

SECTION 1: LABORATORY

To maintain the highest level of quality assurance, records are kept on all media preparation including quality control of prepared media (positive, negative and sterile controls). Additional records are kept of the daily operation of equipment such as incubators, water baths, autoclaves, pH meters and other measuring devices. All instrumentation is calibrated before each use with commercially purchased standards whenever possible. This provides feedback for preventive and/or corrective maintenance that might be required. Further discussion of record keeping is included in Section 3 under each type of equipment. Examples of report forms are found in Sections 6 and 7.

Laboratory Water Quality

Deionized Water

Deionized water is used for the cleaning of glassware and other equipment. Water deionization is accomplished using two resin-filled tanks connected in series. Cartridge filters are located on both upstream and downstream sides of the tanks. Conductivity is monitored continuously by 1-megohm lights after each cylinder.

For proper quality assurance the following records are kept:

- Date of change of cylinder
- Gallons of water processed since last cylinder change
- Standard Plate Count (monthly)
- pH (monthly)
- Conductivity (continuously)
- Chlorine residual (monthly)

The procedure for changing the resin tanks is discussed in the "Instructions for Use" portion of this section of this manual.

Distilled Water

Reagent grade distilled water, which meets DHS requirements, is purchased from a commercially available source. Monthly quality control reports are furnished to and maintained in the lab. This water is used for preparation of all media and solutions.

Buffered Dilution Water

Buffered dilution stock may be purchased in premeasured, sterilized containers. Each shipment will be marked with date of receipt and expiration date. Bottles of stock will be stored in a cool, dark location.

Buffered dilution water may also be prepared from chemicals on hand. If made in the lab, stock solution will be kept in the refrigerator, checked daily for clarity, and discarded if turbid. The date dilution water is made and date it expires will be recorded on the bottles. Any buffers made will be dated after sterilization and discarded when expired.

Sterile Water

Sterile water for dilution of samples is purchased from Baxter in 500ml bottles.

Quality Assurance Procedures For Water Sampling

Sample Collection

Most bacteriological water samples, effluent solids samples, DO samples, and turbidity monitoring are collected by the utility systems repairer/operators and passed to lab personnel. The pH and alkalinity samples are usually collected directly by laboratory personnel. All samples are then prepared and processed by lab employees. Procedures for proper sample collection and testing are included in Section 4.

Sterile, disposable polystyrene vials containing sodium thiosulfate and marked with a 100ml EPA line will be used for sample collection. One sample vial from each lot of 200 will be tested for sterility in the lab by using a Tryptic Soy Broth (TSB), incubating for 24 hours, checking for cloudiness (indicative of bacterial growth).

Sample Preservation and Storage

All samples will be labeled with date, time of collection, chlorine residual, collector's name, water system, sample point, and other pertinent data (flooded stream, chlorinator broken, etc.).

Samples should be inoculated promptly after collection to avoid variance in growth log. If not processed within an hour, samples shall be stored for transport in coolers containing frozen blue ice or equivalent to ensure transport at 0-4°C. Upon receiving in the lab, each sample shall remain refrigerated and be dated, time recorded and initialed.

Delivery of samples and inoculation should be completed within **eight** hours. Therefore delivery should be within six hours and inoculation completed within two hours upon receipt in the lab. In **NO** instance shall the time elapsing between collection and examination exceed 30 hours and the sample shall be refrigerated at all times.

Resample

If any treated sample tubes are excessively turbid (signs of growth without gas) the sample will be invalidated and a resample will be taken from the same sample point within 24 hours.

Repeat Samples

If any treated sample tubes exhibit gas production using MTF or exhibit yellow/fluorescent color changes using Colilert P/A, **three** repeat samples will be taken within 24 hours. The repeat samples will consist of one sample from the original location, one sample immediately upstream, and one sample immediately downstream of the original sample point. Repeat sample points are specified in the manual for each water system. The repeat sample procedure will be initiated by phone or park radio. Additional treated water samples may be required during the month following a confirmed positive potable water sample.

Completed Test

When using the MTF method, double confirmation into BGB for total coliforms and EC broth for fecal coliforms is used. The Colilert P/A method requires no further testing. A log will be kept of all positive potable confirmed samples. These tests confirm the presence of coliform bacteria and provides quality control data.

Reporting and Notification

All results will be recorded and reported to PHS in region and the State. Appropriate personnel within the Parks will be notified of positive potable water samples so that remedial action may be taken.

Quality Assurance Procedures For Media

Preparation Records

A log book of media preparation will be kept with the following information for each batch made: date, media type, reference number, tare weight, volume of water, number of tubes made, pH after sterilization, and positive/negative/sterile control results. A copy of a page of the media log can be found in Section 6.

Diack controls and temperature sensitive tape will be used on each rack of media during an autoclave run. Each use of the autoclave will be recorded. An example of an autoclave run sheet can be found in Section 6.

Positive, negative, and sterile controls will be run on each batch of media made. The type of media will determine bacteria selection for the controls. Positive and negative controls will exhibit a response appropriate for the media being checked. Sterile controls will remain sterile.

Media Storage

Dehydrated media shall be purchased in quantities small enough to be used up within **six** months after opening. Each bottle will be given a reference number when received, and dated when opened and given an open expiration date. All bottles will be inverted upon receipt and between uses to avoid air leakage.

All prepared media tubes will be identified, dated and initialed, and stored as recommended by the manufacturer. Sterilized media tubes with loose fitting caps must be used within two weeks.

Sterilized media stored in tubes with screw caps can be stored three months, out of sunlight, areas of contamination, and excessive evaporation.

Any tubes with bubbles in shell vials and any out-of-date media must be discarded.

SECTION 2: PERSONNEL

Staffing

A professional microbiologist should supervise the testing performed in this laboratory. If this type person is not available, then a professional microbiologist or trained analyst should be available for guidance and assistance. The supervisor should periodically review procedures of all aspects of the lab testing and preparation to identify and eliminate problems. The analyst should be trained in basic laboratory procedures with periodic review by the supervisor.

Safety

All people in the Lab, employees and visitors, may be subjected to a variety of chemical and biological hazards. It is the responsibility of employees to be familiar with this potential and to conduct their work habits in the safest manner.

The Chemical Hygiene Plan details the proper handling of chemicals in the laboratory. Additionally, Material Safety Data Sheets (MSDS) are kept on file and should be reviewed prior to the use of hazardous substances.

Safety equipment available in the lab includes fire extinguishers, acid and base neutralizers, spill containment material, face masks, goggles, respirators, first aid kit, shower and eye wash, an exhaust hood, and ventilators. Employees should be familiar with the location and proper

use of this equipment.

SECTION 3: EQUIPMENT AND MATERIALS

Inventory Log

Upon receipt in the laboratory, each ordered item with a lot number and/or an expiration date will be given a sequential reference number and recorded in the inventory log. Information contained in this log will include reference number, date received, item, quantity, lot number, manufacturer's expiration date, open date, open expiration date, and disposal date and amount. The reference number will be transferred to the proper logbook when the item is put into use. Bottles of powdered media and cases of reagent supplies will be given individual reference numbers when ordered in multiple amounts. A copy of the inventory log page can be found in Section 6.

Equipment

VWR Incubators (2) @ 35° C, 1 @ 20° C, 1 @ 5° C
Market Forge Steam Pressure Sterilizer Autoclaves (2)
Precision Water Baths @ 44.5° C with gable covers (2)
Quebec Colony Counter
Microscope – Dissecting/ Compound
Hot Plate Stirrers (4) of various sizes
Beckman pH Meter with two probes and a temperature compensating probe
Ohaus B300 Analytical Balance
Mettler AE100 Balance Top Loader
Orion 162 Conductivity Meter
YSI Oxygen Meter
Monitek Turbidimeter
Milton Roy Spectrophotometer
VWR Vortexer
Resin bead Deionized Water system
Automatic Pipettors (2)
Micro pipettes (5) of various volume ranges
Certified Thermometers, immersion and non-immersion
Thermometers: Water bath, incubator, and autoclave (0.1° C divisions)
Cole-Parmer Oven
Plas-Labs Desiccating Chamber
Vacuum Pump
Imhoff Cones (4)
Ultraviolet Sterilizing Cabinet
Idexx Quanti-tray Sealer
Wheaton Unispense
VWR Standard Heatblock

Glassware and Miscellaneous

Test Tubes, culture, disposable - 16X125, 18X150
Test tubes, culture, reusable - 16X125, 18X150
Test tube caps – 16 and 18 mm
Culture tubes, screwtop with lids
Shell Vials, short form flat bottom - 1/4 and 1/2 dram
Petri dishes, plastic, disposable, sterile
Pipettes, disposable - 10ml and 1.0ml (0.1 ml divisions)
Pipette jars (2), plastic, lined with disposable autoclave bags
Sample vials with sodium thiosulfate, sterile, disposable
Test tube racks, plastic, aluminum
Inoculating loops, needles
Loop, needle holders
Bunsen Burner
Borosilicate Glassware: beakers, flasks, graduates
Sterility indicators: Diack controls, tape
Bromthymol Blue pH indicator
Disinfectants: foam, spray, EtOH

Chemicals

In Chemical/Media Cupboard:

BOD Polyseed
Calcium sulfate, anhydrous
Formazin
Glycerin
Nitrification Inhibitor
Nochromix
Potassium chloride
Sodium chloride solution-1000mg/L
Sodium chloride solution- 491mg/L
Sodium hydroxide, pellets
Sodium Hydroxide solution – 5N
Sodium thiosulfate solution- 0.025N
Starch Indicator
Alkaline Iodide-Azide
BOD nutrient buffer pillows (3L)
BOD nutrient buffer pillows (300ml)
BOD standard solution (Glucose/Glutamic acid)
Colilert Snap Pack
Colilert Color Comparator
DPD Free Chlorine
DPD Total Chlorine
Manganese Sulfate
Sulfamic Aid

In Fire Resistant Cabinet:

Acetone
Ammonium Hydroxide
Chloroform
Ethanol
Methanol, absolute
Propane, cylinders
Potassium cyanide
Gram Stain Kit

In Corrosive Cabinet:

Hydrochloric acid - 5N
Hydrochloric acid - 10% solution
Potassium Iodide Solution 100g/l as KI
Sodium hydroxide - 1N
Sulfuric Acid – 19.2N
Sulfuric acid - 0.2N
Sulfuric Acid – 0.02N

Media

Brilliant Green Bile broth 2%, dehydrated
EC medium, dehydrated
Lauryl Tryptose broth, dehydrated
Plate Count Agar, dehydrated
Tryptic Soy broth, dehydrated

Biological Reagents

In refrigerator:

BD Bactrol Plus cultures
Sterikon bioindicator spore vials
Quanti-cult kit

Specifications and Quality Control

Operation and service manuals for all equipment are available in the laboratory.

Refrigerator, Incubators, Water Bath

The lab includes a refrigerator maintained at $1-5^{\circ}\text{C}$, a BOD incubator set at $20 \pm 0.5^{\circ}\text{C}$, two coliform incubators set for $35 \pm 0.5^{\circ}\text{C}$, and two water baths maintained at $44.5 \pm 0.2^{\circ}\text{C}$. Each of these contains a thermometer graduated in 0.1°C increments. Each thermometer bulb will be immersed in liquid (water/glycerin or mineral oil). A daily log of the temperature, read to 0.1°C , shall be kept. All entries will be dated.

Every six months thermometers will be calibrated against a certified thermometer, which meets NIST standards. Records of each calibration will be kept. An example of a daily temperature/biannual calibration record is found in Section 6 of this manual.

Incubators will be cleaned inside monthly with a disinfectant. Water baths will be drained and cleaned weekly. The refrigerator will be cleaned and defrosted as necessary.

Autoclave

The autoclave should reach sterilization temperature within 15 minutes. Total time media should be exposed to temperature and pressure is 45 minutes.

Each autoclave run shall be recorded in the logbook and checked for sterility using Diack controls, sterile indicator tape and maximum recording thermometers. Each autoclave run will have date, items sterilized, and sterilization time, time in and out, elapsed time, maximum temperature, maximum pressure and appropriate quality control checks recorded. A maximum reading thermometer will be used to check autoclave runs weekly or "when used" for the unit that is infrequently used.

A clock accuracy check will be done monthly. Sterikon bioindicator spore ampules will be used to check sterility monthly. Two ampules will be used per autoclave. These plus an unautoclaved control will be incubated for 48 hours at 60° centigrade in a heatblock to check for no growth. An example of the autoclave record is included in Section 6.

Autoclaves will be cleaned monthly or more frequently if necessary. Procedures for operating and adjusting an autoclave are given in a following section.

pH Meter

The pH meter shall be calibrated prior to each use using two fresh buffers at least two pH units apart. The standard buffers used are 4.01 and 10.00. A pH 7.00 standard will be used to check the calibration. Standards shall be given a reference number when received. Each use of the pH meter will be noted on the record sheet, an example of which is included in Section 6. Procedure for proper use of the pH meter is found in the following section.

Pipettes

Any broken pipettes or pipette tips shall be discarded. Pipettes with indistinct graduations will not be used. Ten-ml pipettes shall be used for delivering 10ml aliquots and 1ml pipettes used for 1.0 and 0.1ml aliquots.

Pipettes used for water samples shall be of borosilicate glass and the disposable and sterile plugged type. Any used pipettes will be autoclaved (30 minutes) before being discarded.

Culture Tubes and Shell Vials

Culture tubes shall be borosilicate glass. Tubes should be large enough that the media plus sample will not fill the culture tubes over 2/3 full.

The shell vial for gas collection shall be at least one half the diameter of the culture tube, i.e. ½ dram for 18x150mm tubes and ¼ dram for 16x125mm and screwtop culture tubes. Vials will be autoclaved and discarded after use.

After cleaning in the dishwasher, culture tubes will be checked with bromthymol blue to assure that they are free of detergent residue.

Instructions for Use of Equipment

Deionized Water System

The deionized water system should be checked daily for leaks and to insure that the conductivity lights are lit. When a conductivity light goes out it indicates that the resin in the treatment tank is no longer delivering water with a conductivity of less than 1 microSieman. It is time to change tanks.

Refrigerator, Incubators, Water Baths

These are reliable pieces of equipment that need very little maintenance. It may be necessary to seasonally adjust the thermostats to maintain proper temperatures. Make only slight adjustments to the thermostats, as they are very sensitive. Manuals are on file for this equipment.

Autoclave

Mark all items to be autoclaved with temperature sensitive tape. Loads of media being sterilized prior to use should also be monitored with a sterilization indicator (Diack). Load autoclave with materials to be sterilized. Close drain and fill chamber with deionized water to the indicated water line. Close and completely latch the door.

There is a toggle switch labeled "Exhaust Selector" in the middle of the top panel. This determines the rate that the pressure is relieved from the autoclave at the end of the sterilization period. If media is being sterilized, this switch **must** be in the "Slow" position, otherwise the media will boil over making a mess of the autoclave and resulting in a loss of the media. The "Fast" position can be used if liquids are not involved.

To turn the autoclave on, rotate the timer to the correct setting, usually 15 minutes (new media) or 30 minutes (used media, pipettes, petri dishes, inoculating loops, or other waste). Start timer. During the autoclave cycle, check to be sure that it has reached the correct pressure (16 psi) and temperature (121°C).

At the end of the autoclave run, open the door carefully. Do not put ungloved hands above the door, as escaping steam is hot enough to burn. Put on gloves to remove autoclaved items. Open drain. If any media has spilled onto the autoclave rack, rinse it off. Wipe rack with sponge. An additional autoclave tray is stored in the cupboard below the autoclave.

Quality control checks for the autoclave include autoclave tape on each run, tape and Diack control on each media run, temperature checks weekly, clock checks monthly, and Sterikon

bioindicator checks monthly. Record all data in the autoclave logbook. An example of the record sheet is found in Section 6 of this manual.

pH Meter

The following standardization procedure is the easiest method for operating the instrument in the pH mode. Required equipment and supplies are: pH electrode and reference electrode (or a combination electrode), an **Automatic Temperature Compensator (ATC)** probe, three buffers, and several beakers. Buffers must be appropriate to the instrument being used and to the expected pH to be measured.

Turn on the meter. Begin by depressing the CLEAR Key to clear the instrument, if necessary. Rinse the electrode(s) with distilled water, or pH 4 buffer, and blot dry using laboratory grade tissue (Kimwipes). Do not rub or wipe the probe as this can create electrical charges that interfere with the performance of the instrument. Immerse the electrode in pH 4 standard.

Depress the STANDARD Key. The instrument recognizes this entry as the first standard. The actual pH reading, which changes as the electrode(s) stabilize, varies depending upon analytical conditions and upon the electrode used. Both the STD 1 Symbol and STD 1 Value flash.

Shortly, the AUTO Symbol will begin flashing. This flashing indicates that the Auto Read Function of the instrument has detected that the electrode(s) are nearing stabilization. STD 1 Symbol and Value continue flashing. When input from the electrode(s) is stable, Auto Read will lock, the instrument will automatically standardize on the pH value of 4.00 and the STD 1 Symbol will appear in Display along with 4.0. Record pH value and indicated temperature (°C) on the pH Meter Log.

Now rinse the electrode(s) with distilled water or pH 10 buffer, blot, and immerse in pH 10 buffer.

Depress the STANDARD Key again. The instrument recognizes this entry as the second standard. Once again, pH value displayed will change as the electrode(s) stabilize. The STD 1 Value is on and the STD 1 Symbol flashes; the STD 2 Symbol and Value both flash.

The AUTO Symbol will again flash after a short time indicating that the electrode(s) are approaching stabilization. STD 1 and STD 2 Symbols behave as in the preceding stage. When input from the electrode(s) is stable, Auto Read will lock, the instrument will standardize on the pH value of 10.00 pH buffer at the measured temperature. Record pH indicated on the pH Meter Log. The STD 2 Symbol will be on, along with 10 (the approximate value of the second buffer). The STD 1 Symbol and STD 1 Value also remain on. When both STD Symbols and STD Values are on, the instrument has completed two-point standardization with the indicated standard buffers. Check the calibration of the instrument with pH 7.00 buffer. Press the pH key and read the value when the auto lock stops flashing.

The instrument is now ready to make a pH measurement. Rinse the electrode(s) with distilled water or the selected sample, blot (do not wipe), and immerse in the sample. Press the pH key and read the value when auto lock stops flashing. The measured temperature will also be displayed on the screen.

This sequence is repeated for all sample measurements. That is, rinse the probe, blot dry, immerse in sample, depress the pH key, wait for Auto Read to lock, and note the pH value. All calibration are recorded in the pH log book. An example of the pH Meter Log is found in Section

6 of this manual. An operation manual is on file in the laboratory.

Scales

There are two electronic scales in the laboratory, an Ohaus B300 and a Mettler AE100. Since the Ohaus is used much more frequently, instructions for its use will be given.

Turn on the Ohaus by depressing the "Power" button. The scale will go through a series of automatic checks, then stabilize on "0.00g". If you simply want to weigh an item, place it on the scale and read the value given in grams. Maximum weight is approximately 320g.

To weigh dried media or chemicals, first place a weighing boat on the scale. When the scale has stabilized, depress the "Tare" bar. This restores a zero value, making it easy to measure out the desired amount. Now pour media or chemical into the boat.

For only a small amount of media, you can place a beaker (up to 600ml size) on the scale, re-zero it, and measure directly into the beaker.

Turn off the scale by depressing the power button. Always clean the pan and scale immediately after each use.

Operation of the Mettler scale is similar. Prior to using it, look at the bubble in the front right corner of the platform to check for level. Turn the rear legs of the scale to restore levelness.

The Mettler scale must be checked for accuracy, weekly. Use a weight close to the amounts weighed and record on the data sheet being used. The Ohaus will be calibrated monthly with a series of weights and recorded on a record sheet. An example is found in Section 6.

Dishwasher

The dishwasher is similar to many used in homes. Basic instructions are printed on the front. First, load glassware or other items to be washed onto the racks. Add detergent. When using the "Heavy" cycle (the most commonly used), add **one** scoop of detergent to cup 2 and a lesser amount to cup 1. Instructions for other cycles are embossed on the detergent dispenser. Close door and slide latch to the right to lock.

Select the proper options. The usual setting is:

- Steam Action - on
- Final Rinse - distilled
- Power Dry - heat

Select the desired cycle, usually "Heavy". Push the "Start" button. It will take about two hours to run a load of glassware through the dishwasher. Make sure that the DI water system is on while running the dishwasher.

When unloading the dishwasher, check one item (perhaps something small like a test tube) with bromthymol blue to insure that all traces of detergent have been rinsed from the glassware. Let a few drops of the indicator dye run down the inside of a **dry** item. If the dye remains yellow, the dishes are thoroughly rinsed. If the indicator turns blue, detergent has not been completely rinsed away. In this case, check to make sure that all "Options" are correctly set and that the DI water system is on, and run the load through again. Record bromthymol blue test results on the "Glassware Rinse Water Record". A master of this sheet is in Section 6 of this manual.

Oven

The oven is designed for drying at temperatures < 200°C. Operates with an on-off switch and a rheostat to control temperature. There is a vent on the oven, which should be kept open for desiccation. There is a manual on file for the oven.

Quebec Colony Counter

Prior to using the colony counter, wipe the glass surface and the magnifying lens clean of any dust. Zero the counter by depressing the button under the numerals. Turn on the light. Place a petri dish on the glass grid. Swivel the electric contact so that it is contacting the agar, preferably in a portion of the plate that does not disturb any colonies. The contact is on a magnetized base and can be moved to any location on the counter. The contact can also be moved within the base by loosening the set-screw.

- ◆ Adjust the magnifying lens by moving it in and out.
- ◆ Count colonies by touching each one with the pen-like contact. As the contact touches the agar it completes an electrical circuit and advances the counter.
- ◆ Record the total when all colonies have been counted in the standard plate count record book.
- ◆ Reset the counter.
- ◆ After using the counter turn off the light and wipe the contacts with alcohol.

Procedures for doing a Heterotrophic Plate Count are covered in Section 4 of this manual.

Spectrophotometer

The manual for this instrument is on file in the laboratory.

Operation

- ◆ Turn on spectrophotometer at lower left rear panel. Display example
 1. For 1 min. 301 SPEC
 2. Then 555 0.474*abs

Let warm up for 30 minutes.

Set optimal wavelength for solution to be tested.

This should be the transmittance minimum and the absorbance maximum for this solution.

(e.g. residual chlorine + DPD reagent = 515nm)

(wavelength $1\lambda=1\text{nm}=1\text{ nanometer} = .000000001\text{m}$)

- ◆ Press [5][1][5][GO TO λ] 1. Reads 515 0.625*abs

The instrument is in absorbance mode unless otherwise specified.

Set the test mode desired - transmittance, absorbance or concentration.

- ◆ Press [sec. func.][conc] 1. Reads 511 0.625*conc

For concentration mode testing, enter the factor (1/ab) to relate absorbance to concentration.

When the factor is an unknown value, proceed as follows:

- ◆ Add 3 ml. Blank solution to cuvette
- ◆ Insert cuvette into sample compartment with clear sides in front to back orientation
- ◆ Close door completely
 - ◆ Press [auto zero] 1. Reads 515 0.673*conc
2. Reads 515 0.000*conc
- ◆ Remove blank
- ◆ Insert cuvette with 3 ml. known standard
- ◆ Close door and Key in value e.g.[0.25]
- ◆ Press [sec. func.][conc] 1. Reads 515 0.250*conc
- ◆ To check factor and press [sec. func.][factor] 1. Reads Fct 3.549
- ◆ This factor is shown only when key held down
- ◆ Release both keys simultaneously to return to reading
- ◆ If [2nd] remains on display, hit [sec. func.] again

To test unknowns, proceed as follows:

- ◆ Remove standard solution
- ◆ Insert cuvette with 3 ml. unknown solution 1. Reads 515 ???*conc
- ◆ Read value as soon as numbers stabilize (usually instantaneously unless the value is very high)
- ◆ Readings may slowly drift over time due to monochloramines
- ◆ Repeat steps 1 through 3 for each additional unknown
- ◆ Factor will remain in place for an entire set of unknowns
- ◆ You may change between test modes with a sample in the compartment without disturbing the factor
- ◆ (for instance, to go to %T for high or low concentrations).

Notes

Optimal conditions exist if:

- ◆ Wavelength chosen is at the absorbance maximum for the solution being tested.
- ◆ Range of concentrations falls between 20-50% transmittance (10-70%T good).
- ◆ Standard solution concentration is in midrange of good slope of curve and also in general ranges of unknowns being tested.

False readings may occur if:

- ◆ Compartment door not completely closed (stray light).
- ◆ Fingerprints or dust are on the clear sides of the cuvette in the laser path.
- ◆ Bubbles or water spots are on the clear sides of the cuvette.
- ◆ Particles are not completely in solution.
- ◆ Samples have concentration values too high or too low.

Use standby mode to extend lamp life but leave instrument power on when not in immediate use by pressing [sig av][sec. func.][diag4].

➤ Relationship at absorbance maximum $C = A/ab$

Spectrophotometer Chlorine Standards

To make known chlorine standards

- ◆ Choose a standard value near the midrange of the expected values.
- ◆ Use the following formula to determine the amount of standard needed.
- ◆ $\frac{\text{desired concentration} \times \text{total volume}}{\text{chlorine concentration in kit}} = \text{volume standard to add}$

e.g. $\frac{0.25\text{mg/L} \times 25\text{ml}}{62.6\text{mg/l}} = 0.1\text{ml mg/L}$

- ◆ Add ddH₂O to a beaker. 24.9ml.
- ◆ Add one pack total DPD reagent and mix.
- ◆ Add chlorine standard from kit. 0.1ml.
- ◆ Let color develop for 3 minutes.
- ◆ Take 3ml of this solution and place in cuvette.
- ◆ Follow directions on spectrophotometer hints page.
- ◆ To compare results, take 10ml to use in colorimeter.

Chlorine Standard Sample Calculations

Desired Concentration mg/L	ml's ddH ₂ O	ml. standard at 62.6mg/L
0.100	24.96	0.040
0.250	24.9	0.100
0.500	24.8	0.200
0.750	24.7	0.300
1.000	24.6	0.400
1.250	24.5	0.500
1.500	24.4	0.600
1.750	24.3	0.700
2.000	24.2	0.800
2.250	24.1	0.900
2.500	24.0	1.000

Turbidimeter- Portable

A Hach 2100P turbidity meter is on hand in the lab. Cleanliness of the sample vials is critical when measuring low turbidities such as encountered in the water systems. Fill a sample cell to the line with a representative sample. Wipe the vial dry and clean with a soft, lint-free cloth. Do not touch the clean cell. Apply a thin layer of silicone oil to minimize the effects of any scratches on the sample vial. With the turbidimeter on a flat sturdy surface, turn it on and insert the sample cell. Orient the mark on the cell toward the mark on the instrument and close the cover. Press the "Read" button. Record the turbidity after the lamp symbol turns off.

Gelex standards are available in the lab to check the calibration of the instrument. Formazin is also available to do a recalibration if necessary. The recalibration procedure is detailed in the manual, which is on file in the laboratory.

Turbidimeter

- ◆ Turn on power and lamp switches
- ◆ Allow machine to warm up for thirty minutes
- ◆ Set range switch at 2 NTU for most readings
- ◆ Adjust zero knob to give a .00 reading on the display
- ◆ Place standard vial in the compartment and replace the cover (1 NTU standard)

- ♦ solution replaced monthly)
- ♦ Set the range switch to the next higher value than the standard value (2 NTU)
- ♦ Using the standardize knob adjust the reading on the display to match the standard value
- ♦ Record the zero and standard values in the turbidimeter log book. A sample of this is found in Section 6.
- ♦ Remove the standard vial and insert the sample vial and replace cover
- ♦ Set the range switch to maximum value without over-ranging
- ♦ Record the value of the sample
- ♦ Repeat steps 8, 9, and 10 for each subsequent sample
- ♦ Remove the final sample, replace cover and turn off the lamp and power switches.

Pocket Colorimeter

A Hach Colorimeter test kit is provided to test chlorine residuals in collected water samples, both treated and effluent. Prior to actual bacteriological sample collection, fill both vials to the 10ml mark with water from the sample tap.

For treated samples, add 1 packet of DPD-Free Chlorine reagent powder to one of the vials, and shake well.

- ♦ Set the colorimeter on a flat surface.
- ♦ Place dried blank vial in compartment with diamond facing toward screen.
- ♦ Cover with removable lid (this prevents light from effecting reading).
- ♦ Press **ZERO**.
- ♦ Immediately place the dried sample vial into compartment with diamond facing the screen and cover with lid.
- ♦ Press **READ** and record value on lab slip.

For effluent samples, use 1 packet of DPD-Total Chlorine reagent powder.

Rinse out vials and dry before placing in kit to avoid moisture damage to the instrument. The colorimeter will automatically turn off between uses.

Dissolved Oxygen Meter

The lab uses a YSI 58 dissolved oxygen (DO) meter with YSI 9505/9510-stirrer probe. Check the membrane and remove any condensation with a kimwipe. The instrument should be turned on (to "0.01mg/l") at least ½ hour prior to use in order to recondition the probe. Calibration with water saturated air must be done following this warm-up period. With the probe suspended in a BOD bottle with 2-3cm of water turn the selector knob to "Zero". Adjust to zero, if necessary. Select "Temperature" and note value. Turn selector knob to "0.01mg/l" and calibrate to the altitude-adjusted reading for that temperature. A table of temperature and altitude corrected O₂ readings are kept with the instrument. Record all calibration readings in the DO meter logbook. A sample page is found in Section 6.

If the instrument is giving unreliable readings, you may do one of three things to correct it depending on the length of time since last adjustment. The instrument must be turned off and the stirrer removed for all three procedures. You may remove the membrane cartridge and refill it with O₂ probe solution to get rid of bubbles. You may also gently sand the end of the probe to remove deposits and reuse the same cartridge. Or you may replace it with a new cartridge filled with O₂ solution if it hasn't been changed lately.

To take a DO measurement set the selector switch on "0.01mg/l" and immerse the probe into the sample. Turn on stirrer, if appropriate. When meter stabilizes record reading in the BOD worksheet book. A sample of this worksheet is found in Section 6. The manual and other literature on dissolved oxygen measurements are available in the lab.

Conductivity Meter

Conductivity is the ability of water to conduct electricity. It serves as a rough measurement of the amount of dissolved minerals in a sample.

- ◆ Turn on machine.
- ◆ Check calibration of the cell if necessary by measuring the value of the known standard at 25°C and adjusting the cell constant to give the correct standard value at that temperature. Alternatively, measure the value of the known standard at room temperature and adjust the cell constant to give the correct value from the chart at that temperature. For both of these methods, the temperature coefficient is set at zero. The cell constant will change with time. Adjust the constant if it varies +/- 0.5% from the standard value. Record calibration checks in the conductivity meter logbook. A sample is found in Section 6.
- ◆ If the constant requires a sizable adjustment, the probe may need cleaning or even replatinizing.
- ◆ Check chosen parameters of operation.
- ◆ Press the C key to check the cell constant (0.970 for initial value of probe)
- ◆ Press the TC key if necessary to set the temperature function to nLF.
- ◆ Press the TC key if necessary to set the reference temperature to Tref 25.
- ◆ Check to see if the auto range indicator is on (reads ARng in display area)
- ◆ Rinse probe with DI water, drain and place in sample to be tested along with the temperature probe.
- ◆ Read conductivity value by pressing X key and record on lab sheet.
- ◆ Repeat steps 5, and 6 for each additional sample to be tested.
- ◆ To store probe, rinse and place in DI water to cover electrodes.
- ◆ Do not store temperature probe in water.
- ◆ Turn off machine.

There is a manual for the conductivity meter on file in the laboratory.

Volumetric Dispenser

The lab uses a Wheaton Unispence to dispense media into tubes. The on/off switch is located on the back of the instrument. A few seconds after turning on the screen reads "Manual / 0". The manual mode can be changed to "Automatic" by pressing the labeled button. Pressing "Select" moves to the next menu item "Volume" which should read 10ml. Next is "Delay", the amount of seconds between each aliquot. Most users find 0.5-2.0 seconds to be the most convenient. The next menu item is "Tube size" which is 6.0mm. Pressing "Sel" one more time brings up "Count". Enter the number of tubes to be dispensed and press "Enter". Press "Auto/Man" until "Auto" and the number of tubes to be dispensed appear on the screen. Press "Start" to begin dispensing.

Each type of media should be dispensed through its own tube. When tubes are changed the instrument must be recalibrated. To calibrate, place an empty 250ml beaker on a scale and zero. Dispense 100ml of DI water and reweigh. The weight in grams is close to the volume in milliliters. If the volume being dispensed is more than 1.0ml (1.0g) off, enter the delivered

volume and press "Vol calb". Empty the 250ml beaker, re-zero, and dispense another 100ml. Weigh again. Repeat procedure until the desired volume is being dispensed. Rinse tubing with DI water after each use.

Heatblock

To use the heatblock for Sterikon bioindicator testing, preheat it to 60°C and record temperature on record sheet. After autoclaving 2 ampules/autoclave, place these along with an unautoclaved control ampule in the heatblock slots for 48 hours. Record results in the autoclave logbook and turn off the heatblock. The autoclaved ampules should remain purple showing no growth, while the control should turn yellow indicating growth.

SECTION 4: PROCEDURES

Many of the procedures outlined in this section of the Lab Manual are taken from Standard Methods for the Examination of Water and Wastewater, 18th Edition (1992). Other reference material that may be of use includes:

- Microbiological Methods for Monitoring the Environment, Water and Wastes (EPA-600/8-78-017) (1978)
 - Difco Manual, Tenth Edition (1984)
 - Distribution System Bacteriological Sampling Control and Guidelines, AWWA (1978)
-
- ✓ Effluent samples will be analyzed by 15 tube MTF (pp22-25)
 - ✓ Source water samples will be analyzed by Colilert Quanti-tray and HPC (p26; p27)
 - ✓ Well samples will be analyzed by Colilert Quanti-tray and HPC (p26; p27)
 - ✓ Filtered samples will be analyzed by HPC (p27)
 - ✓ Drinking water samples will be analyzed by Colilert P/A (p25)

These and other applicable materials are kept on file in the laboratory.

Water Lab Chores

DAILY:

- Check and record temperatures on all incubators twice a day
- Check DI water condition
- Check pH probe's KCl level
- Autoclave any large amount of contaminated wastes
- Clean all surfaces in the lab with EtOH
- Drain and clean the autoclave, if used
- Log any missing, broken or needed supplies

WEEKLY:

- Transfer stock TSB cultures
- Compile any necessary orders
- Prepare media for the following weeks' samples (2 wk. Shelf-life) and record in media log book
- Drain and clean the water baths
- Autoclave all wastes present in lab on Friday

MONTHLY:

- Clean all incubators with EtOH
- Change membrane cartridge on DO meter
- Start new standard cultures in TSB tubes (monthly)
- Compile any necessary monthly orders
- Damp mop lab floors
- Recalibrate balances
- Run standard plate count of DI water
- Prepare assorted media and agar as needed
- Distribute vials as needed to sub-districts
- Distribute water and wastewater log sheets as needed to sub-districts

QUARTERLY:

- Check calibration of thermometers in incubators
- Perform alkalinity tests on water sources

YEARLY:

- Send master thermometer to be calibrated
- Arrange for seasonal water sampling training

AS NEEDED:

- Sterility checks on sample vial and Quanti-tray lots
- QC checks on Colilert reagents

Sample Collection

Sample Point Selection

- ✓ Choose a faucet that will provide a representative sample of the water circulating in the distribution system. A faucet supplied from a main distribution pipeline is best. Avoid sampling from a dead end main where the water may be old and stagnant. A distribution system map is a very helpful reference when selecting sampling locations.
- ✓ Select faucets that are in frequent use. The pipes supplying a faucet that has not been used for weeks or months contain stagnant water and sediment, which may provide a breeding ground for bacteria. This material is difficult to flush out and a sample from such a faucet may not truly indicate the quality of the water in the system at the present time.
- ✓ Inspect the faucet before collecting the sample and avoid any faucet that is dusty, dirty, or corroded. Inside faucets are desirable because they are likely to be cleaner than an outside faucet, but they have the disadvantage of being more inaccessible. Therefore, many water system operators sample from faucets on the outside of buildings and this practice has proven satisfactory when the faucet is carefully chosen.
- ✓ Avoid a faucet that leaks water around the stem. The leaking water may run down the outside of the faucet and contaminate the sample by dripping into the bottle. This is a frequent cause of positive samples. For the same reason avoid faucets where water "curls" back up onto the threads at the mouth of the faucet.
- ✓ Do not use a faucet that is close to the ground where splashing spray could get into the bottle. Neither should shrubbery or tall grass surround the faucet. Dust and bacteria on the vegetation are stirred up, getting to the faucet and could contaminate the sample.
- ✓ The sample faucet should discharge downward. It is nearly impossible to obtain a satisfactory sample when the faucet discharges vertically upward and the sample is questionable when the faucet discharges at an upward angle or to the side. Drinking fountains and water coolers that produce a smooth stream at an upward angle are sometimes satisfactory for sampling but must be chosen carefully. Most fountains have a spring-loaded faucet that makes sample collection especially awkward and inconvenient.
- ✓ Never collect a sample from a hose or any other temporary attachment fastened to the faucet.
- ✓ Do not collect samples from fire hydrants, corporation stops, or plug valves. It is usually impossible to obtain a smooth flow of water from such valves.
- ✓ Do not use a faucet that is wrapped with insulation to protect against freezing.
- ✓ Kitchen and bathroom faucets are frequently equipped with aerators and/or screens. DO NOT sample from these faucets. If no alternative is available, remove the aerator and screen and let the faucet flow for several minutes before collecting the sample. Be suspicious of swing-type faucets. Contamination could be sucked into the faucet through the swing connection.
- ✓ Be sure to sample the cold water faucet, not the hot water.
- ✓ Avoid faucets that are supplied through a water softener, a charcoal filter, or any other type of household filter or purification device. These devices frequently become breeding grounds for bacteria and other organisms that may actually contaminate the water flowing through it.

Use of Sample Vials

1. Identify source by writing on the cap with a waterproof felt-tip pen.
2. Turn on tap to full flow for 3-5 minutes to flush line back to main.
3. Reduce flow to pencil size stream.
4. Remove seal and open vial. DO NOT touch inside of top or inside of vial when opening.
5. Grasp vial and insert into flow stream with upward motion.
6. Fill to the EPA 100ml line.
7. Remove from flow stream. DO NOT let water flow touch hand.
8. Replace cap tightly.
9. Check seal effectiveness by inverting vial and checking for visible leaks. While vial is inverted, give it a couple of shakes to remove any water that has collected on rim.
10. Complete lab sheet and send with sample vial.
11. Dispatch sample to lab or pick-up point by usual means in an ice chest with blue ice.
Samples should reach the lab no more than **six** hours after collection, if at all possible.

A memorandum detailing how to fill out information on sample bottles and lab slips is found in Section 7 of this manual.

Media Preparation

Careful media preparation is necessary for meaningful bacteriological testing. Attention must be given to the quality, measuring, mixing and sterilization of the ingredients. The purpose of this care is to assure that if the bacteria being tested for are indeed present in a sample, every opportunity is present for their development and ultimate identification. Much bacteriological identification is done by noting changes in the medium; consequently, the composition of the media must be standardized. Much of the tedium of media preparation can be avoided by purchase of dehydrated media (Difco, BBL, or equivalent). The operator is advised to make use of these products or, if only a limited amount of testing is to be done, consider using tubed, prepared media.

Quality Control

All glassware must be thoroughly cleaned using a suitable detergent and hot water (70°C), rinsed with hot water (80°C) to remove all traces of residual detergent, and finally rinsed with distilled water **12 TIMES**. This is accomplished during the run cycle of the dishwasher.

Use only distilled water, which has been tested and found free from traces of dissolved metals for preparation of culture media.

For each batch of media that is made, the following checks will be run: a sterile, a positive (+), and a negative (-). These will be inoculated as soon as possible after the sterilized media has cooled. The pH also will be checked after autoclaving and recorded in the media logbook.

Stock Cultures

In order to run the above quality check, five different stock cultures are kept on hand.

Enterobacter aerogenes
Escherichia coli
Pseudomonas aeruginosa

To start a new set of cultures, prepare 100ml of Tryptic Soy Broth (3.00g of dehydrated media in 100ml of water). Fill each of five disposable culture tubes (18x150mm) with 15ml of prepared media and pipette remaining 25ml of TSB into a 100ml beaker. Cover containers and autoclave at 121°C for 15 minutes (45-min. cycle). When cooled, record pH of media in beaker.

Label and date each tube. Take one dehydrated bacteria disk from its stock bottle and drop into the corresponding tube. This is most easily done by heating an inoculating needle in a flame and, while hot, touching one of the disks. The disk will stick to the hot needle and can be brushed off into the culture tube. To avoid contamination, sterilize the needle between different bacteria. If using the Bactrol Plus cultures, take 0.25ml sterile TSB from culture tube, mix gently in vial and add contents back into tube.

Every week to ten days, prepare a new batch of Tryptic Soy Broth as directed above. Label and date the tubes. Transfer the cultures from the old tubes into the new ones by dipping an inoculating loop into the old culture and swirling it in the new one. Discard the loop after each tube has been transferred. Autoclave and discard the old cultures and tubes.

After several transfers (once a month) discard the stock cultures and start fresh ones as directed above.

Preparation of Media

As discussed above, media must be prepared in glassware that is thoroughly washed and rinsed. This is to avoid any possible contamination of the media with chemicals that might promote or retard bacterial growth. Similarly, use only **reagent** grade water.

Determine the amount of media you will be preparing. Remember to add 50ml to the total - 20ml for the pH check and 30ml for the three quality control tubes. Choose a beaker of sufficient volume so it will not be more than **half** full.

Determine the weight of dehydrated media needed by multiplying the volume desired (in **liters**) times the weight of media required to prepare one liter. For example, you want 600ml of EC media. It takes 37.00g of dehydrated media to make one liter of EC broth. Since you want only 600ml of prepared media, multiply 37.00g by 0.6 (600ml = 0.6liter). You need to measure out 22.20g ($37.00 \times 0.6 = 22.20$) of dehydrated media.

Weigh out the correct amount of media, using the Ohaus scale and a weighing boat. Put the dehydrated media into the appropriate beaker, add a stirring stone, and the correct volume of water. If opening a new container of media, be sure to date it. Invert the media container when placing it back on the shelf.

Place the beaker on a hot plate. Turn on the stirring bar so that it is rotating nicely. Turn on the heat to a fairly low setting to speed up the dissolving of the media. Check to be sure that no undissolved media is sticking to the sides of the beaker.

While media is dissolving, set up tubes in racks. Add inverted vials if necessary. Pipette media into culture tubes, cap and autoclave as directed below.

After cooling, check for the presence of bubbles in vials for those tubes that have them. Discard any tubes with bubbles. Check to be sure that caps on screw-top tubes are tight. Culture tubes with loose-fitting caps can be stored in a cool, dark cupboard for up to two weeks. Screw-cap tubes can be stored under similar conditions for up to three months. Label and date all media prior to storage.

The following is a list of the most commonly used media and the amounts for each. Remember:

- USE ONLY REAGENT (DI) WATER
- RECORD THE DATE WHEN BOTTLE IS OPENED
- INVERT MEDIA BOTTLE AFTER REMOVING NEEDED MEDIA

Lauryl Tryptose Broth (LTB)

1x 35.60g/1000ml
17.80g/500ml
8.90g/250ml

2x 71.20g/1000ml
35.60 g/500ml
17.80 g/250ml

Sterilize 15 min. at 121°C, 15 psi

Brilliant Green Bile (BGB)

1x 40.00g/1000ml
20.00g/500ml
10.00g/250ml

Sterilize 15 min. at 121°C, 15 psi

EC

1x 37.00g/1000ml
18.50g/500ml
9.25g/250ml

Sterilize 15 min. at 121°C, 15 psi

Standard Plate Count Agar (SPCA)

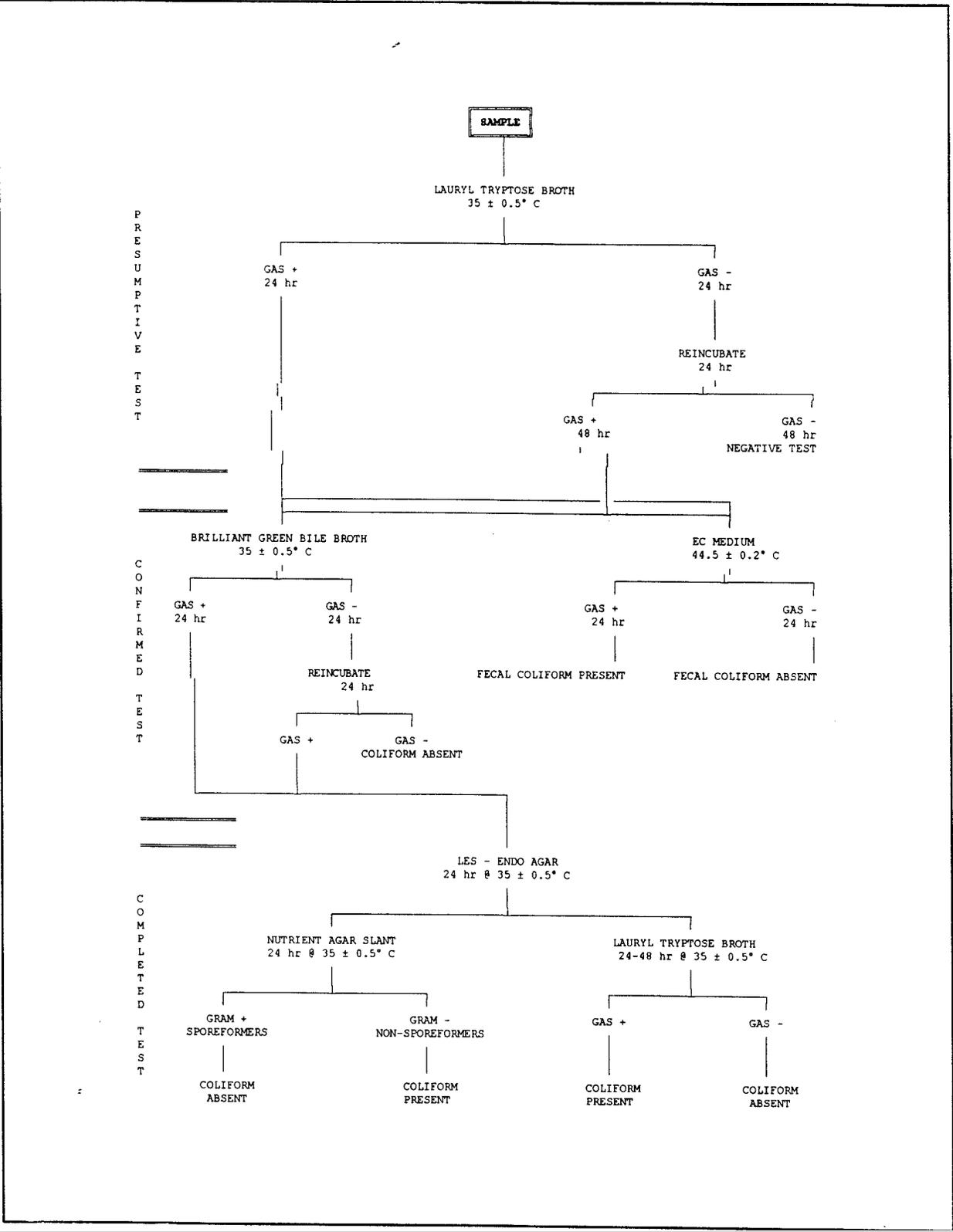
1x 23.50g/1000ml
11.75g/500ml
5.87g/250ml

Sterilize 15 min. at 121°C, 15 psi

Tryptic Soy Broth (TSB)

1x 30.00g/1000ml
15.00g/500ml
7.50g/250ml

Sterilize 15 min. at 121°C, 15 psi



Multiple Tube Fermentation Process

Test for Coliform Bacteria

Much of the information in this section of the manual is taken from Standard Methods..., Edition 18, Section 9221 (p. 9-45) and Microbiological Methods for Monitoring the Environment, Water and Waste, p. 114. It is strongly recommended that the beginning laboratory technician study these sources in addition to the following discussion. Even experienced technicians may want to review this material from time to time.

General Discussion

The test for coliform bacteria is used to measure the suitability of a water for human use. The bacteria detected by this test are normally found in the intestinal tract of humans and other mammals. These bacteria are therefore present in sewage, numbering as many as 1,000,000 per milliliter. Coliform bacteria are considered to be harmless. However, their presence does indicate the possible presence of other pathogenic organisms. As a consequence, when coliform bacteria are found, the water is suspected of being polluted by human waste discharge.

Depending on the use of the water, standards are established for the numbers of coliform bacteria permissible in a given volume of that water. For example, a safe bathing water standard would not be as strict as a safe drinking water standard. The test is not only useful in determining the bacterial quality of a finished water, but also can be used by the operator in the treatment plant as a guide in achieving a desired degree of treatment.

The test for coliforms is a three-step process consisting of the presumptive phase, the confirmed phase, and the completed phase. The entire process is outlined in Figure 2.

Multiple-tube Fermentation Technique

Coliform bacteria are detected by placing an aliquot of the sample in Lauryl Tryptose Broth (LTB). The coliform bacteria are those which will grow in this medium producing gas by fermenting available sugars at 35°C within 48 hours. Thus, to detect these bacteria, the operator need only inspect fermentation tubes for gas. Coliform bacteria also change the pH of the media. An alternative method for detecting the growth of coliform bacteria involves adding bromocresol purple to the LTB media. A change in color to yellow indicates increasing acidity and the possible presence of lactose fermenting bacteria. In this lab, the use of fermentation tubes is the preferred method.

In practice, several fermentation tubes are used for each sample. Treated water samples may be tested by adding 10ml samples to ten tubes of double strength LTB. Effluent samples are subjected to a 15 tube dilution series (five tubes at each of three decimal dilutions). The most frequently used series involves 10ml, 1.0ml and 0.1ml aliquots of sample.

Recording of Most Probable Number

An estimation of the coliform bacterial density can be determined from the number of positive tubes in each series. This estimation is referred to as the **Most Probable Number (MPN)** and is reported as the number of coliform group organisms per 100ml of sample. It must always be kept in mind that this is strictly a statistical figure and not a precise enumeration.

Tables of MPN's are kept accessible in the laboratory and as Figure 3 in this manual. Additional tables with 95% confidence levels can be found on pages 9-49 and 9-50 of Standard Methods..., 18th Edition. A glance at the 95% confidence level will reveal how imprecise the MPN is.

To determine the MPN, record the number of positive tubes, then look up the number on the chart. For the ten-tube test of treated water samples, this is straightforward. For the fifteen tube dilution series it becomes a bit more complicated. Record the positive tubes for each five-tube dilution separately and report them starting from the largest sample to the smallest. Usually, the dilution series will consist of aliquots of 10ml, 1.0ml and 0.1ml. If all five of the 10ml tubes turn positive (presence of gas), three of the 1.0ml tubes, and one of the 0.1ml tubes are positive, this is reported as 5-3-1. This gives a MPN value of 110 from the chart.

The quantities indicated at the heads of the columns are for the common 10.0-1.0-0.1ml series of aliquots. The same values may be used in computing the MPN in larger or smaller portion plantings in the following manner: if, instead of 10.0-1.0-0.1ml portions, a combination of 100.0-10.0-1.0ml is used, the MPN is recorded as 0.1 times the value given in the tables. If, on the other hand, portions of 1.0-0.1-0.01ml are planted, the value recorded would be 10 times that given in the tables.

The general formula for determining the MPN value is:

$$\text{MPN}/100 \text{ ml} = \text{MPN value (from table)} \times (10/\text{largest volume tested})$$

When more than three dilutions are employed in a decimal series, the results from only three consecutive dilutions are used in determining the MPN. To select which three dilutions to use, the highest dilution (smallest sample volume) which gives positive results in all five tubes tested (no lower dilution giving any negative results) and the next succeeding higher dilutions should be chosen. The results at these three dilutions should then be used in computing the MPN. For a complete discussion on determining MPN when more than three dilutions were tested, see Standard Methods..., 18th Edition, page 9-49 and following.

Presumptive Phase

The presumptive phase uses ten tubes of double strength LTB for each treated water sample or a fifteen tube dilution series for each effluent sample. Prepare the double strength LTB [LTB (2X)] as directed above and pipette 10ml portions into 18x150mm culture tubes with inverted ½ dram vials in the bottom. Cap tubes. Prepare single strength LTB [LTB (1X)] as directed above and pipette 10ml portions into 16x125mm tubes with inverted ¼ dram vials in the bottom. Cap tubes. Autoclave all tubes at 121°C for 15 minutes. After cooling, check the vials for bubbles (does not happen often). Discard if bubbles present.

For each treated water sample set up a row of ten LTB (2X) tubes. Label the first tube of each row with the lab number (and location, if desired) of the sample to be tested. For each raw, filtered or effluent sample set up a row of five LTB (2X) tubes and two rows of five LTB (1X) tubes. Label the first tube of each row with the lab number of the sample. Also indicate the amount of sample being tested on the 1X tubes.

Shake each sample bottle approximately 25 times. Add 10ml of sample to each of the treated tubes (a total of 100ml of sample). For the dilution series add 10ml of sample to each of the 2X tubes. Switch to a 1.0ml pipette. Add 1.0ml of sample to each of the first row of 1X tubes and 0.1ml of sample to each of the second row of 1X media (a total of 55.5ml of sample).

Place the inoculated tubes into a 35°C incubator. After 24 ± 2 hours, check all sample tubes for the presence of gas. Record results on the lab slip. Treat all positive tubes to the confirmatory phase discussed below. Return all other tubes to the incubator. After 48 ± 3 hours (from start

of test), check again. Record results and transfer all positive tubes. This completes the presumptive phase of the test.

Autoclave all LTB tubes for 30 minutes at 121°C to destroy any bacteria growing in the cultures. Discard medium and vial in each tube. Wash tubes in the dishwasher.

Notification of Positive Test

If any **treated** (drinking water) samples are positive during the presumptive phase, notify the appropriate people in the Park, at Region and at the Public Health Service. A list of people and phone numbers is found in Section 1 of this manual.

Repeat Sample vs. Resample

If any treated samples are positive, **three** repeat samples must be obtained immediately. These samples should come from the positive sample point, plus one upstream and one downstream sample, if possible. Repeat sample points are specified in the Water Systems Manual. Additionally, there must be **five** samples from this water system during the month following a positive test. This will mean additional sampling for the smaller systems.

If any treated samples exhibit turbid (excessively cloudy) tubes during the presumptive test, a resample from the same sampling point must be obtained immediately. Resampling will continue until the turbidity problem is resolved.

Notification and resampling procedures are given in the flow chart found in Figure 1 of Section 1.

Confirmed Phase

Cultures from each positive tube from the presumptive phase will be transferred to tubes of BGB and EC media. Prepare media as directed above and pipette 10ml portions into screw-top culture tubes with inverted ¼ dram vials. Autoclave at 121°C for 15 minutes. After cooling, check for bubbles in vials.

A culture from each positive LTB tube is transferred to both a BGB and an EC tube. Label each tube with the lab number, original sample amount (not necessary for treated samples), and number of tube in series. Dip a sterile swab or inoculating loop at least 3-5mm into the LTB tube and swirl it in an EC tube. Repeat to inoculate a BGB tube. Always transfer to the EC tube first, then the BGB. Autoclave and discard medium and vials in all LTB tubes.

When all transfers are done, incubate the BGB tubes at 35°C. Check after 24 ± 2 hours and 48 ± 3 hours for the presence of gas. EC tubes go into the water bath at 44.5°C. Make sure the water level is deep enough to at least cover the media in the tubes. Incubate for 24 ± 2 hours only and check for the presence of gas. Record results on the lab slip. The MPN for the BGB tubes is recorded as total coliforms, while the MPN for the EC tubes is for fecal coliforms.

At the end of this phase, autoclave and discard all EC and BGB tubes. Screw-top culture tubes are not reusable.

Completed Phase

Double confirmation into BGB media for total coliforms and into EC broth for fecal coliforms (see Section 9221E of Standard Methods, 18th Edition, pp.9-52 to 9-53) is used. Consider positive EC broth elevated temperature (44.5C) results as a positive completed test response for fecal coliforms. (See Section 9221 B.3 on page 9-47 of Standard Methods, 18th Edition)

MPN Calculation Chart

0-0-0	<2	3-0-0	8	5-0-0	23	5-3-3	170
0-0-1	2	3-0-1	11	5-0-1	30	5-4-0	130
0-1-0	2	3-1-0	11	5-0-2	40	5-4-1	170
0-2-0	4	3-1-1	14	5-1-0	30	5-4-2	220
		3-2-0	14	5-1-1	50	5-4-3	280
1-0-0	2	3-2-1	17	5-1-2	60	5-4-4	350
1-0-1	4			5-2-0	50	5-5-0	240
1-1-0	4	4-0-0	13	5-2-1	70	5-5-1	300
1-1-1	6	4-0-1	17	5-2-2	90	5-5-2	500
1-2-0	6	4-1-0	17	5-3-0	80	5-5-3	900
		4-1-1	21	5-3-1	110	5-5-4	1600
2-0-0	4	4-1-2	26	5-3-2	140	5-5-5	>1600
2-0-1	7	4-2-0	22	0	<1.1	5	6.9
2-1-0	7	4-2-1	26	1	1.1	6	9.2
2-1-1	9	4-3-0	27	2	2.2	7	12.0
2-2-0	9	4-3-1	33	3	3.6	8	16.1
2-3-0	12	4-4-0	34	4	5.1	9	23.0
						10	≥23.0

Colilert Test

Colilert Reagent is used for the simultaneous detection and confirmation of total coliforms and *E. coli* in water. Colilert utilizes nutrient indicators that produce color and/or fluorescence when metabolized by total coliforms and *E. coli*. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 CFU/100ml within 24 hours with as many as 2 million heterotrophic bacteria/100ml present. The presence/absence test is used for drinking water samples and the quanti-tray enumeration procedure is used for raw water samples.

Presence/Absence Test Procedure

- ◆ Carefully separate one Snap Pack from the strip taking care not to accidentally open adjacent pack.
- ◆ Tap the Snap Pack to ensure that all of the Colilert powder is in the bottom part of the pack.
- ◆ Open one pack by snapping back the top at the scoreline.
- ◆ Add the reagent to the water sample in a sterile, transparent, non-fluorescent vessel.
- ◆ Aseptically cap and seal the vessel.
- ◆ Shake until dissolved.
- ◆ Incubate for 24 hours at 35°C ±0.5°C.
- ◆ Read the results at 24 hours. Compare each results against the comparator dispensed into an identical vessel.
 - If no yellow color is observed, the test is negative. Check vessel for fluorescence by placing a 6 watt 365nm UV light within 5 inches of the sample in a dark environment. If fluorescent, indicates *shigella*-type organisms.
 - If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If color is not uniform, mix by inversion then recheck.
 - If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (but no more than 28 hours total). If the sample is coliform positive, the color will intensify. If it does not intensify, the sample is negative.

- If yellow is observed, check vessel for fluorescence by placing a 6 watt 365 nm UV light within 5 inches of the sample in a dark environment. Be sure the light is facing away from your eyes and toward the vessel. If fluorescence is greater or equal to the fluorescence of the comparator, the presence of *E. coli* is confirmed.

Quanti-Tray Enumeration Procedure for 100ml Sample

- ◆ Pour the sample reagent mix from step 6 above directly into the tray avoiding contact with the foil tab, and then seal the tray according to Quanti-Tray instructions.
- ◆ Incubate for 24 hours at 35°C ±0.5°C.
- ◆ Follow the same interpretation directions from step 8 above to count the number of positive wells. Refer to the MPN table provided with the Quanti-Tray to determine the Most Probable Number (MPN) of total coliforms (yellow wells) and *E. coli* (fluorescent wells) in the sample. The color and fluorescence of positive wells may vary.

Procedural Notes

- ◆ If an inoculated Colilert sample is inadvertently incubated over 28 hours, the following guidelines apply: Lack of yellow is a VALID NEGATIVE TEST. A yellow color after 28 hours is not a valid and should be repeated or verified.
- ◆ Some water samples containing humic materials may have an innate color. If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.
- ◆ Use sterile water, not buffered water for making dilutions. Colilert is already buffered. Always add Colilert to the proper volume of diluted sample after making dilutions.
- ◆ Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.

Quality Control Procedure

Quality control should be conducted on each lot of Colilert, or more often as regulations require. Inoculate sterile water (100ml) with the Quanti-Cult or American Type Culture Collection (ATCC) bacteria listed below. Follow the above test procedure, and compare test results to the expected results below.

<u>Quanti-Cult Organism</u>	<u>ATCC #</u>	<u>Expected Result</u>
<i>E. coli</i>	25922 or 11775	yellow, fluorescent
<i>Klebsiella pneumoniae</i>	31488	yellow, not fluorescent
<i>Pseudomonas aeruginosa</i>	10145 or 27853	clear, not fluorescent

Heterotrophic Plate Count

The Heterotrophic Plate Count (HPC) is a method to estimate the total number of live heterotrophic bacteria present in a sample. There are several variations of this test which are covered in Standard Methods..., 18th Edition, Section 9215, page 9-32. The most commonly used method in this lab is the pour plate method using Standard Plate Count Agar (SPCA).

Preparation of Plates

Prepare SPCA and pipette 10-12ml portions into screw-top culture tubes. Autoclave for 15 minutes at 121°C, then store in 5°C refrigerator until needed. To use, place desired number of tubes in a beaker and fill with water. Bring to a boil to completely melt the agar. Allow to cool in the water to 44-46°C. At this temperature the agar is still liquid but does not present a severe

thermal shock to any bacteria in the sample.

Prepare two (duplicate) plates for each sample. Label the plates with lab number, sample location, date and amount tested. Pipette sample, usually 1.0 or 0.1ml (never more than 2.0ml of sample), into petri dish. The goal is to have 30-300 colonies per sample. This may require diluting some samples. Never allow more than 20 minutes between time that the sample is placed in the petri dish and agar is added. Uncap tube of agar and pour into petri dish, lifting lid as little and as quickly as possible. Thoroughly mix agar and sample by gently swirling dish in a figure-eight pattern. Avoid splashing agar up sides or onto lid of dish. Allow agar to cool and solidify.

When cool, **invert** plates and place in $35 \pm 0.5^\circ\text{C}$ incubator for 48 hours. If plates must be stored for a longer time before being counted, remove from incubator and place in 5°C refrigerator. Every effort should be made to count plates after 48 hours. As a control, duplicate air plates will be run with each batch.

Counting Colonies

Since colonies can grow from a single bacterium or clumps or chains of bacteria, the number of colonies is reported as colony forming units (CFU) per ml of sample.

After 48 hours, count all colonies with the Quebec colony counter and record counts as CFU/ml. If any plates have no colonies, record as < 1 CFU/ml. If the number of colonies is greater than 300 but less than 10 colonies/cm² (570 colonies with plastic petri dishes), count colonies in 13 representative squares and multiply by 5 (65cm² glass dishes) or 4.4 (57cm² plastic dishes) and record as **estimated** CFU/ml. If there are significantly more than 10 colonies/cm², count four representative squares, average count per square, and multiply by 65 for glass plates or 57 for plastic plates. Record as **estimated** CFU/ml.

If some sample volume other than 1.0 ml is used, compute the heterotrophic plate count in CFU/ml by multiplying the average number of colonies per plate by the reciprocal of the dilution (volume) used. For example, if only 0.1 ml of sample is plated, multiply the count by 10 ($1/0.1 = 10$).

Round-off and record only the left-hand two digits, e.g. 142 = 140, 1763 = 1800, 52 = 52. Round up with "5", e.g. 265 = 270. Average plate counts for each sample. Record plate counts on the lab slip and in the log.

Total Alkalinity

Apparatus: micropipetters
pH meter
Stirrer

Reagents: A. 1N H₂SO₄ (28 ml conc. H₂SO₄ diluted to 1 L)
B. 0.2N H₂SO₄ (200 ml 1N H₂SO₄ diluted to 1 L)

- ◆ Measure 100ml of sample into a clean 250-ml beaker with small stirring stone.
- ◆ Place pH probe in the beaker. Determine and record initial pH.
- ◆ Titrate with 0.2N H₂SO₄ to a pH of 4.50. Add small amount of H₂SO₄ to sample. Stir and determine resulting pH. As target pH nears, use very small amounts of acid.
- ◆ Record amount of acid used to reach a pH of 4.50.

- ◆ Calculation for total alkalinity as calcium carbonate in mg/L:

$$\text{Alkalinity (mg/L)} = \left(\frac{\text{mlH}_2\text{SO}_4 * N * 50,000}{\text{mlSample}} \right)$$

or substituting the values used in the above titration:

$$\text{Total alkalinity (mg CaCO}_3\text{/L)} = \text{ml titrant} \times 100$$

- ◆ Record values on lab slip.

Solids Testing

Total Dissolved Solids

A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated to dryness in a weigh dish and dried to constant weight at 180°C. The increase in dish weight represents the total dissolved solids.

Apparatus:

Glass-fiber filter disks w/o organic binders
Filtration apparatus
Suction Flask
Drying Oven 180 ± 2°C

Procedure:

- ◆ Insert filter disk with wrinkled side up into filtration apparatus.
- ◆ Apply vacuum and wash disk with three successive 20ml aliquots of DI water.
- ◆ Continue suction to remove all traces of water.
- ◆ Discard washings.
- ◆ Heat clean dish to 180 ± 2°C for one hour in an oven.
- ◆ Store in desiccator until needed.
- ◆ Weigh immediately before use.
- ◆ Choose sample volume to yield between 2.5 and 200mg dried residue.
 - ◆ If more than ten minutes are required to complete filtration, increase filter size or decrease sample volume but do not produce less than 2.5mg residue.
- ◆ Filter measured volume of well-mixed sample through glass-fiber filter.
- ◆ Wash with three successive 10ml aliquots of DI water, allowing complete drainage between washings, and continue suction for about three minutes after filtration is complete.
- ◆ Transfer filtrate to a weighed evaporation dish and evaporate to dryness on a steam bath.
- ◆ If filtrate volume exceeds dish capacity add successive portions to the same dish after evaporation.
- ◆ Dry dish for at least one hour in an oven at 180 ± 2°C
- ◆ Cool in a desiccator to balance temperature, and weigh.
- ◆ Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until weight loss is less than 4% of previous weight or 0.5 mg, whichever is less.

Calculation:

$$\text{Total dissolved solids (mg/L)} = \left\{ \frac{(A - B) * 1000}{mlSample} \right\}$$

where: A = weight of dried residue + dish (mg)

B = weight of dish (mg)

Total Suspended Solids

A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103-105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, the difference between the total solids and the dissolved solids may provide an estimate of the total suspended solids. In sample preparation, exclude large floating particles or submerged agglomerates of nonhomogeneous materials if it is determined that their inclusion is not desired in the final result. Because excessive residue on the filter may form a water-entrapping crust, limit the sample size to that yielding no more than 200mg residue. For samples high in dissolved solids thoroughly wash the filter to ensure removal of the dissolved materials. Prolonged filtration times resulting from filter clogging may produce high results owing to excessive solids captured on the clogged filter.

Apparatus: Same as for total dissolved solids
Watch glass

Procedure:

Insert filter disk with wrinkled side up into filtration apparatus. Apply vacuum and wash disk with three successive 20ml aliquots of DI water. Continue suction to remove all traces of water. Discard washings.

Remove filter from filtration apparatus and transfer to a watch glass as a support. Dry at 103-105°C for 1 hour. Cool in the desiccator to balance temperature and weigh. Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until weight loss is less than 0.5mg between successive weighings. Store in desiccator until needed. Weigh immediately before use.

Filter measured volume of well-mixed sample through glass-fiber filter. Wash with three successive 10ml aliquots of DI water, allowing complete drainage between washings. Continue suction for about three minutes after filtration is complete. Carefully remove filter from filtration apparatus and transfer to a watch glass as a support. Dry for at least one hour in an oven at 103-105°C, cool in a desiccator to balance temperature, and weigh. Repeat cycle of drying, cooling and weighing until a constant weight is obtained or until weight loss is less than 4% of previous weight or 0.5mg, whichever is less.

Calculation:

$$\text{Total suspended solids (mg/L)} = \left\{ \frac{(A - B) * 1000}{mlSample} \right\}$$

where: A = weight of filter + dried residue (mg)

B = weight of filter (mg)

Settleable Solids

Settleable solids in surface and saline waters as well as domestic and industrial wastes may be determined and reported on either a volume (ml/L) or a weight (mg/L) basis.

Apparatus: For volumetric determination - Imhoff cones
For gravimetric determination - All equipment for suspended solids and a glass vessel with a minimum diameter of 9 cm.

Procedure:

Volumetric: Fill an Imhoff cone to the 1 liter mark with a well-mixed sample. Settle for 45 minutes. Gently stir sides of cone with a rod or by spinning and let settle 15 minutes longer. Record volume of settleable solids in the cone as ml/L. If the settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from volume of settled solids. The practical lower limit of measurement depends on sample composition and generally is in the range of 0.1-1.0ml/L. Where a separation of settleable and floating material occurs, do not estimate the floating material as settleable matter.

Gravimetric: Pour a well-mixed sample into a glass vessel. Let stand quiescent for one hour. Without disturbing the settled or floating material, siphon 250ml from center of container at a point halfway between the surface of the settled material and the liquid surface. Determine total suspended solids (mg/L) of this supernatant liquor (see above). These are the nonsettleable solids.

Calculations:

$$\text{Settleable solids (mg/L)} = A - B$$

Where: A = Total suspended solids (mg/L)

B = Nonsettleable solids (mg/L)

Biological Oxygen Demand (BOD)

Supplies & Materials

300ml BOD bottles
1ml & 10ml serological pipette
BOD pillow for 3 liters, 300ml
4 liter carboy with spigot
Glucose Glutamic Acid Ampule 300mg/l
Polybac Seed Pillow
Distilled water
Dissolved oxygen Meter
Pipetmen and tips
pH meter
Potassium iodide solution 100g/l as KI
Sulfuric acid 0.02N
Sodium thiosulfate solution 0.025N
Starch indicator solution

Dilution Water Preparation

- Collect 3 liters of distilled water in carboy.
- Add contents of 1 3L BOD pillow.
- Mix carboy vigorously for 1 min.
- Let stand 48 hours in incubator @ 20°C.
- **DO NOT REMIX** when you go to use the water.

PolyBac Seed

Add Polybac seed to appropriate amount of BOD nutrient buffer solution. This is usually between 500 and 1000ml. buffer depending on the strength of the seed. Ideal DO uptake of seed water should be 0.6 to 1.0mg/L. Stir and aerate the mixture for at least one-hour, but not more than six hours. Turn seed flask off fifteen minutes before use.

Sample Collection

Collect samples first thing in the morning.
Place samples in 20°C incubator.

IT IS IMPORTANT THAT SAMPLES BE AS CLOSE TO 20°C AS POSSIBLE.

Worksheet Preparation

- ◆ Turn on the Dissolved Oxygen Meter 30 minutes before you are ready to read DO.
- ◆ Fill out the worksheet prior to putting samples in the bottles. (An example of the worksheet is found in Section 6 of this manual.)
- ◆ The top left box is important information. Take the time to fill it out completely and correctly. In the top right box fill in the date the samples come out. With this information one quick glance allows the lab tech to see when the test needs to be read.
- ◆ Fill out the bottle numbers and sample sizes. Complete the dissolved oxygen readings as you read the bottles. Read the bottles as they are on the worksheet, starting with the blank and working down the sheet.

Sample Preparation

- ◆ Determine the pH of each sample to be used.
 - ◆ Adjust pH to 7.00 ± 0.10 @ 20°C, using 10% NaOH or 0.2N H₂SO₄
 - ◆ Be sure to pH enough solution for all dilutions plus dechlorination determination.
 - ◆ Record the initial and adjusted pH values on lab slips.
 - ◆ Dechlorinate the sample if necessary
 - ◆ Take 100ml of pH'ed sample and place in a 250ml flask
 - ◆ Add 10ml KI and 10ml Sulfuric acid and mix
 - ◆ Add 3 droppers full of starch indicator and mix
 - ◆ Titrate to absence of blue color using 0.025N sodium thiosulfate.
 - ◆ Record volume of sodium thiosulfate needed per 100ml of sample on lab slip.
 - ◆ Add calculated volume of sodium thiosulfate to remaining sample.

Bottle Preparation

When filling bottles with dilution water be sure to fill bottle to the bottom of the neck. Do not overfill as sample will be lost. Also be sure to have enough water so that stopper has some liquid around it after it has been placed in bottle. This makes for a complete seal in the bottle.

Blanks

- ◆ Fill one blank with only dilution water.
- ◆ Fill another blank with 20ml Polybac seed and the rest with dilution water.

Glucose Glutamic Acid (QA/QC Test)

- ◆ Fill one bottle with 3ml glucose glutamic acid, 2ml Polybac seed and the rest with dilution water.

- ◆ Fill one bottle with 2ml glucose glutamic acid, 2ml Polybac seed and the rest with dilution water.

Seeded Samples (Secondary & Tertiary Effluent Samples)

- ◆ Fill one bottle with 2ml Polybac seed, the smallest sample volume and the rest with dilution water.
- ◆ Fill one bottle with 2ml Polybac seed, the middle sample volume and the rest with dilution water.
- ◆ Fill one bottle with 2ml Polybac seed, the largest sample volume and the rest with dilution water.

Unseeded Samples (Influent Samples)

- ◆ Fill each bottle with the calculated raw mls and the rest with the dilution water.
- ◆ Sample sizes are dependent on the strength of the raw wastewater.
 - The lower the BOD the larger the sample size.
 - Sample size may be determined by this formula:

$$1200 * \text{expected BOD} = \text{the sample ml.}$$

EXAMPLE: 1200 * 90 = 13 ml.

13ml would become the middle sample volume and one bottle would be a slightly smaller sample size and one bottle would be a slightly larger sample size (three (3) samples total).

EXPECTED BOD CAN BE OBTAINED BY LOOKING AT PREVIOUS BOD WORKSHEETS AND MAKING AN "EDUCATED GUESS" FROM THAT DATA.

Look at the data on previous sheets. Make note of how depletions turned out. Remember you must have at least 2.0mg/L depletion between the initial D.O. and the final D.O. Also you must have 1.0mg/L of final D.O. With this in mind look at sample sizes already used and determine if they should be increased or decreased.

Use the Dissolved Oxygen Meter to measure the DO in each bottle. Record all data and place bottles in 20°C incubator.

Determination of BOD

After the samples have been incubated for 5 days at 20°C, use the DO Meter to measure the final DO in each bottle. Use the following equations to determine the BOD and complete the worksheet.

Worksheet Calculations

Equation 1: *BOD of Seed* (Blank + Seed Bottle or Unseeded Samples)

$$(\text{InitialDO} - \text{FinalDO}) * \left(\frac{300}{\text{ml of Seed}} \right)$$

Equation 2: BOD of Seeded Samples

$$\left\{ (InitialDO - FinalDO) - \left(D * \frac{A}{B} \right) \right\} * \frac{300}{C}$$

A = Depletion DO of Blank + seed

B = ml of seed in Blank + seed

C = ml of sample added to bottle

D = ml of seed in sample

EXAMPLE Equation 1:	Seed = 20ml	
	Initial DO	Final DO
BOD Seed Bottle	7.30	2.70

$$\begin{aligned} (7.30 - 2.70) * \left(\frac{300}{20} \right) &= \text{BOD of seed} \\ 4.60 * 15 &= \text{BOD of seed} \\ 69 &= \text{BOD of seed} \end{aligned}$$

EXAMPLE Equation 2:	Seed = 2ml	Sample = 100ml
	Initial DO	Final DO
Sample Bottle	8.40	1.95

$$\begin{aligned} (8.40 - 1.95) - \left\{ 2 * \left(\frac{4.60}{20} \right) \right\} * \left(\frac{300}{100} \right) &= \text{BOD Sample} \\ \{6.45 - 0.46\} * 3 &= \text{BOD Sample} \\ 17.97 &= \text{BOD Sample} \end{aligned}$$

Glucose Glutamic Acid QA/QC Samples

Use Equation 2, and divide result by 2.

SECTION 6: LABORATORY FORMS

ALKALINITY RECORD

SAMPLE _____ LAB # _____ DATE _____ TIME _____ TEMP _____		SAMPLE _____ LAB # _____ DATE _____ TIME _____ TEMP _____		SAMPLE _____ LAB # _____ DATE _____ TIME _____ TEMP _____	
pH	μl acid	pH	μl acid	pH	μl acid
Total		Total		Total	
μl titrant/10 =		μl titrant/10 =		μl titrant/10 =	
mg/L CaCO ₃		mg/L CaCO ₃		mg/L CaCO ₃	

SEKI WATER QUALITY CONTROL LABORATORY 5 DAY 20C BOD WORKSHEET

ANALYST _____ DATE _____ TIME _____ DATE IN _____			ANALYST _____ DATE OUT _____			
			TIME IN _____ TIME OUT _____			
Polyseed ref. # _____		GGA ref. # _____				
	BOTTLE	SAMPLE SIZE mls	INITIAL DO mg/l	FINAL DO mg/l	DEPLETION DO	DILUTION H2O
BLANK		-----				PASS FAIL
BLANK+SEED		20 ml. seed				
QA/QC (167<BOD<229)		2 GGA + 2 seed				
		3 GGA + 2 seed				
	BOTTLE	SAMPLE SIZE mls	INITIAL DO mg/l	FINAL DO mg/l	DEPLETION DO	BOD mg/l
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						

Final DO must be 1.0 mg/l. Depletion DO must be 2.0 mg/l. Blanks must not deplete more than 0.2 mg/l.

EFFLUENT SOLIDS WORKSHEET

Wt. _____

Date _____

	ASH MT	BUCKEYE	CLOVER	GRANT EFF	GRANT INF
LAB NUMBER					
TIME					
SETTLABLE SOLIDS (ml / L)					
SUSPENDED SOLIDS					
Filter wt.(mg)					
average					
Sample size in mls.					
Filter + dried sample (mg)					
average					
Dried sample wt (mg)					
Total suspended solids (mg/L)					

SEMIANNUAL TEMPERATURE RECORD

INCUBATOR _____ WATERBATH _____ REFRIGERATOR _____

CERTIFIED THERMOMETER _____ INCUBATOR THERMOMETER _____
 CORRECTION FACTOR _____ DATE _____
 CERTIFIED THERMOMETER # _____

MO.	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
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