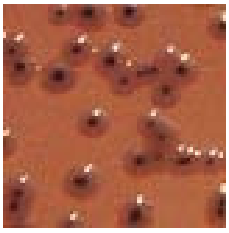
VERIFICATION OF POSITIVE WELLS

Verify at least one positive sample per week by picking up to 10 positive wells and subculturing in SBAP and EMB plates. From each culture from the positive cupules or wells, 5 to 10 colonies should be picked. Perform Gram stain, oxidase, indole and describe the morphology of the colonies on EMB.

E. coli-type organisms are oxidase positive, indole negative and grow on EMB as is described and shown on the following page.

- **Coli-type** colonies are very dark, almost black, when observed directly against the light. By reflected light a green sheen can be seen which is due to the precipitation of methylene blue in the medium from the very high amount of acid produced from fermentation. Those which form this type of colony are methyl red-positive lactose-fermenters such as most strains of *E. coli* and some strains of *Citrobacter*.

- **Aerogenes-type** colonies are less dark. Often a dark center is seen surrounded by a wide, light-colored, mucoid rim – resulting in a "fish-eye" type of colony. Those which form this type of colony are methyl red-negative lactose-fermenters which include most strains of *Klebsiella* and *Enterobacter*.



- **Non-lactose-fermenting** colonies produce no acid from fermentation, so the lighter-colored alkaline reaction is seen.

References:

1. Colilert-18 product insert. IDEXX Laboratories, Inc. 2002.
2. Standard Methods for Examination of water and Wastewater. 18th Ed. APHA. Washington D.C. 1992.
3. John Lindquist. Homepage Pictures–Madison. http//:www.johnlinquist.com

Colilert and Colilert 18 Package Inserts

Colilert: For potable water testing
Colilert-18: For recreational water testing

ACQUISITION, DATA REDUCTION, VALIDATION AND REPORTING

Determining the MPN/100 mL for MTF method

Determine MPN using the 5-tube-3 dilution MPN test chart. The MPN for combinations not appearing in the table, or for other combinations of tubes or dilutions, may be estimated by Thomas' simple formula:

$$\text{MPN/100 mL} = \frac{\text{No. of positive tubes} \times 100}{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}$$

MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE-10 ML. PORTIONS ARE USED

No. of Tubes Giving Positive Reactions out of 5 of 10 mL each	MPN Index Per 100 mL	95% Confidence Limits	
		Lower	Upper
0	<2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.0	3.3	52.9
5	>16.0	8.0	INFINITE

Reference:

Standard Methods for the Examinations of Water and Wastewater 16th edition, 1989, p. 9-77 TABLE 9221:III.

MOST PROBABLE NUMBER (MPN) INDEX FOR FIVE TUBE, THREE DILUTION SERIES

RESULTS (3 Dil Summary)			MPN INDEX PER 100 ml ml OF ORIGINAL SAMPLE USED IN EACH OF 5 TUBES								
Low	Med.	High	10	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
0	0	0			<2	<20	<200	<2,000	<20,000	<200,000	<2.0M
0	0	1			2	20	200	2,000	20,000	200,000	2.0M
0	1	0			2	20	200	2,000	20,000	200,000	2.0M
0	2	0			4	40	400	4,000	40,000	400,000	4.0M
1	0	0			2	20	200	2,000	20,000	200,000	2.0M
1	0	1			4	40	400	4,000	40,000	400,000	4.0M
1	1	0			4	40	400	4,000	40,000	400,000	4.0M
1	1	1			6	60	600	6,000	60,000	600,000	6.0M
1	2	0			6	60	600	6,000	60,000	600,000	6.0M
2	0	0			4	40	400	4,000	40,000	400,000	4.0M
2	0	1			7	70	700	7,000	70,000	700,000	7.0M
2	1	0			7	70	700	7,000	70,000	700,000	7.0M
2	1	1			9	90	900	9,000	90,000	900,000	9.0M
2	2	0			9	90	900	9,000	90,000	900,000	9.0M
2	3	0			12	120	1,200	12,000	120,000	1,200,000	12.0M
3	0	0			8	80	800	8,000	80,000	800,000	8.0M
3	0	1			11	110	1,100	11,000	110,000	1,100,000	11.0M
3	1	0			11	110	1,100	11,000	110,000	1,100,000	11.0M
3	1	1			14	140	1,400	14,000	140,000	1,400,000	14.0M
3	2	0			14	140	1,400	14,000	140,000	1,400,000	14.0M
3	2	1			17	170	1,700	17,000	170,000	1,700,000	17.0M
3	3	0			17	170	1,700	17,000	170,000	1,700,000	17.0M
4	0	0			13	130	1,300	13,000	130,000	1,300,000	13.0M
4	0	1			17	170	1,700	17,000	170,000	1,700,000	17.0M
4	1	0			17	170	1,700	17,000	170,000	1,700,000	17.0M
4	1	1			21	210	2,100	21,000	210,000	2,100,000	21.0M
4	1	2			26	260	2,600	26,000	260,000	2,600,000	26.0M
4	2	0			22	220	2,200	22,000	220,000	2,600,000	22.0M
4	2	1			26	260	2,600	26,000	260,000	2,600,000	26.0M

Standard Methods For the Examination of Water and Wastewater 17th Edition 1989 pp. 9-77 to 9-79

Read 10^{-2} column when determining MPN for tubes with 1, 0.1, and 0.01 ml of sample (sea water testing).

For determining MPN when higher sample dilutions are used (e.g., as with sewage samples); in most cases use the column representing the highest dilution at which positive tubes are seen.

Thomas formula for MPN estimation: $\text{MPN/100 ml} = \dfrac{\text{\# of pos tubes} \times 100}{\sqrt{(\text{ml sample in neg. tubes}) \times (\text{ml sample in all tubes})}}$

MOST PROBABLE NUMBER (MPN) INDEX FOR FIVE TUBE, THREE DILUTION SERIES (Continued)

RESULTS (3 Dil Summary)			MPN INDEX PER 100 ml ml OF ORIGINAL SAMPLE USED IN EACH OF 5 TUBES								
Low	Med.	High	10	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
4	3	0			27	270	2,700	27,000	270,000	2,700,000	27.0M
4	3	1			33	330	3,300	33,000	330,000	3,300,000	33.0M
4	4	0			34	340	3,400	34,000	340,000	3,400,000	34.0M
5	0	0			23	230	2,300	23,000	230,000	2,300,000	23.0M
5	0	1			30	300	3,000	30,000	300,000	3,000,000	30.0M
5	0	2			40	400	4,000	40,000	400,000	4,000,000	40.0M
5	1	0			30	300	3,000	30,000	300,000	3,000,000	30.0M
5	1	1			50	500	5,000	50,000	500,000	5,000,000	50.0M
5	1	2			60	600	6,000	60,000	600,000	6,000,000	60.0M
5	2	0			50	500	5,000	50,000	500,000	5,000,000	50.0M
5	2	1			70	700	7,000	70,000	700,000	7,000,000	70.0M
5	2	2			90	900	9,000	90,000	900,000	9,000,000	90.0M
5	3	0			80	800	8,000	80,000	800,000	8,000,000	80.0M
5	3	1			110	1,100	11,000	110,000	1,100,000	11.0M	110.0M
5	3	2			140	1,400	14,000	140,000	1,400,000	14.0M	140.0M
5	3	3			170	1,700	17,000	170,000	1,700,000	17.0M	170.0M
5	4	0			130	1,300	13,000	130,000	1,300,000	13.0M	130.0M
5	4	1			170	1,700	17,000	170,000	1,700,000	17.0M	170.0M
5	4	2			220	2,200	22,000	220,000	2,200,000	22.0M	220.0M
5	4	3			280	2,800	28,000	280,000	2,800,000	28.0M	280.0M
5	4	4			350	3,500	35,000	350,000	3,500,000	35.0M	350.0M
5	5	0			240	2,400	24,000	240,000	2,400,000	24.0M	240.0M
5	5	1			300	3,000	30,000	300,000	3,000,000	30.0M	300.0M
5	5	2			500	5,000	50,000	500,000	5,000,000	50.0M	500.0M
5	5	3			900	9,000	90,000	900,000	9,000,000	90.0M	900.0M
5	5	4			1,600	16,000	160,000	1,600,000	16.0M	160.0M	1,600.0M
5	5	5			≥1,600	≥16,000	≥160,000	≥1,600,000	≥16.0M	≥160.0M	≥1,600.0M
											M=Million

Standard Methods For the Examination of Water and Wastewater 17th Edition 1989 pp. 9-77 to 9-79

Read 10^{-2} column when determining MPN for tubes with 1, 0.1, and 0.01 ml of sample (sea water testing).

For determining MPN when higher sample dilutions are used (e.g., as with sewage samples); in most cases use the column representing the highest dilution at which positive tubes are seen.

Thomas formula for MPN estimation: $\text{MPN/100 ml} = \dfrac{\text{\# of pos tubes} \times 100}{\sqrt{(\text{ml sample in neg. tubes}) \times (\text{ml sample in all tubes})}}$

4.9 Calculation of MPN Value

The calculated density of the Confirmed or Completed Test may be obtained from the MPN table based on the number of positive tubes and reactions in each dilution.

4.9.1 Table II-C-4 illustrates the MPN indices and 95% Confidence Limits for general use.

4.9.2 Table II-C-5 shows the MPN indices and limits for potable water testing.

4.9.3 Three dilutions are necessary to formulate the MPN code. For example in Table II-C-4 if five 10 mL, five 1.0 mL, and five 0.1 mL portions are used as inocula and positive results are observed in five of the 10 mL inocula, three of the 1.0 mL inocula, and none of the 0.1 mL inocula, the coded results of the test are 5-3-0. The code is located in the MPN Table and the MPN index of 79 per 100 mL is recorded.

4.9.4 When the series of decimal dilutions is other than 10, 1.0 and 0.1 mL, select the MPN value from Table II-C-4 and calculate according to the following formula:

$$\text{MPN (From Table)} \times \frac{10}{\text{Largest Quantity Tested}} = \text{MPN/100 mL}$$

As an example, five out of five 0.01 mL portions, two out of five 0.001 mL portions, and zero out of five 0.0001 ml portions from a sample of water, gave positive reactions. From the code 5-2-0 in MPN Table (Table II-C-4), the MPN index 49 is adjusted for dilutions:

$$49 \text{ (From Table)} \times \frac{10}{0.01} = 49{,}000$$

The final corrected MPN Value = 49,000/100 mL.

4.9.5 If more than the above three sample volumes are inoculated, the three significant dilutions must be determined. The significant dilutions are selected using the following rules:

Only three dilutions are used in the code for calculating an MPN value. Example: From a sample of water, five out of five 10 ml portions, two out of five 1.0 mL portions, and zero out of five 0.1 ml portions gave positive results.

Therefore, MPN/100 mL =

$$\frac{7 \times 100}{(3.5) \times (55.5)} = 50.22$$

(a) 4.10 Reporting Results: Report the MPN value for water samples on the basis of 100 mL of sample. Report the MPN values of solid type samples on the basis of 1 gram of dry weight sample.To obtain the proper three dilutions select the smallest sample volume giving all positive results and the two succeeding lesser sample volumes. See Table II-C-6, Test 1 and 2.

(b) If less than three dilutions show positive tubes, select the three highest sample volumes which will include the dilutions with the positive tubes. See Table II-C-6, Test 3.

(c) If there are positive tubes in the dilutions higher than these dilutions selected, positive results are moved up from these dilutions sample volume to increase the positive tubes in the highest dilution selected. See Table II-C-6, Test 4.

(d) There should be no negative results In higher sample volumes than those chosen. However, if negative tubes are present, e.g., 4/5, 5/5, 3/5, and 0/5 the highest sample volume with all positive tubes must be used along with the next two lower sample volumes. See Table II-C-6, Test 5.

(e) If all tubes are positive, choose the three highest dilutions. See Table II-C-6, Test 6.

(f) If all tubes are negative, choose the Three

(g) If positive tubes skip a dilution, select the highest dilution with positive tubes and the two lower dilutions. See Table II-C-6, Test 8.

(h) If only the middle dilution is positive, select this dilution and one higher and lower dilution. See Table II-C-6, Test 9.

4.9.6 A number of theoretically possible combinations of positive tube results are omitted in Table II-C-4 because the probability of their occurrence is less than 1%. If such unlikely tube combinations occur in more than 1% of samples, review the laboratory procedures for errors and note sample types. Collect fresh samples for analyses.

4.9.7 The MPN can also be computed for each sample based upon the number of positive and negative Presumptive, Confirmed or Completed tests, and the total number of milliliters tested (10). MPN/100 mL =

$$\frac{\text{No. of Positive Tubes} \times 100}{\text{(No. of mL in Neg. Tubes)} \times \text{(No. of ml in all Tubes)}}$$

Reference:

Microbiological Methods of Monitoring the Environment Water and Wastes, EPA. 1978.

TABL. 3-4

Most Probable Number Index and 95% Confidence Limits for Five Tube, Three Dilution Series (8, 9)

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence Limits		No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence Limits	
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each		Lower	Upper	5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each		Lower	Upper
0	0	0	<2			4	2	1	26	9	78
0	0	1	2	<0.5	7	4	3	0	27	9	80
0	1	0	2	<0.5	7	4	3	1	33	11	93
0	2	0	4	<0.5	11	4	4	0	34	12	93
1	0	0	2	<0.5	7	5	0	0	23	7	70
1	0	1	4	<0.5	11	5	0	1	31	11	89
1	1	0	4	<0.5	11	5	0	2	43	15	110
1	1	1	6	<0.5	15	5	0	2	43	15	110
1	2	0	6	<0.5	15	5	1	0	33	11	93
						5	1	1	46	16	120
2	0	0	5	<0.5	13	5	1	2	63	21	150
2	0	1	7	1	17						
2	1	0	7	1	17	5	2	0	49	17	130
2	1	1	9	2	21	5	2	1	70	23	170
2	2	0	9	2	21	5	2	2	94	28	220
2	3	0	12	3	28	5	3	0	79	25	190
						5	3	1	110	31	250
3	0	0	8	1	19	5	3	2	140	37	340
3	0	1	11	2	25	5	3	3	180	44	500
3	1	0	11	2	25	5	4	0	130	35	300
3	1	1	14	4	34	5	4	1	170	43	490
3	2	0	14	4	34	5	4	2	220	57	700
3	2	1	17	5	46	5	4	3	280	90	850
3	3	0	17	5	46	5	4	4	350	120	1,000
4	0	0	13	3	31	5	5	0	240	68	750
4	0	1	17	5	46	5	5	1	350	120	1,000
4	1	0	17	5	46	5	5	2	540	180	1,400
4	1	1	21	7	63	5	5	3	920	300	3,200
4	2	1	26	9	78	5	5	4	1600	640	5,800
4	2	0	22	7	67	5	5	5	≥2400		

TABLE II-C-6

Selection of Coded Results, Five Tube Series

Test	Positive Tubes/ml Sample Volume 10	1.0	0.1	0.01	0.001	0.0001	Code
1	5*	3	0	0	0	0	5-3-0
2	5	5	4	0	0	0	5-4-0
3		4	1	0	0	0	4-1-0
4	5	5	4	1	1	0	5-4-2
5	4	5	3	0	0		5-3-0
6		5	5	5	5	5	5-5-5
7		0	0	0	0	0	0-0-0
8		4	0	2	0	0	4-0-2
9		0	1	0	0	0	0-1-0

*Underlines indicate positive tube series selected for code.

AVAILABILITY AND INTERPRETATION OF RESULTS

Reporting sea water samples (AB411):

Results greater than the following values must be called immediately to the Department of Environmental Health at 338-2386 or 748-2386.

Total coliforms	> 10,000 MPN/100mL
Fecal coliforms	> 400 MPN/100mL
Enterococcus	> 104 MPN/100mL

Record in “Water Notification” log book

- The Colilert method is used for analysis of **potable waters. Results are available within 24 hours** and are expressed as the presence or absence of coliforms and *E. coli*.

- All **other water samples**, including sea waters and sewage, are tested using the multiple tube MPN method and results are expressed as the Most Probable Number. No reports are given before the test has been incubated for the required time of 24 hours $\pm$ 2 hours and 48 hours $\pm$ 3 hours. **Final results are available within 4 days**.

- Environmental health staff is welcome to consult with lab staff regarding interpretation of results when needed.

- All questions from owners or the public concerning samples, results, and interpretations are routinely referred to Environmental Health Services. It is not appropriate to refer these individuals to the lab unless by special arrangement in advance.

REPORTING

The MPN for total and fecal is determined from the MPN charts and recorded on the appropriate line on the front of the laboratory slip. For potable water samples, the slips are marked "coliforms present" or "coliforms absent" and "*E. coli* present" or "*E. coli* absent". Results are entered into the computer and reviewed and initialed by the Senior or Supervising Microbiologist. The slips are date/time stamped out and mailed to the appropriate person.

All completed reports and QC records must be kept for 5 years.

INTERNAL QUALITY CONTROL CHECKS AND CORRECTIVE ACTIONS

MEDIA QUALITY CONTROL/ASSURANCE PROCEDURE OUTLINE

Below is a list of equipment and systems with their required periodic checks or maintenance. Unless otherwise specified these procedures will be performed by assigned Laboratory Assistants under the supervision of the Senior Lab Assistant. All results must be recorded on appropriate quality control logs, reviewed by the Supervising or Senior Microbiologist at the end of each month. Any problems with the quality control checks must be brought to the immediate attention of the Supervising or Senior Public Health Microbiologist for corrective action. All corrective action must be documented.

I. RO/DI WATER SYSTEM Q.C.

A. Daily Checks:

1. Water Hardness Test (See Hach Water Hardness Test Procedure)
2. Free Chlorine Test (See Hach Free Chlorine Test Procedure)
3. Water Pressure at Gauges 3 to 11
4. Primary DI Light
5. UV Light
6. Salt Level Check
7. Storage Tank Level Check
8. Conductivity (hand-held meter)

- RO/DI Water system QC Manual checked monthly. Manual kept in Water Room.

B. Monthly: (Under direction of Senior or Supervising Public Health Microbiologist)

1. pH Check
2. Plate colony count

- pH check and plate colony count recorded in Water QC book in Water section and checked monthly

C. Yearly: (Usually done in May under direction of Supervising or Senior Public Health Microbiologist)

1. Trace Metal Analysis
2. Inhibitory Residues on Glassware
3. Bacteriologic Quality of Reagent Water

- All recorded in Water QC book in Water section.

II. AUTOCLAVE Q.C.

- Autoclaves should be checked for proper temperature by using "sterile tape" with each item.

- Once a month a maximum registering thermometer should be used to monitor the sterilization temperature. Once a month the sterilization time cycle must be monitored with a second timing device and documented. Once a month spore strips or "Kilit" vials will be tested. The above will be documented on lab form I-17a.

- A log must be maintained to include the date, items sterilized and total elapsed time from start to removal of media. Total elapsed time from start to removal of media from the autoclave should be no longer than 45 minutes. The autoclave printout must be saved as a record of the time in, time out, elapsed time, sterilization time, and temperature maintained.

A. Daily:

1. Clean Drain Filter and Sterilization Chamber
2. Record Time started, ending and elapsed time, temperature and contents for each run on Sterilization Log
3. Check Printout to Verify Cycle; Time/Temperature
4. Place Sterilization Indicator Tape Check on each item

B. Monthly:

1. Spore strips or Kilit vials
2. System Temperature Check with Autoclave Maximum Reference Thermometer.
3. Timer verification
4. Autoclave Quality Control is checked and filed in Autoclave QC Manual kept in the Hallway bookcase

III. DRY STERILIZING OVEN

STERILIZING OVENS

Hot air sterilizing ovens must maintain a constant temperature of 160-180ºC for a minimum of 2 hours. Records of all items being sterilized, the total sterilization time and temperature must be kept.

A. Daily:

1. Record Temperature
2. Daily Sterilization Log: Time, Elapsed time, Temperature and Contents of each Run

B. Monthly:

1. Spore strips

- Sterilizing oven Quality Control is recorded and filed in Autoclave QC Manual kept in the Hallway bookcase

IV. THERMOMETER Q.C.

A. Semi-annually:

1. Check laboratory Thermometers and calibrate against Certified Thermometer. This is usually done in January and July of each year for the Water section and in January for all the other thermometers.

   Record results in Thermometer Q.C. Log book located in the Media Section. Record the difference between the readings with a numbered "flag" on each thermometer.

   Record results in Pipette Tracker version 3.2 software. The data is stored in the Pipette Tracker book located in the Q.A. Coordinator's Office.

B. TEMPERATURE RECORDING

INCUBATORS AND WATER BATHS

All thermometers used for microbiological work are cross-checked for accuracy against an NIST certified thermometer twice-a-year and documented. Any corrections must be marked on each thermometer and the corrected temperature must be on the log sheet.

Incubators must be maintained at 35 $\pm$ 0.5ºC. Coliforms water baths must be maintained at 44.5 $\pm$ 0.2ºC. Temperatures should be monitored twice daily at a minimum of 4 hours apart.

A. Daily:

1. Record Refrigerator/Freezer Temperatures
2. Check Walk-in Refrigerator/Incubator Temperatures and High-Low Charts

- Record filed in Thermometer/MLA Pipette QC Log in Serology

B. Weekly:

1. Change Temperature Charts on Recording Thermometers

- Temperature charts checked monthly. Charts are filed in the office

V. LABORATORY CLEANING DUTIES

A. Monthly:

1. Follow Lab Cleaning Checklist and turn in completed Checklist to Supervising Microbiologist with Monthly Reports

VI. WATER SYSTEM Q.C. CHECKS

In order to maintain the correct water pressure and water quality, specific checks must be followed in accessing the systems function.

Procedure:

1. Record readings of the pressure gauges labeled P-3 and P-4. Pressure should be between 20-60 psi. Record the difference of P3-P4. This difference should be between 0-10.

2. Record pressure at gauge P-5 (**Acceptable range = 150-220**).

3. Record pressure at P-7. Close valve V-23 and read P-8 (**acceptable range = 30-50**). Record the difference (**acceptable range = 5-15**).

4. With V-23 still closed, record P-9 (**range 30-50**). Record the difference of P-8 minus P-9 (**range 0-10**).

5. Open V-23 and close V-22. Read gauge P-10 (**range 30-50**) and P-11 (**range 20-40**). Record the difference of P-10 minus P-11 (**range 0-10**). Open V-22.

6. Check that the primary DI light, located by the tanks by the door is on.

7. Check the Continental Western Water Quality Meter (Ohm meter), mounted on the north wall, **reading should be >10 MegOhms, the green light indicating "Above Setpoint" should be illuminated.** Conductivity is also checked using a hand-held meter. See procedure on following pages.

8. Check to see that the UV light is ON in the line sterilizer.

9. Visually check the salt level (**should be at least one-half full)** in the water softener and the level of water in the storage tank. Add salt if needed.

10. **RECORD ALL READINGS IN THE WATER SYSTEM Q.C. NOTEBOOK LOCATED IN THE WATER ROOM.** Completed QC sheets are kept in a file folder in the office. Records must be kept for 5 years.

11. Once a year, under the supervision of the Supervising or Senior PHM the laboratory water must be analyzed for trace metals, bacteriologic quality and inhibitory residues on glassware. Procedures for these tests are found in the "Water Procedure Manual".

    NOTE: If any problems are discovered while performing the above procedures or the system is not functioning properly, immediately notify the Supervising or Senior PHM.

VII. **pH METER CALIBRATION AND FUNCTION CHECK**

The pH meter is expected to maintain calibration for at least eight hours. To establish that the pH meter is stable over an extended period of time the following procedure must be performed.

Materials:

Mettler Toledo SevenEasy pH Meter S20
Reference Buffer pH 7.0
Reference Buffer pH 4.0
Reference Filling Solution

Procedure:

1. If meter is disconnected, allow one hour for warm-up. Meter should be left in STD mode when not in use.

2. Verify, visually, that the reference filling solution is adequate. Replace filling solution if level is low.

3. Place electrode in a reference pH 7.0 buffer solution. For maximum precision, stir solution throughout the measurement.

4. Set the instrument slope at 100%.

5. Turn the function switch to the pH mode.

6. Using the CALIB. designation, set the display to the buffer value at the temperature being measured.

7. Remove the electrode from the buffer solution and rinse with distilled water.

8. Once a week calibrate the meter by using two buffers as follows:

    a) Repeat the procedures above from steps 1 to 7.
    b) Place electrode in 4.0 pH reference buffer and turn the FUNCTION switch to pH.
    c) Turn % Slope knob until the pH of the second buffer, at the temperature being measured, is displayed.
    d) Remove the electrode and rinse with distilled water.

9. Keep the electrode in pH 7.0 buffer when not in use.

10. Record all results in the Media Prep log book. Completed QC sheets are kept in a folder in the office. Records must be kept for 5 years.

<u>Reference:</u> Mettler Toledo SevenEasy pH Meter S20. Mettler Toledo: A Guide to pH Measurement.

THERMOMETER Q.C. AND CALIBRATION

The accuracy of the thermometers used in the Public Health Laboratory must be verified twice a year (usually January) for all thermometers used in the laboratory and again (usually in July) for all thermometers used in the water section. They are calibrated against a NBS-certified thermometer. There are two certified thermometers used: one partial immersion used for water baths and one total immersion for incubators. Records are kept in Thermometer Log book located in Media Section.

Procedure (Q.C. Check):

1. Check the thermometer carefully for cracks in the capillary tube.

2. Check the mercury column for separation. If separated, replace with a new one.

3. If the thermometer appears in good order, check it against a thermometer of known accuracy (NBS- certified).

Procedure (Calibration Check):

1. All thermometers in use by the laboratory have a numbered "flag" wrapped around the top. These designated numbers correspond to the number in the Thermometer Q.C. log sheet.

2. To check the accuracy of the thermometer; place the NBS-certified thermometer next to thermometer to be calibrated and leave it there until stabilized (15-30 minutes).

3. Record the temperatures of the two thermometers on the log sheet and calculate the difference correction value + or - required for specified thermometer).

4. Record the difference between readings (correction value) on the number "flag" of each thermometer.

5. Give results to Supervising Microbiologist for review.

6. Add the "+" difference correction value to the actual reading of the thermometer and subtract the "-" difference correction value from the actual reading of the thermometer before recording the value on the temperature charts.

Note: Any thermometer that varies from the certified thermometer by more than 1ºC should be replaced.

Reference: CAP, "Evaluation, Verification and Maintenance Manual, Fourth Ed., 1989.

MEDIA PREPARATION RECORDS

Daily:

Record Lot Numbers of Reagents and Media used, including the final pH, sterilizing time and temperature on the Media Log Sheet. Turn report in with the Monthly Reports to the Supervising Microbiologist

MEDIA PREPARATION

For media preparation, a log must be maintained with the following information:

1. Preparation date
2. Preparer
3. Weight of dried media used
4. Volume of media prepared
5. pH of media after sterilization
6. Type of media prepared
7. Lot number of the dried media
8. Expiration date of the dried media from the date the new bottle is opened

MEDIA STORAGE

All dried media must be stored in a cool, dark contamination free area preferably with a maximum temperature less than 30ºC. Each bottle of dried media must be dated when received and opened. Opened bottles of dried media have a six (6) month expiration date from the date opened, and should have the expiration written on each container.

Prepared media in tubes with loose fitting caps are not to be held more than two (2) weeks. Media in screw caps are held not longer than three (3) months. Each rack of prepared media must be labeled with the type of media, date made and expiration date. Prepared media may be kept in the dark at room temperature.

The Senior Laboratory Assistants perform a monthly inventory on chemicals and dried media. Records are kept in the Logbook in Media Room.

LABELING:

All media and reagents should be labeled with the name of medium/reagent, the concentration when applicable, date prepared, date expires and initials of preparer.

MEDIA PERFORMANCE:

Positive, negative and sterility checks must be performed on each batch of media and reagents. Results are recorded in the Water QC notebook. Organisms to be used are: *E. coli (EPA 0691), Enterobacter aerogenes (EPA 10658)* and *Pseudomonas aeruginosa (ATCC 27853).* These control cultures are subcultured monthly on BHIA and stored at 2-8ºC.

MEDIA DISPENSING PUMP:

Check the accuracy of volumes dispensed with a graduated cylinder each day of use. Record results on media preparation log.

BALANCES:

Check balances monthly with class S certified weights. Record results on form titled "Monthly QC Top Loading Analytical Balances Report." Use the weights shown in the following table:

Balance	Weights to be Checked and Recorded
Mettler PL1501S	0.1, 50.0, and 150.0 gm
Voyager	0.1, 50.0, and 150.0 gm
Mettler AE160	0.1, 50.0, and 150.0 gm

KILIT VIAL OR DUO SPORE/TEMPERATURE CHECK
PROCEDURE FOR AUTOCLAVE Q.C.

To establish that the Autoclaves are functioning properly and providing correct sterilization a Kilit ampule test should be performed each month. The sealed ampules contain suspensions of spores of *Bacillus stearothermophilus* in culture medium containing bromcresol purple as an indicator. Chemical indicator strips are used on each item to verify performance. A high registering thermometer is placed in the autoclave each month to verify that the autoclave reaches the proper temperature.

Procedure:

1. Place an unopened Kilit ampule and autoclave thermometer in the autoclave and run a cycle (121ºC/15 minutes).

2. After autoclaving, remove ampules and incubate in a specially designated Kilit 56-60ºC Syphilis Serology waterbath. Include a non-autoclaved control Kilit ampule along with the autoclaved ampule to serve as a positive growth control. Record the thermometer reading on the autoclave QC form.

3. Observe the ampule at 48 hours for the appearance of growth (turbidity) and a yellow color, indicating failure of the sterilization process. If no color change occurs within 72 hours, hold for a total of 7 days, examining periodically growth. Notify Supervising PHM if test ampule turns yellow.

Note: Ampules remaining purple indicate that sterilization has been accomplished, provided that the non-heated control shows growth.

4. Record final results on a Lab form I-17 (see Appendix A), and give to Supervising Microbiologist.

5. Completed forms are kept in a notebook in the media section. Records must be kept for 5 years.

Reference: Becton Dickinson, BBL Autoclave Control (Kilit Ampules), Cat. # 12018.

AUTOCLAVE AND STERILIZER CLEANING AND TEMPERATURE VERIFICATION

To maintain the autoclave and sterilizer systems in good working order they must be cleaned periodically as outlined below. It is also important to validate the correct temperature of the systems by using an autoclave maximum thermometer.

Procedure (Autoclave):

Daily:

1. Remove tray and sweep chamber.
2. Check drain filter and remove any foreign material.

Weekly:

1. Clean exterior surface with metal polish.
2. Scrub interior surface with stainless steel or scouring pad.
3. Rinse interior with water after cleaning.

Monthly:

1. Check seal and gasket integrity.
2. Place an autoclave maximum thermometer into the autoclave and run along with a regular cycle (record on the Kilit Q.C. chart).

Procedure (Dry Oven Sterilizer):

Weekly:

1. Clean exterior surface with mild detergent.
2. Clean inside with a stainless steel or a soft pad.
3. Remove and clean shelves with a mild cleaning solution.

Note: If the temperature readings with the Autoclave Maximum reading thermometer do not correspond to the chart temperature reading, or if any problems with the Autoclave or sterilizer systems occur, immediately notify the Senior or Supervising Microbiologist.

DUO-SPORE: AUTOCLAVE QUALITY CONTROL

PRINCIPLE:

The DUO-SPORE system is used as a biological indicator for checking the efficiency of the autoclaves. The organisms used for testing are *Bacillus subtilis* var. Niger and *Bacillus stearothermophilus*. Growth should occur in the unsterilized control vial (containing the control strip in broth) within 48 hours, with no growth in the autoclaved vial (containing the autoclaved strip in broth).

PROCEDURE:

1. Remove the 2 test strips from the pocket within the DUO-SPORE pack and sterilize according to normal procedure.

2. After sterilization aseptically unseal strip using sterile forceps, remove both strips (test and control), and place them into individually labeled tubes of tryptic soy broth.

3. Incubate tubes at 55ºC for seven days.

4. Observe daily for growth in the unsterilized control.
   Note: Growth should occur within 48 hours in control vial, with no growth occurring in the sterilized vial.

5. Record results on the Media I-17A form (Autoclave Quality Control).

If growth does not occur in the control vial within specified time or if the sterilized vial contains growth, notify Supervising Microbiologist immediately.

Reference:

"DUO-SPORE: Instructions for using Biological Indicators to Determine Efficiency of Gas, Steam, or Dry Heat Sterilization", no date provided. Propper Manufacturing Co. Inc., Long Island City, New York 1101.

DRY HEAT STERILIZER USE AND QC PROCEDURE

1. All items in the dry heat sterilizer should be held at a temperature of 160-180°C for a minimum of 2 hours (120 minutes).

2. All the items should be recorded on form "DRY HEAT STERILIZER OVEN LOG" with each batch sterilized.

3. The temperature of the oven should be recorded with each batch using a thermometer placed inside the oven.

4. A sterilization indicator (Proper Manufacturing "Temptube", or similar) should be used with each batch and should give the proper reaction to indicate the correct sterilization conditions were met.

5. Each month a spore strip should be placed in a glass petri dish and held in the oven for 2 hours at a temperature $\geq$160°C. Then the strip should be placed in TSB, along with the second tube containing the control strip, and incubated at 37°C (55°C if steam autoclaved) for up to 7 days. Results should be recorded on form I-17a and kept with the autoclave monthly QC forms.

6. Report any problems or QC failures to the Senior Lab assistant and Supervising Microbiologist.

References:

1. *Standard Methods for the Examination of Water and Wastewater.* 18th Ed. American Public Health Association Washington D.C. 1992.

2. Duo-Spore sterilization indicator procedure. Propper Manufacturing Co., Inc. Long Island City, New York 4/94.

DAILY pH CHECK OF GLASSWARE

PURPOSE: To determine the pH of any residue that may be on glassware after completion of the laboratory procedure for cleaning, rinsing and drying.

EQUIPMENT: HACH Chlorine and pH Test Kit.

PROCEDURE:

1. Take a clean, rinsed and dried piece of glassware, preferably a bottle or beaker.
2. In one vial of the HACH test kit collect 10 ml of deionized water from the tap. This is the water that is used in the final rinse of the glassware in the washer.
3. Turn on the pH meter.
4. Using the second vial in the kit that contains the standard as a blank, wipe the outside of the vial and zero the meter.
5. Wipe the outside of the first vial and insert into the meter and take the reading.
6. Pour the 10 ml of collected deionized water from the first vial into the bottle or beaker that is to be tested. Shake and swirl the water over all of the sides several times to make certain that the surfaces are being covered.
7. Pour this water back into the first vial.
8. Zero the meter again with the blank (second vial).
9. Remove the blank and insert the first vial after wiping the sides. Take the pH reading.
10. A passing reading is in the range of pH 5.5 to 7.5.
11. Record the reading on the sheet for pH Check of Glassware.
12. If the reading is out of limits, notify a Senior Laboratory Assistant or Supervisor.

DETERMINATION OF CONDUCTIVITY OF PURIFIED WATER FOR MEDIA PREPARATION

Introduction

The conductivity of the laboratory water is determined daily by using the Digital Conductivity Meter. This is a precision instrument that has a microcomputer. It has four calibration points to ensure the complete accuracy over the entire measurement range. All calibration data is saved when the unit is turned off. It has both automatic and manual temperature compensation. The Unit displays results in conductivity in micromhos/cm=microsiemens/cm.

Operating techniques

1. Use very clean beakers.

2. Avoid cross contamination between measurements by rinsing the probe in deionized/distilled water and by rinsing the probe in the solution to be tested.

3. The solution temperature should remain constant during the readings. The ideal temperature is 25.0ºC.

4. Make certain to sustain flow through the probe (or move the probe through the solution in a stirring motion) while making your readings. Stirring helps prevent polarization, ensures that the solution is well mixed and helps to maintain a uniform temperature within the solution.

5. Pure water will pick up contaminants from the air in a relatively short time. For the measurement of very pure water, consider shielding the measuring container.

6. When finished using the probe, always rinse it in distilled water and stored in distilled water. Solutions which are allowed to dry on the probe will eventually block out active sites on the internal platinum electrode. The surface area will be reduced and a new probe will be required. Do not touch the internal platinum electrode, if the surface is damaged linearity will be affected, specifically in the high conductivity readings, and difficulty may be found in achieving high readings.

Calibration with Known Standard

1. Erase any existing calibration data.

    a. Press the CHECK key, “CHK” will appear on the top right corner of the display.
    b. Press and hold the ENTER key for 10 seconds, “0” zero will be displayed to the far left of the display to indicate that all calibration data has been cleared.
    c. Return to the measuring function desired by pressing the MODE key.

2. Insert the probe into a small beaker containing a known standard solution, Traceable Conductivity Calibration Standard # 23226-589.

3. Press the MODE key the place the unit in the measurement function desired, such as conductivity (µS/cm).

4. Press the arrow keys to adjust the value on the display to the value of the solution. “CAL” will blink on the display. NOTE: Each press of the arrow key increases/decreases the display by 1 digit. To rapidly increase/decrease the display, press and hold down the arrow key, the least significant digit is changed until ten digits have been counted, then the next significant digit, etc.

5. With the correct value on the display, press the ENTER key to enter the value as a calibration point. The instrument is now calibrated.

6. Rinse the probe with distilled water.

7. To read a sample, follow Operating Techniques.

Taking Measurements

1. Press the On/Off key to turn the unit on

2. Place the unit in the MODE required, such as conductivity, by pressing the MODE key.

3. Place the probe in a clean beaker that contains the sample solution (the lab deionized water).

4. Read the answer on the digital display while stirring the probe in the solution. Reading should be < 1 µmhos/cm (or microsiemens/cm) at 25ºC. If reading is out of acceptable range, notify supervisor immediately.

5. Record the reading on the daily media work sheet.

Reference:

Manual of Traceable Conductivity, Resistivity, TDS, Salinity Concentration Meter Instructions, 1993 Control Company.

PERFORMANCE AND SYSTEMS AUDITS
and Corrective Action

QC/QA FORMS FOR WATER BACTERIOLOGY

1. RO/DI system daily log sheet:
   Form used by Lab assistant staff to record daily and monthly water system QC values.

2. Fecal coliform waterbath temperature form:
   Form used by all staff to record the daily fecal coliform waterbath temperature and control results.

3. Incubator temperature recording form:
   Form used by all staff to record daily incubator temperatures.

4. Media preparation log forms and daily pH recording form:
   Form used by Lab assistant staff to record lot numbers of media, amounts used, ph of media, and autoclave time and temperature. Daily pH meter calibration is recorded on the back side of the form.

5. Colilert verification form:
   Form used by all microbiologists to record verification of 10% of the ONPG positive (yellow) samples and all MUG positive (fluorescing) samples.

All forms are reviewed monthly by the Supervising Microbiologist.

Any corrective actions performed because of QC failures or values "out-of-range" should be recorded on these forms and the Senior or Supervising microbiologist informed.

Completed forms on the autoclave are kept in the Autoclave QC manual as well as performance tapes of each run.

Completed thermometer charts are kept in a file in the office.

Files are kept in the cabinet in Media. These files include completed media records, water room parameters, and autoclave QC.

All public health microbiologists performing water analysis are required to read the QA Water Manual annually before working in the section. This is documented in the manual entitled “Responsibilities of CLIA-Regulated Personnel.”

PREVENTIVE MAINTENANCE

PREVENTIVE MAINTENANCE:

Preventive maintenance checks are performed on all laboratory equipment semi-annually. This includes the autoclaves, balances, pH meters and incubators.

The laboratory reagent-grade water system is serviced monthly.

Maintenance records and copies of maintenance contracts with scope of work are stored in the lab office.

ASSESSMENT OF PRECISION AND ACCURACY

ASSESSMENT OF PRECISION AND ACCURACY:

All results are reviewed and checked for accuracy by the Senior or Supervising Microbiologist.

Precision and accuracy are also assessed by yearly proficiency testing from recognized ELAP organizations.

A. Media dispensing pump:

   Check the accuracy of volumes dispensed with a graduated cylinder each day of use. Record results on media preparation log.

B. Labeling:

   All media and reagents should be labeled with the name of medium/reagent, the concentration when applicable, date prepared, date expires and initials of preparer.

NOTE: All forms are reviewed monthly by the Supervising Microbiologist. Any corrective actions performed because of QC failures or values “out-of-range” should be recorded on these forms and the Senior or Supervising microbiologist informed.

CORRECTIVE ACTION

CORRECTIVE ACTION:

All complaints, problems, or reporting errors are documented and reviewed using the Laboratory Problem/Error log forms. Corrective actions are also noted.

Any corrective actions performed because of QC failures or values "out-of-range" should be recorded on the QC form for that parameter and the Senior or Supervising Microbiologist informed.

QUALITY ASSURANCE REPORTS

QUALITY ASSURANCE REPORT FORMS

Computer locations for all forms used to record QA and QC data are found in Appendix A.

Completed QC and QA records are stored for 5 years in the following locations:

- Temperature Charts: top drawer of lateral file cabinet in Office room 6407
- Pipet calibration records: Recorded on Pipette Tracker software on laptop PC in QA Coordinator's office
- Records of notification of Environmental Health staff of positive water test results: Water Notification Log binder, on top of 36" high counter in Room 6430
- Records for autoclaves, dry sterilizing oven, and analytical balances: Autoclaves, Dry Ovens, and Analytical Balances QC binder in bookcase in hallway opposite door to Room 6477
- Water purification system records and records for preparation of media, stains, and reagents are filed in the black file cabinet in hallway opposite door to Room 6477

The following records are stored in the Water QC Binder on the counter in Room 6430:
Water media bacteriologic QC
Quanti-Tray sealer QC
Calibration temperature QC for autoclaves
Glassware pH checks
Verification by culture for potable water tests
Colilert verification log (new lot numbers)
E. coli verification by culture from Colilert wells
Enterolert verification log (new lot numbers)
Enterococcus confirmation by culture from Enterolert wells
Water purification system plate county QC
Bacteriological Quality of Water test records
Culture verification QC organisms in water
Laboratory problem reports
Glassware inhibitory residues test
Chemical analysis of purified water
Colilert and Quanti-Tray certificates
Unsatisfactory specimen log

References:

1. Standard Methods for the Examination of Water and Wastewater. 1995, 19th Edition, APHA..
2. Standard Methods for the Examination of Water and Wastewater. 1971, 13th Edition, APHA. (MPN table for 3-tube dilution series).
3. Microbiological Methods for Monitoring the Environment. Water and Wastes. EPA-600/8-78-017, December 1978, USEPA.
4. USEPA Manual of Methods for Virology. EPA-600/4-84-013, February 1985.
5. Improved Membrane Filter Technique for Enumeration of Escherichia coli and Enterococci. Microbiological Criteria Workshop, February 1985 held at Newport Beach, CA. Published by USEPA, Washington, DC 20460.

APPENDIX A

Schedule of duties and Computer locations of forms:

Pipette calibration: see Pipette Tracker software on laptop ITE00114168

1.	Each batch	Daily	Weekly	Monthly	Semi-annually	Annually	Use Form located at:
Staff to review the procedure and quality assurance manuals within 2 weeks of assignment, then annually						X	Record in "Responsibilities of CLIA-Regulated Personnel" book located in the Office
Temperatures of incubators, waterbaths		X (a.m., p.m.)					S:\PHS\Lab\WP51\FORMS\Temp. Charts-BSC Charts
Conductivity of purified water		X					S:\PHS\Lab\WP51\FORMS\WATER ANALYSIS FOR PARKER STEAM BOILER.doc
Resistivity (reciprocal of conductivity)		X					S:\PHS\Lab\WP51\FORMS\WATER ANALYSIS FOR PARKER STEAM BOILER.doc
Total Chlorine		X					S:\PHS\Lab\WP51\FORMS\ WATER ROOM DAILY LOG SHEET.doc
Water pressure, primary DI light, UV light, salt level, storage tank level		X					S:\PHS\Lab\WP51\FORMS\ WATER ROOM DAILY LOG SHEET.doc
Hardness using the HACH Hardness Test Kit		X					S:\PHS\Lab\WP51\WATER\Water QC worksheets\ pH check of glassware.doc
pH and QC of pH meter		X					S:\PHS\Lab\WP51\WATER\Water QC worksheets\ pH check of glassware.doc
SPC (standard plate count)				X			S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Q.C PLATE COUNT WATER ROOM.doc
Heavy Metals						X	Performed by City Lab, filed under Chemical Analysis section.
Bacteriologic quality of laboratory test water when conditions change or annually						X	S:\PHS\Lab\WP51\WATER\Water QC worksheets\BACTI QUALITY OF WATER.doc
Glassware Inhibitory Residues test when conditions change or annually						X	S:\PHS\Lab\WP51\WATER\Water QC worksheets\ TEST FOR INHIBITORY RESIDUES ON GLASSWARE.doc

2.	Each batch	Daily	Weekly	Monthly	Semi-annually	Annually	Use Form located at:
9.0 mL Buffered dilution water, 90 mL DI water sterility QC	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Water Media QC form.doc
IDEXX sample containers QC each lot	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Water Media QC form.doc
Sample cup or vial sterility QC	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Water Media QC form.doc
Accuracy of the 100 ml mark on Colilert containers	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Water Media QC form.doc
Colilert reagent for potable waters performance QC	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ COLI_CONFIR.DOC
Enterolert reagent performance QC	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ ENTEROLERT QC.doc
Enterolert positive well verification			X				S:\PHS\Lab\WP51\WATER\Water QC worksheets\ENT_CONFIRMATION.DOC
Colilert-18 for seawaters (if used) performance QC	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Colilert 18 QC.doc
In-house prepared media (tubes, bottles, and plates)	X						S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Water Media QC form.doc

Charts for periodic recordings:	**Use Form located at:**
Dry heat oven chart daily or when used	S:\PHS\Lab\WP51\FORMS\ Dry Heat Sterilization Oven Chart.doc
Confirmation of MTF for Sea Waters	S:\PHS\Lab\WP51\WATER\ Confirmation of MTF for Sea Waters log.doc
Quantitray Sealer QC	S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Quantitray Sealer QC.doc
Drinking Water Results Notification Log	S:\PHS\Lab\WP51\WATER\Water QC worksheets\ DRINKING WATER RESULTS NOTIFICATION LOG.doc
Calibration of temperatures for autoclaves	S:\PHS\Lab\WP51\WATER\Water QC worksheets\ Calibration of Temperatures for Autoclaves.doc
Record Duospore/Kilit results for autoclaves	S:\PHS\Lab\WP51\MEDIA\I-17a Autoclave-Dry oven QC rev 2-07.doc
Requisition and Recording forms:	
Water Bacteriology requisition form	S:\PHS\Lab\WP51\FORMS\Lab Requisition Forms\Water Bacteriology.doc
Recording results of sea water/sewage	S:\PHS\Lab\WP51\WATER\Water QC worksheets\Water Worksheet