SWOFFER INSTRUMENTS, INC.

MODEL 2100 SERIES CURRENT VELOCITY METERS

*Instructions for Operation and Maintenance of 2100 Indicator**

INDICATOR FEATURES and OVERVIEW

The *Model 2100* Current Velocity Meter provides stream current measurements from 0.1 to 25 feet per second by reading directly in feet or meters per second. Velocity is shown on a liquid crystal display readout and units of measure are selected by a toggle switch located inside the battery compartment at the back of the indicator.

The display has three averaging periods as selected by the rotary switch. These averaging periods range from a minimum time of about 5 seconds to a maximum time of about 90 seconds in the feet per second mode. The liquid crystal display holds the average velocity for the most recent update period until the end of the next period when a new average velocity will appear in the display and hold.

The *Model 2100* Indicator is powered by a single 9-volt battery, which also supplies power to the photo-diode and the photo-transistor in the sensor. A 2-inch (50 mm) propeller drives a rotor containing two fiber-optics bundles. The rotation of these fiber-optics bundles gates infrared light from the photo-diode to the phototransistor creating a pulse rate that is proportional to the propeller RPM. The pulses are counted and stored then compared to a quartz crystal oscillator and processed to display velocity.

The *Model 2100* Indicator can be calibrated in the field and corrections can be made for optimum accuracy at the velocities most often encountered. Velocities below about 1.5 feet per second require some minor calibration adjustments, which will be discussed later in the CALIBRATION section. PLEASE READ IT CAREFULLY.

The battery compartment at the back of the indicator can be opened by use of the four thumbscrews at the corners of the case back. Space in the compartment is provided for both the operating battery and a spare plus there are cutouts in the foam lining for those accessories required for the particular Model being used.

The *Model 2100* Indicator is water sealed at the case front and at the bottom in the battery compartment. The battery compartment itself is not watertight however, and can fill with water if the indicator is immersed. The indicator will float even with the battery compartment filled because the foam lining is closed-cell and will not absorb moisture except on its surface.

The electrical connector between the sensor and the indicator is water-resistant only when mated. The connector is keyed (mate the two sides carefully) and locks with a twist of the fluted collar.

A neck strap is furnished with the *Model 2100* and it clips to the loop located at the bottom end of the battery compartment cover on the back of the indicator. Make sure that all four battery compartment screws are snug before suspending the meter from the battery back. Thumb tight is all that is necessary.

* Separate instructions are provided for operating the specific type of sensor and wand shipped with the 2100 Indicator

QUICK OPERATING INSTRUCTIONS FOR THE MODEL 2100

All *Model 2100* instruments regardless of the complete Model Number operate in the following manner:

1. Remove the sensor protection cap (if used) and install the propeller rotor using the Rotor Installation Wrench (1/16" Allen wrench). This set screw in the side of the sensor body need only be snug to the shaft, do not over-tighten it ! Damage to the sensor can result. The set screw is a 6-32 x 3/16" stainless steel socket head set screw with a nylon patch to prevent back out.

2. Connect the Sensor Wand to the *Model 2100* Indicator by using the twist lock electrical connector. The connector is keyed so align it carefully, press the two halves together and twist the collar.

3. Rotate the Indicator selector switch to the **CALIBRATE** position. The display should read about **186*** (feet per second mode) or **610*** (meters per second mode, 2" (50 mm) propellers only.) *Important Note: see page 4, *Calibration of the Model 2100 Indicator*. Change to whichever unit of measure is wanted by use of the FEET/METERS toggle switch located inside the battery compartment.

4. Rotate the selector switch to the **COUNT** position. Spin the propeller and confirm that the indicator reads increasing counts (sensor output pulses) as the propeller spins. There should be four counts per revolution. (The spin test is described later in the calibration instructions).

5. Rotate the selector switch to the minimum update time. (First position from the left hand **OFF** position).

6. Place the sensor in the stream with the propeller facing into the flow.

7. Press and release the **RESET** button to zero the display.

8. The next figure, which appears on the display, will be the stream velocity. That velocity will remain on the display until the next update period ends. The figure in the display will always be the velocity of the last averaging period. (The indicator **does not** provide a "moving average" nor instantaneous "real time" velocity.

*For specific instructions for your *Model 2100* see the documentation for the wand assembly that came with your current meter.

CARE AND MAINTENANCE OF THE MODEL 2100 INDICATOR

Should the indicator fall into the stream, open the battery compartment as soon as possible and dry the battery terminals and the cable connections. Since the foam lining is closed-cell, water will not absorb into more than just its surface. Allow the compartment to air dry or use a hair dryer if available before replacing cover. If you can see evidence of water inside the Indicator (inside window or on the LCD) immediately disconnect the battery and make all attempts to dry the indicator. You must remove the calibration adjustment screws and drain as much water as possible then place the indicator in an extremely dry location and under very warm temperature (not to exceed 150° F) for several days. Contact Swoffer for further details about a water incursion. No permanent damage should result, however the operation of the Model 2100 Indicator may be temporarily compromised with water on the circuit board.

Always make sure that the calibration adjustment cover screws located at the bottom end of the indicator are tightly fitted. These provide DIRECT ACCESS TO THE CIRCUIT BOARD and if loose, will allow water to enter the indicator.

Clean the indicator only with a solution designed for plastics. The indicator lens is made of acrylic and can be easily scratched if an abrasive cloth is used. The indicator case is ABS and there are many chemicals that act as solvents for ABS, severely affecting its appearance if they make contact. Use caution when cleaning.

Periodically check the condition of the pins and sockets in the connector. Keep the contacting surfaces clean and bright and make sure the pins are not bent and that the sockets still fit the pins snugly. Although the connection cable is rugged, avoid sharp bending and re-bending and **DO NOT SUSPEND THE WAND OR INDICATOR BY THE CABLE**.

Extreme temperatures will effect the *Model 2100* Instrument. At below freezing temperatures the liquid crystal display becomes sluggish making response time slow. Some "ghosting" of unused digit segments may also be noticed. This condition is only temporary and the instrument will operate normally after temperatures rise to normal operating levels.

The battery is also affected by low temperatures and may not have enough power to bring the calibration numbers up to the correct level for accurate measurement. Check calibration number frequently when working in low temperatures. For best results keep the indicator close to the body inside your coat during operation in the cold. Keep extra batteries in your coat and exchange them often.

High temperatures and direct sunlight will also effect the operation of the *Model 2100*. DO NOT LEAVE THE INDICATOR IN A CLOSED VEHICLE EXPOSED TO THE SUN. Cover the indicator and avoid prolonged exposure of the liquid crystal display to ultra-violet rays. Ultra-violet will eventually degrade the display requiring its replacement.

High temperatures may also cause the indicator electronics to give erroneous readings due to pulse-count-timing errors.

In short, keep the Model 2100 Indicator within the recommended operating temperatures for optimum results.

NORMAL OPERATING TEMPERATURE	**77°F (25°C)**	
MIN. TEMP (FOR RELIABLE OPERATION)	**-14°F (-25.56°C)**	
MAX. TEMP.	**180°F (82°C) @ LESS THAN 15%**	**RELATIVE HUMIDITY**
	120°F (49°C) @ LESS THAN 95%	**RELATIVE HUMIDITY**

BATTERY CONDITION AND ITS EFFECTS ON THE MODEL 2100

The battery, which powers the Model 2100, is a single alkaline type 9-volt transistor cell. This battery powers both the indicator electronics and the sensor photo-diode/transistor circuitry. **The indicator alone draws very little current, however the sensor circuit (if allowed to remain on constantly) can drain a fully charged battery in a short time.** Depending on your use of the *Model 2100*, a fresh battery can last as long as several months or as short as a few days. To conserve battery life be sure to return the selector switch to one of the "OFF" positions after each measurement has been taken and always have a fresh spare battery on hand.

One way to determine battery strength is to check the Calibration Number held by the indicator. Rotate the selector switch to the calibrate position. If the displayed calibration number is much less than when you set it then the battery should be replaced. **NOTE:** This test is only valid with the sensor connected to the indicator and the rotor positioned so that the optics trigger an “on” condition. Only then is maximum battery drawdown achieved.

In no case should you use a battery with less than 5 volts remaining. Below the threshold of about 5 volts the battery will operate the indicator electronics **but not the sensor circuit too**. The sensor will not be able to transmit a signal to the indicator or will transmit only an occasional signal, which will give erroneous readings. This is also relative to the manufacturing date of the Model 2100 sensor. Sensors produced later than mid 1992 require much less power for operation than earlier units and can be used at lower voltages.

Mid-stream sensor “wink out” can usually be avoided by occasionally checking the sensor output and replacing the battery if necessary. Spin the propeller while the indicator is in the COUNT mode. If the display counts up the sensor pulses as the rotor spins then the battery can be considered acceptable. There are four counts per revolution. (One revolution of the propeller must produce 4 counts on the LCD).

Errors in measurements due to battery voltage drop (and subsequent CALIBRATION NUMBER drop) will be in direct percentage proportion to the difference between the "ideal" calibration number and the displayed calibration number.

CALIBRATION OF THE MODEL 2100 CURRENT METER

The *Model 2100* Current Meter is designed to be easily calibrated by the user. This calibration must be done with each Rotor you use. *The calibration numbers recommended by SWOFFER INSTRUMENTS, INC. are not necessarily correct for all measuring situations, therefore for optimum accuracy the user must calibrate the rotors before use and at or near to the velocities expected to be encountered.*

If very accurate velocity measurements are required then you must calibrate your *Model 2100* system and check the calibrations often. The instructions below should be followed very carefully for reliable measurements using the *Model 2100*.

> **IMPORTANT NOTE:** "Calibrating a sensor" is actually calibrating a particular propeller rotor for use with the *Model 2100* Indicator. If you use more than one propeller-rotor assembly you must check the calibration for *each* rotor assembly and adjust the Indicator Calibration Numbers accordingly as you switch from one propeller assembly to another. A calibrated rotor assembly will produce the same velocity results on any Model 2100 sensor as long as the calibrated Indicator and rotor assembly are paired

Calibration numbers correctly matching a rotor assembly to a 2100 indicator are especially important at the lower velocities (1.5 FPS and lower) and can vary greatly depending on many factors; bearing surface condition in the rotor, make-up of the water being measured (amount of suspended particulates), any damage to the propeller, rotor, shaft, thrust-bearing nut, etc. Calibrate your rotors !

What a calibration number is:

The Model 2100 rotors produce four pulses per revolution. Each of the four fiberoptic "eyes" in the rotor triggers an electrical pulse from the sensor. These pulses are called "Counts" and are read by the Model 2100 Indicator. The Indicator uses these counts, measuring the number of them against an internal timer to determine velocity. The two calibration numbers therefore represent the number of counts a specific rotor produces as it travels through 10 feet and 10 meters of still water. When the sensor is *stationary* and water is moving past the propeller, a specific number of counts produced in a specific amount of time determines velocity when you know how many counts are produced per foot or meter (pitch). Calibration numbers then, can also be referred to as Pitch.

Although rotor/propeller combinations are "similar" they are not necessarily "identical" and therefore each may have a slightly different Calibration Number. Always remember that the Calibration Numbers shown on the Indicator's display represent the Calibration Numbers for a single rotor assembly only. Double check any and all rotor assemblies used for a measuring job and make sure that each is within your accepted tolerance for calibration variation (i.e. "error"). Only go out into the field with specific knowledge of each rotor assembly's calibration number. It is essential that the calibration number shown on the 2100 Indicator display matches the calibration number of the rotor that is attached to the sensor before relying on any readings.

THE SPIN TEST : The Rotor Assembly (**2100-A21**) should spin very freely when held in the vertical position (propeller pointing up) and simply blow lightly on the propeller. If it does not, clean the bore of the Rotor and the surface of the Rotor Shaft thoroughly.
One method to determine an acceptable level of low-velocity performance by a particular Rotor Assembly is to perform a "Spin Test":
Install the Rotor on the sensor, connect the sensor to the Indicator, and place the Indicator in the **COUNT** mode. With the propeller pointing straight up blow very hard straight down on the propeller. *At the instant you stop blowing* hit the **RESET** key on the indicator and allow the rotor to coast to a stop. A rotor, that will perform to the low velocity limits of its design, produces counts on the indicator of at least 400.

CHECKING AND CHANGING CALIBRATION OF THE MODEL 2100

Before applying corrections to the *Model 2100* rotate the selector switch to the **CALIBRATE** position. A figure will appear in the display and will be either the FEET calibration number or the METERS calibration number depending on the position of the FEET/METERS switch (located in the battery compartment). For many measuring applications using the 2" (50 mm) propeller the calibration numbers will be *about*:

FEET	**=**	**186**
METERS	**=**	**610**

If the displayed figures are much lower than these figures the first thing to check is the battery. A weak battery can allow the indicator calibration numbers to "drift" downward slightly and will cause errors in measurements. Be sure to connect the sensor to the indicator when confirming battery strength. Always keep a *full charge* 9-volt battery in the compartment as a spare. The next thing to check is your calibration for the rotor. It may be that the calibration number is lower than the above values because the rotor to be used has a lower cal. Number. When using the 3" (70 mm) propeller the Cal nos. are 130 for feet and 426 for meters). Be sure before relying on any readings.

NOTE: It is important to note that errors in measurements due to Calibration Number variation will be in direct percentage proportion to the difference *between the ideal (correct) Calibration Number for any rotor assembly and the number that the indicator displays.*
Example: If the ideal number is 186 for a particular rotor assembly and the Indicator-displayed number is 184 then the velocity error due to calibration error will be about 1%.

To determine a reliable calibration number for your *Model 2100* perform the following: This is something you **must** do if you are working with slow flows (below about 1.5 FPS) and for measurements taken in very shallow streams. NOTE: This procedure as described is performed for readings in feet per second with a conversion to meters after.

Mark a straight course of 10 to 20 feet in length in a body of calm, current-free water along which the sensor can be towed by walking the course (a shorter length course can be satisfactory as well; just multiply your counts from the short course so that the results equal equivalent counts for 10 feet and 10 meters). A swimming pool or dock into a quiet lake serves well. Rotate the selector switch to the **COUNT** position. If the display does not show all zeros press and release **RESET**. (The decimal point does not show in the count mode.)

Place the sensor in the water a few feet before the beginning of the course, 6 to 12 inches below the surface. Begin walking the sensor through the course at a rate close to that which you will be measuring. (If shallow flows are to be encountered try to duplicate those conditions when making calibration checks.) Using the wand rather than the propeller as a guide, press and release the **RESET** button at the instant the wand enters the course. The indicator will begin counting the number of sensor pulses as you walk. At the instant the wand leaves the course press and release the **START/STOP** button. The display now shows (and will hold) the number of pulses generated through the course length. Several passes through the course in both directions are recommended to develop a reliable average figure. Press and release **RESET** each time a run is completed to re-zero the display.

Determine the average number of pulses generated through the course. If your course length is not 10 feet, compute the number of pulses that the sensor would generate if the course were exactly 10 feet. This will be the CALIBRATION NUMBER that the *Model 2100* Indicator should hold for accurate measurements with that rotor assembly in feet per second:

$$\textbf{FEET CAL. No.} = \frac{\textbf{10 x AVERAGE No. OF PULSES}}{\textbf{COURSE LENGTH (IN FEET)}}$$

This number can then be multiplied by 3.281 (the number of feet in one meter) to determine a calibration number for meters. NOTE: If calibrating directly for meters and the course length is in meters disregard this multiplier.

Next, rotate the selector switch to the **CALIBRATE** position. Put the **FEET/METERS** switch (in battery compartment) in the FEET position and the indicator will display the Calibration Number it presently holds for measuring in Feet Per Second. With a good battery it should be 180-186 [2” (50 mm) props only]. If your derived Calibration Number is different from the number displayed you must change the calibration number by using the **CAL ADJUST** screw(s) at the bottom end of the indicator. To access the **CAL ADJUST** screws first remove the cover screws (black plastic fillister-head screws).
USING ONLY A JEWELER'S SCREWDRIVER (to prevent damage to the adjustment screw) rotate the screw clockwise to increase the displayed number and counterclockwise to decrease the number. Do the same for the Meters calibration number. Each calibration adjustment screw is a 15-turn potentiometer with very fine resolution and plenty of latitude for normal adjustment given a full charge 9-volt battery.

REPLACE THE ADJUSTMENT COVER SCREWS AFTER MAKING CALIBRATION CORRECTIONS. INDICATOR IS NOT WATER RESISTANT WITHOUT THESE COVER SCREWS IN PLACE !

Note and store *with the Model 2100 Indicator* your new Calibration Number(s). Every time the instrument is used the calibration numbers and rotor assembly(s) that generated it must be confirmed (rotate switch to **CALIBRATE**) before relying on readings. Be sure to check the calibration number with the sensor connected to the indicator to achieve maximum battery current draw.
Remember that a calibration number is correct only for the rotor assembly that generated it. Each propeller & rotor used may have a different calibration number.

IMPORTANT: Errors in measurements due to Calibration Number variation will be in direct percentage proportion to the difference between the ideal (correct) Calibration Number and the number that the indicator displays.

MODEL 2100 SERIES CURRENT METER-INDICATOR FUNCTION SWITCH

OFF & OFF

Redundant positions. Cuts all power from battery to the indicator and the sensor.

VELOCITY

Three display averaging switch positions; from the minimum display update time to the medium to the maximum. The LCD displays and holds the stream velocity for the previous update time until the next averaging period is completed. Update times vary with the selection of FEET or METERS and also vary with the value of the calibration numbers held by the indicator. *Approximate* update times are as follows:

	FEET	METERS
MIN	10 SEC	1.5 SEC
(Med)	20 SEC	6.0 SEC
MAX	90 SEC	30.0 SEC

START/STOP

Used mainly when calibrating. Begins and ends **COUNT** function. Display will hold data until **RESET** is pressed.

RESET

Resets the display to zero. Will operate in any rotary switch position (except OFF). Used to begin timing functions at "time zero" (i.e. the first reading after the rotary switch has been shifted to a new position may not be accurate. Use of the **RESET** switch will eliminate "first averaging period" timing errors.

CALIBRATE

LCD will show the figure that the indicator holds as the "Calibration Number". The **FEET-METERS** switch in the battery compartment is used to change the displayed number from feet to meters. See CALIBRATION INSTRUCTIONS.

COUNT

Indicator counts and displays the number of sensor output pulses generated. Used when calibrating the *Model 2100* and checking sensor output.

FEET/METERS

A toggle switch located inside the battery compartment changes the indicator readout between meters per second and feet per second.

CALIBRATION ADJUST

Removing the plastic, fillister-head screws at the bottom end of the indicator provides access

to the Calibration Adjustment Screws. With the rotary switch in the **CALIBRATE** position the displayed figure can be altered by turning the adjustment screws. Clockwise rotation increases the calibration number. Use only a jeweler's screwdriver when making adjustments to prevent damage to the calibration adjustment screws. The plastic screws must be replaced after adjustments to preserve the water-resistance of the Indicator.

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Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedure for Measuring and Calculating Stream Discharge

Version 1.1

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Signatures on File

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
10-05-2011	1.1	Revised mean gage height calculation methods in rapidly fluctuation stage conditions.	6.9	J.R. Shedd
		Changed field procedure for assigning quality ratings to discharge measurements.	6.11	
		Updated citations and reference to 2010 edition of EAP Safety Manual.	9.3	
		Removed duplicate paragraph 6.8.3 and renumbered following paragraphs in proper sequence through rest of section.	6.8	
		Added double spacing between paragraphs 6.15.1 and 6.15.2,	6.15	

1.0 Purpose and Scope

1.1 This standard operating procedure describes the techniques and methods used to measure and calculate stream discharge in a variety of measurement conditions.

2.0 Applicability

2.1 The procedures presented in this document apply primarily to stream discharges measured with mechanical instruments and acoustic velocimeters. However, some sections including but not limited to; Cross Section Selection, Measurement Rating Guidelines, and Assessing the Control also apply to discharges measured with acoustic profilers.

3.0 Definitions

3.1 Midsection Method – A widely used technique for calculating stream discharge, the midsection method involves the calculation of discharge in individual measurement cells in a cross section.

3.2 Cross Section – The measurement cross section is a vertical plane extending from either stream edge, up from the stream bottom to the surface. Depth measurements and velocity samples are taken at about 30 predetermined verticals across the cross section.

3.3 Vertical – The vertical is one of a series of measurement points through the cross section where depth and velocity are measured.

3.4 Primary Gage Index – The primary gage index is the base gage for the station and is directly referenced to the recording gage. The primary gage index is the most stable and reliable gage at a site. All other gages at a station are considered secondary.

3.5 Control – The physical features of a stream that controls the relationship between stage and discharge at a gage site.

3.6 Point of Zero Flow – The point of zero flow is the elevation of the lowest point of a control structure. Flow stops when stage reaches the point of zero flow elevation.

4.0 Personnel Qualifications/Responsibilities

4.1 Personnel using this document will have the training necessary to operate current meters and related equipment. Staff will have knowledge of field and safety procedures associated with the collection of stream flow information.

4.2 Users of this document will typically work in the Environmental Specialist or Hydrogeologist job classifications.

4.3 No special certifications are required.

5.0 Equipment, Reagents, and Supplies

5.1 A standard English four foot, top set wading rod measures vertical depths in streams shallow enough for wading. A velocity meter attached to the top set wading rod measures current speed. The rod is designed to set the current meter at 0.2, 0.6, and 0.8 of the total stream depth.

5.2 The Sontek® FlowTracker® Acoustic Doppler Velocimeter® is used in the majority of wading measurements. The basic components of the FlowTracker® include the under water probe containing the acoustic elements, the probe cable, keypad and controller (Burks, 2009). All of these components attach to a wading rod.

5.3 The primary mechanical current meter used in wading measurement applications is the Swoffer®, Inc. Model 2100® flow meter kit. The flow meter kit consists of a model 2100® digital indicator, sensor cable, and a two inch rotor assembly (Holt, 2009).

5.4 In the case of measurements from a bridge using mechanical equipment, a bridge board or bridge crane is used. Three types of sounding reels are available; USGS Type A and B reels, and a Hydrological Services® Pty Ltd San Winch®. The Swoffer® flow meter kit and the Hydrological Services® OSS-B® current meter are the mechanical instruments used in bridge measurements. The Hydromate® counter registers velocities for the OSS-B1 current meter (Holt, 2009).

Figure 1 Bridge board equipped with a USGS Type A reel. (Photo by Washington Dept. of Ecology)

Figure 2 Bridge crane equipped with a USGS Type B reel. (Photo by Washington Dept. of Ecology)

5.5 Shore operated cableway discharge measurements require the use of a removable sounding reel, permanently installed bearing posts, main tow cable, pulleys, trolley, and related hardware.

5.6 Discharges measured from a boat require specially made equipment. This equipment includes a crosspiece, retractable boom, and a nose piece. A Kevlar® tag line maintains the position of the boat along a cross section.

Figure 3 A boat measurement conducted with necessary equipment attached to a Kevlar® tag line. (Photo by Washington Dept. of Ecology)

5.7 Columbus sounding weights, ranging from 15 to 100 pounds are attached to the cable of the sounding reels in bridge, cableway, and boat measurements.

5.8 Copies of form number 040-56 (Appendix A) for recording discharge notes are kept in a suitable field notebook. These forms are printed on Rite in the Rain™ paper for outdoor durability.

5.9 A fiberglass measuring tape or marked cable is stretched across the measurement cross section or bridge span.

5.10 Good quality hip or chest waders are worn by staff conducting discharge measurements by wading.

5.11 As mandated by the Environmental Assessment Program's Safety Manual (EAP, 2010) an approved personal flotation device must be worn when working in areas where the danger of drowning exists, such as on the water, over the water, or alongside the water.

6.0 Summary of Procedure

6.1 Preparation

6.1.1 Prior to departure complete a Field Work Plan.

6.1.2 Review the equipment checklist (Appendix B) and ensure all field and safety equipment are in the vehicle. Make sure all station information, instructions, and

field forms are on hand. Test all stream discharge measuring equipment for proper operation.

6.1.3 Ensure vehicle is equipped to handle the driving conditions you may encounter. Make sure vehicle operates properly before leaving. Check oil level, fluid levels and tire pressure.

6.2 Measurement Notes

6.2.1 It is important that field staff record clear, detailed notes prior to, during, and after the measurement. Future evaluations of a measurement depend on complete and thorough notes. Record the following information at each discharge measurement:

6.2.1.1 Name of station and station number.

6.2.1.2 Party (person conducting measurement listed first, person recording notes listed second).

6.2.1.3 Date and time measurement started and ended using 24 hr clock in Pacific Standard Time.

6.2.1.4 Note the start and end time along with REW (right edge of water) or LEW (left edge of water). Right and left edges of water are oriented looking downstream.

6.2.1.5 Identify the type of instrument and record the instrument number. If using mechanical equipment, note the propeller or fan number.

6.2.1.6 Record pre and post calibration readings if applicable (Holt, 2009).

6.2.1.7 Record the gage height from the primary and secondary gage indices at the beginning and end of the measurement. Additionally, observe and note the gage height at least once during the course of the measurement to determine if stage is changing.

6.2.1.8 Record the variability in gage height observations, i.e. the range of water level bounce i.e. +/- n hundredths of a foot of variability.

6.2.1.9 At stations equipped with continuously recording data loggers, record the time beside the corresponding vertical in the notes every 15 minutes. These time observations are important in determining the mean gage height if the stage is changing significantly during the measurement. In the case of stations without a continuous recorder the gage height is manually observed and noted when stage is changing rapidly.

6.2.1.10 Record the water temperature particularly when using acoustic instruments.

6.2.1.11 At the end of the measurement indicate the rating of the measurement in the space provided on the note sheet.

6.2.1.12 When measurement statistics are available in electronic form, record the width, area, average velocity, and maximum depth. Also include the number of verticals, wetted perimeter and discharge.

6.2.1.13 Record velocity uncertainty, depth uncertainty, and overall uncertainty in the space provided on the note sheet when these statistics are available.

6.2.1.14 Thoroughly describe channel and control conditions.

6.2.1.15 Describe any situation which might affect the accuracy of the measurement or affect the stage and discharge relationship.

6.2.1.16 Measure and record the point of zero flow if possible.

6.2.1.17 Prior to submittal of notes for review, the measurement number, and initials of person compiling notes are entered in the space provided on the note form.

6.2.1.18 The person reviewing the measurement enters their initials in the space provided on the note form.

6.2.1.19 Record vertical locations, depths, and velocities on the back side of the measurement note form. It is important to record velocities in the proper two tenths, six tenths, or eight-tenths column.

6.3 Selecting the Correct Instrument to Conduct a Discharge Measurement

6.3.1 Not all available measurement equipment is designed for use in all measurement situations.

6.3.2 Acoustic Doppler Current Profilers (ADCP) makes an attractive instrument choice because of their convenience and relative ease of deployment. If used appropriately ADCPs provide a more complete and accurate measurement.

6.3.3 However, they are not used in every situation. The following are circumstances in which an ADCP may not be an appropriate instrument choice:

6.3.3.1 Poor depth distribution.

6.3.3.2 High Turbulence.

6.3.3.3 Aerated water.

6.3.3.4 Soft or vegetal covered substrate.

6.3.3.5 Moving bed situations.

6.3.3.6 Debris or fish in the water.

6.3.3.7 Extremely clear water with very little or no turbidity or entrained material.

6.3.4 As a matter of practice, field staff should review the quality of past ADCP measurements at a site. Consider using another type of instrument or changing measurement locations if there were problems with past measurements.

6.3.5 Refer to The Teledyne RD Instruments® (TRDI) StreamPro® Standard Operating Procedure (Shedd, et al, 2008) or the WinRiver II Quick Start Guide (TRDI, 2007) for detailed information regarding the proper use of current profilers.

6.3.6 In circumstances where use of an ADCP is inappropriate, use either an acoustic velocimeter or a mechanical instrument.

6.3.7 FlowTracker® velocimeters are preferred over mechanical equipment because of their accuracy, automatic discharge calculation capabilities, statistical presentation of data quality parameters, and lack of moving mechanical parts. The acoustic velocimeter is versatile and can be used in most wading measurement applications. However like the ADCP, the velocimeter will not work in all measurement situations.

6.3.8 Boulders, logs or other large structures collectively referred to as boundaries cause interference with acoustic signals. This interference precludes velocity measurements with velocimeters. Avoid cross sections with significant boundary structures.

6.3.9 Very clear water with very little or no turbidity is another condition in which velocities cannot be measured with these instruments.

6.3.10 Highly turbulent or aerated water can also prohibit the use of the velocimeter.

6.3.11 Mechanical equipment will perform in almost all situations except in velocities less than about 0.10 feet per second. Mechanical instruments will register a velocity in situations where an acoustic instrument may not. However, the same factors that preclude the use of acoustic instruments may also limit the reliability of mechanical equipment.

6.3.12 If a velocimeter cannot be used because of channel and flow conditions, attempt to find a more favorable measurement location. Use mechanical equipment only when circumstances preclude the use of acoustic equipment.

6.4 Selecting a Cross Section

6.4.1 Prior to conducting a stream discharge measurement field staff make considerable efforts to locate the best available measurement cross section. If the stream cannot be waded and an Acoustic Doppler Current Profiler (ADCP) cannot be used, the measurement must be made either from a bridge, cableway, or a boat.

6.4.2 In the case of ADCP and wading measurements the selection of a suitable cross section cannot be over emphasized. The quality of equipment or the ability of the individuals conducting the measurement cannot overcome the limitations in measurement quality caused by a poor cross section. The choice of cross sections is obviously limited when measuring from a bridge, cableway, or boat.

6.4.3 Field staff look for the following characteristics in an ideal measurement cross section (Rantz, 1982):

6.4.3.1 A stream channel relatively straight with parallel edges upstream and downstream of the cross section.

6.4.3.2 Defined edges on both sides of the cross section.

6.4.3.3 A channel of uniform shape.

6.4.3.4 A channel free of vegetative growth, large cobbles, and boulders.

6.4.3.5 A cross section free of eddies, slack water, and turbulence.

6.4.3.6 A cross section with depths greater than 0.5 feet.

6.4.3.7 Velocities greater than 0.5 feet per second and distributed evenly through the cross section.

6.4.3.8 A cross section relatively close to the gaging station control to avoid the inflow of tributaries and differences in relative flow between the control and cross section during periods of changing stage.

6.4.4 Meeting all of the selection criteria is often not possible. Field staff should choose the best available cross section based on these characteristics.

6.5 Dividing the Stream Channel into Segments

6.5.1 After locating a satisfactory cross-section, stretch a measuring tape or marked tag line across the measurement cross section. The tape or tag line extends across the channel perpendicular or normal to the direction of flow. Limit the number of cells with oblique flow angles, i.e. cells with current angles not perpendicular to the cross section.

6.5.2 Note the width of the stream channel at the cross section and divide into measurable segments or cells. Divide the cells such that approximately five percent and no more than ten percent of the total flow is within any one segment. In most cases divide the cross section into approximately 30 segments. Position verticals closer together where flow is more concentrated and velocity variation or bottom irregularities are greatest.

6.5.3 The width of a measurement segment should not be less than three tenths of a foot.

6.6 Measuring Velocity

6.6.1 Measure velocity at each pre-determined vertical across the stream. Although velocity is evenly distributed in ideal cross sections, there can be significant variability in stream velocity throughout a less than ideal cross section. Velocity varies horizontally across the cross section and vertically through the water column. Velocity also naturally pulses in streams at any single location over short periods of time.

6.6.2 *Horizontal Velocity Variation*

6.6.2.1 Channel geometry, substrate, and other stream features cause horizontal variability between stream segments. Field staff can minimize measurement uncertainty due to horizontal velocity variation by applying these guidelines:

6.6.2.1.1 Divide the stream cross-section into about 30 segments. If previous measurements show uniformity of the cross section and an even velocity distribution, fewer verticals may be used (Rantz, 1982).

6.6.2.1.2 Concentrate the distribution of segments where discharge is highest and in areas where significant velocity variation can be identified.

6.6.2.1.3 Increase the number of verticals in vicinity of bridge piers.

6.6.3 *Vertical Velocity Variation*

6.6.3.1 In most natural stream conditions, a logarithmic relationship exists between velocities through the water column. Typically velocity increases in the upper portion of the water column and decreases near the bottom. Address vertical velocity variability within a segment using one of the following methods, depending on measurement conditions.

6.6.3.1.1 Six tenths method: Sample velocities at sixth tenths of the depth from the water surface. Assume velocity samples at six tenths of depth represent the average velocity through the water column up to 1.5 feet. Use the six tenths method in stream segments less than 1.5 feet in depth. Use the six tenths method at all depths when stage is fluctuating rapidly and the measurement must be made quickly.

6.6.3.1.2 Two point method: Employ the two point method in streams with depths greater than 1.5 feet. Velocities are sampled at two tenths and eight tenths of the depth, and the results are averaged.

6.6.3.1.2.1 When measuring from a bridge, cableway, or boat, the two point method is used in depths greater than 2.5 feet.

6.6.3.1.3 Three point method: When a logarithmic relationship does not exist between strata of velocities through the water column, use the three point method.

6.6.3.1.3.1 Presume a non logarithmic relationship exists when the two tenths velocity is less than the eight tenths velocity or the two tenths velocity is greater than two times the eight tenths velocity.

6.6.3.1.3.2 The three point method consists of velocity samples at two tenths, six tenths, and eight tenths of depth. Average the six tenths sample against the mean of the eight tenths and two tenths velocity samples, thus weighting the six tenths sample as half of the calculated velocity for the segment.

6.6.4 *Single-Point Velocity Variation*

6.6.4.1 Stream velocities in natural conditions tend to pulse over time at the same stage. These fluctuations compound the effects of horizontal and vertical velocity variability at fixed locations in the stream. Apply the following guidelines to address single point velocity variability:

6.6.4.1.1 Take 40 second velocity samples to address variations in velocity over time at a single measurement point.

6.6.4.1.2 Measure 40 second velocity samples at each measurement point except when stage is rapidly fluctuating, or when velocities less than 0.5 feet per second exist when measuring with mechanical instruments.

6.6.4.1.3 In circumstances when stage is fluctuating, take single 20 second velocity samples to complete the discharge measurement quickly.

6.6.4.1.4 When using mechanical equipment, increase the sample time to 60 seconds when velocity is less than 0.5 feet per second.

6.7 Adjusting Velocities of Oblique Flow Angles

6.7.1 Pay close attention to the direction of flow when using mechanical current meters. The velocity of the current normal or perpendicular to the cross section must be determined to calculate discharge correctly.

6.7.2 The following procedure does not apply to SonTek® velocimeters as they are always pointed perpendicular to the cross section. SonTek® velocimeters automatically calculate and report velocity as normal to the cross section (Burks, 2009).

6.7.3 Ecology's Freshwater Monitoring Unit's mechanical instruments consist of horizontal axis type current meters. In wading measurements mechanical meters should be pointed into the current when the angle of the current, relative to the perpendicular of the cross section is greater than 15 degrees. When the meter is suspended by a cable the meter will automatically point into the current.

6.7.4 At any measurement vertical, when a mechanical meter is pointed into an oblique current greater than 15 degrees from the perpendicular of the cross section, multiply the registered velocity by the cosine of the angle. This calculation yields velocity normal to the cross section. An approximate 3.5 percent difference exists between registered velocity at a 15 degree current angle and the calculated velocity normal to the cross section. A 20 degree angle results in a greater than 6 percent difference between registered and calculated velocity normal to the cross section.

6.7.5 Determine the cosine in the field with the use of the Ecology Discharge Measurement Note sheet number 040-56. On the back side of the form locate the dot in the center of the left margin. Cosine values are on the right side of the page as well as the top and bottom.

6.7.6 Hold the note sheet horizontally with the dot over the measuring tape if wading, or the edge of a bridge rail if conducting a bridge measurement. Position the note sheet such that the long edge of the sheet is parallel to the direction of the current. The measuring tape or bridge rail edge will intersect the cosine value on the right edge, top, or bottom of the sheet. Multiply the cosine value times the registered velocity to obtain the velocity normal to the cross section.

6.7.7 Oblique angles should be measured and velocities adjusted at all verticals where current angles are greater than 15 degrees.

6.7.8 In many instances when measuring discharge from a bridge, the entire stream channel is at an oblique angle to the cross section defined by the bridge. In this case multiply the raw average velocity of the measurement by the cosine of the angle between current direction and the cross section.

6.8 Measuring Discharge When Stage Is Fluctuating Rapidly

6.8.1 Field staff should streamline measurement methods to obtain a more representative gage height when stage changes rapidly. Some accuracy in the measurement is sacrificed. However the error caused by changing flow patterns in rapidly changing stage situations is greater than the loss of accuracy in a streamlined discharge measurement (Buchanan and Somers, 1969).

6.8.2 Generally, stage is considered rapidly changing when stage changes at a rate greater than 0.1 feet per hour. Be aware that measurements conducted during or after storm events, impoundment or diversion activities, or seasonal snow melt periods are the most likely times to encounter rapidly changing stage conditions.

6.8.2.1 Measure velocities using the six-tenths method regardless of depth.

6.8.2.2 If the six tenths method is not possible, use either the two tenths method, subsurface method, or the surface method.

6.8.2.3 Reduce velocity sampling time to 20 seconds.

6.8.2.4 Reduce the number of verticals to 15 or 20.

6.8.2.5 At sites with continuous stage height recorders, record the time every 15 minutes on the note sheet corresponding to the vertical measured at that time.

6.8.2.6 In the case of non-recoding stations, manually read and note gage heights. Observation times are recorded on the note sheet next to the corresponding vertical.

6.8.2.7 This record of stage and corresponding observation times are used in the calculation of a weighted mean gage height.

6.9 Calculating Mean Gage Height When Stage Is Fluctuating Rapidly

6.9.1 The mean gage height of a discharge measurement and the discharge value itself comprise the two plotted coordinates used to establish a rating. An accurate determination of mean gage height is as important as the accuracy of a discharge measurement.

6.9.2 At continuously recording stations the stage record during the time of the measurement is checked against the recorded times on the measurement sheet. Check the record for the amount of fluctuation in stage during the measurement.

6.9.3 If changes in stage occurred during the measurement and the changes are uniform and less than about 0.1 feet, mean gage height is determined by calculating the average of the beginning and ending gage height readings (Buchanan and Somers, 1969). Use judgment in determining the allowable change in gage height where a simple mean gage height is calculated. Consider the percentage of discharge the change in stage represents at smaller streams.

6.9.4 If the change in stage is greater than 0.1 feet or the change is not uniform a weighted mean gage height for the measurement is calculated. Two methods are used to obtain a weighted mean gage height. The first, called Partial Discharge Weighting is only appropriate for use with discharges measured with mechanical equipment or acoustic velocimeters. The second method, Time Weighting, is

used with all measurement equipment including Acoustic Doppler Current Profilers. When mechanical equipment or acoustic velocimeters are used both methods are employed and the separate results averaged to obtain a weighted mean gage height.

6.9.4.1 With the Partial Discharge Weighting method, the discharge between recorded times during the measurement, the mean gage height for the corresponding time periods, and the total measured discharge are used to compute a mean gage height. The Partial Discharge Weighting formula is

$$H = \frac{q_1h_1 + q_2h_2 + q_3h_3 \ldots . + q_nh_n}{Q}$$

where
H = mean gage height
Q = total discharge = $q_1 + q_2 + q_3 \ldots . + q_n$
where $q_1 + q_2 + q_3 \ldots . + q_n$ = discharge measured during time interval 1, 2, 3, ….*n* and
$h_1 + h_2 + h_3 \ldots . + h_n$ = average gage height during time interval 1, 2, 3,….n.

6.9.4.1.1 In figure 4 an example of a partial discharge weighting calculation is presented. The gage height (h) is recorded every 15 minutes from 13:30 to 14:30. The average gage height (Avg h) is calculated between each 15 minute interval. The sum of the discharges of each cell (q) measured in the 15 minute interval is computed. Each of these discharges are summed to give Sum Q = 76.37. The product of each Avg. h and q is calculated and the products summed to give Sum (avg. gh) * (q) = 135.43. To calculate the mean gage height H, divide (avg. gh) * (q) by sum Q in the form $\frac{135.43}{76.37}$ to give a mean gage height of 1.77 feet.

Example:

Time	h	Avg. h q	(Avg. h) X (q)	
13:30	1.94			
		1.92	***16.1***	**30.91**
13:45	1.90			
		1.70	***24.31***	**41.33**
14:00	1.49			
		1.67	***20.99***	**35.05**
14:15	1.85			
		1.88	***14.97***	**28.14**
14:30	1.92			

Sum q = 76.37 **Sum (avg. gh) * (q) = 135.43**

$$H = \frac{135.43}{76.37} = 1.77\ feet$$

Figure 4 Example of partial discharge weighting calculation method.

6.9.4.2 The Time Weighting method uses the mean gage heights between the noted times, the duration of those times, and the total time of the measurement. The Time Weighting formula is

$$H = \frac{t_1h_1 + t_2h_2 + t_3h_3 \ldots . + t_nh_n}{T}$$

where
H = mean gage height
T = total time for the measurement, in minutes = $t_1 + t_2 + t_3 \ldots . t_n$,
$t_1,\ t_2,\ t_3 \ldots . t_n$ = duration of time intervals between GH observations, and
$h_1, h_2, h_3 \ldots . h_n$ = average height during time interval 1, 2, 3, ….n.

6.9.4.2.1 In the example below the average gage heights (Referred to as Avg. (h)) from the discharge weighting example are used. The product of each time interval of 15 minutes Time interval (t) and Avg, (h) are calculated to give h * t. The four 15 minute time intervals in the example are added to give a total of 60 minutes. Each product of h * t are summed to give a total of 107.55. The summed products of h * t are divided by the total time in the form $\frac{107.55}{60}$ to give a mean gage height of 1.79 feet.

Example:

Avg. (h)	Time interval (t)	h * t
1.92	15	28.80
1.70	15	25.50
1.67	15	25.05
1.88	15	28.20
Total	60	107.55

$$H = \frac{107.55}{60} = 1.79\ feet$$

Figure 5 Example of time weighted calculation method.

6.9.5 Studies by the United States Geological Survey indicate the Partial Discharge method tends to overestimate the mean gage height and the Time Weighted method underestimates the mean gage height. For that reason it is recommended that both methods are computed and the two results averaged (Rantz, 1982). Keep in mind both methods are only applied to measurements where mechanical instruments or acoustic velocimeters were used. In the above examples the mean stage for the measurement using the results of both methods is 1.78 feet.

6.10 Measurement and Calculation Techniques When High Velocities Preclude Depth Soundings or Conventional Velocity Observations

6.10.1 If velocity is too great to make depth soundings or obtain conventional velocity samples, three alternative methods make discharge estimations possible. These are the two tenths method, the subsurface velocity method, and the surface velocity method.

6.10.2 *Two Tenths Velocity Method*

6.10.2.1 Calculation of velocity using the two tenths depth method involves sampling velocity at 0.2 of the depth from the surface and applying a coefficient derived from the point to mean velocity ratio. The point to mean velocity ratio refers to the calculated or estimated ratio between the measured velocity at two tenths depth, and the mean velocity of the entire water column.

6.10.2.2 In circumstances where depths can be reliably sounded but velocity cannot be sampled at 0.6 or 0.8 of depth because of drift debris or other reasons, sample velocity at 0.2 of the measured depth.

6.10.2.3 In situations where soundings are not possible, depths can be estimated when a reliable standard cross section or some knowledge of the bottom contour is available (Buchanan, Somers, 1969).

6.10.2.4 Coefficients applied to two tenths depth velocities are most reliable when derived from velocity curves from the specific location as opposed to published velocity curves. Develop a velocity curve between two tenths velocities and true mean velocities by recalculating past measurements. Recalculate complete measurements or measurement segments where 0.8 and 0.2 depth velocity samples were measured, using only the 0.2 velocity in the calculation. By plotting the true mean velocities versus the two tenths velocities for each measurement a mathematical relationship can be derived.

6.10.2.5 Studies conducted by USGS indicate that for a given cross section the relationship between the two tenths and true mean velocities remains constant or varies uniformly with stage (Rantz, 1982).

6.10.2.6 USGS Water Supply Paper 2175 (Rantz, 1982) provides a vertical velocity graph and table of point to mean velocity ratios. This graph and table were developed through intensive study of vertical velocity curves. If an insufficient number of measurements have been conducted to derive a reliable relationship at a specific site, the replicated graph (figure 6) and table 1 may be used to calculate point velocities. The point velocity is derived from the table by dividing the measured velocity by the ratio of point velocity to mean velocity. For example the ratio applied to a velocity measured at 0.2 of depth is calculated in the form $\frac{V}{1.149}$

where v is the measured velocity and the denominator of 1.149 is the ratio of point velocity to mean velocity at 0.2 of depth found on table 1.

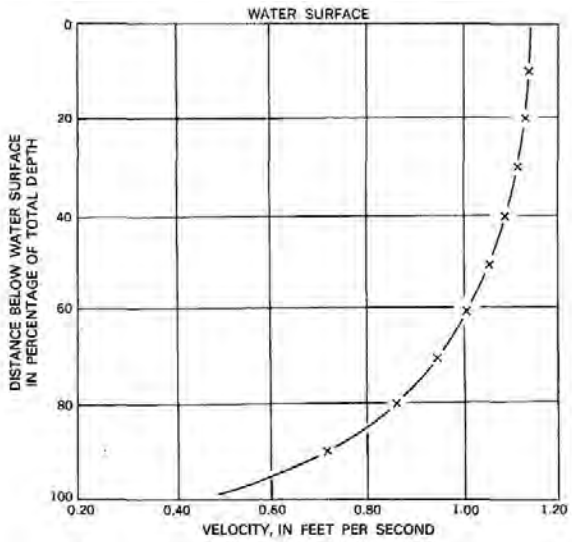

Figure 6 Graphical representation of standard vertical velocity curve developed by USGS. (Illustration from USGS Water Supply Paper 2175, pg. 133.)

Table 1 Point to mean velocity ratios for standard vertical velocity curve.
(Table from USGS Water Supply Paper 2175, pg. 133.)

Ratio of observation depth to depth of water	Ratio of point velocity to mean velocity in the vertical
0.05	1.160
.1	1.160
.2	1.149
.3	1.130
.4	1.108
.5	1.067
.6	1.020
.7	.953
.8	.871
.9	.746
.95	.648

6.10.3 *Subsurface Velocity Method*

6.10.3.1 Use the subsurface velocity method if measuring or estimating depth is not possible or practical.

6.10.3.2 Sample velocities at an arbitrary depth at least two feet below the surface.

6.10.3.3 Determine depths by soundings made at a later measurement when the flow has receded. Calculate the difference between the gage heights of the two measurements to estimate depths and meter elevations at the time of the subsurface velocity measurement. Compare cross sections of previous measurements with the cross section of the latest measurement to determine if the cross section has shifted (Buchanan, Somers, 1969).

6.10.3.4 With the known depths of the current meter, the ratio of point of velocity observation to vertical depth is computed.

6.10.3.5 As previously described, point velocity to mean velocity ratios can be obtained from velocity curves developed for the specific site. Use the graph in figure 6 and table 1 if a velocity curve specific to the site is not available.

6.10.3.6 A simple example of this application follows: Based on soundings subsequent to a subsurface velocity sample at a certain vertical, the depth at the time of the sample is calculated at 10 feet. Velocities were sampled at 3 feet below the surface. The ratio of point of velocity observation to vertical depth is $\frac{3feet}{10feet} = 0.3$. Table 1 shows the ratio of point velocity to mean velocity in the vertical at 0.3 of depth is 1.13. Calculate the adjusted velocity at 0.3 of depth in the form $\frac{V}{1.13}$ where v is the measured velocity and the denominator 1.13 is the ratio of point velocity to mean velocity.

6.10.4 *Surface Velocity Method*

6.10.4.1 If obtaining velocities below the surface is not practical or possible, the surface velocity method may be used. Lower the meter to the water surface to sample the velocity. The coefficient used to calculate mean velocity is 0.85 (Rantz, 1982). Alternatively the sampled velocity can be divided by 1.18.

6.11 Rating a Measurement

6.11.1 At the conclusion of each measurement, field staff apply a rating of the measurement on a scale from excellent to poor. This field rating forms the basis of the final quality code assigned to the measurement by the station's principle investigator.

6.11.2 Field staff base the measurement rating on observed conditions as well as quality analysis statistics available in the field. Acoustic velocimeters' and current profilers' operating systems offer comprehensive analytical packages.

6.11.3 The desirable characteristics of a measurement location serves as the basis for rating the physical conditions of the channel and cross section. Field staff observe the degree in which each of these characteristics influence the quality of the measurement.

6.11.4 Take into account factors such as the proximity of bridges and other structures to the cross section. These structures can effect velocity distribution and uniformity of depth (Rantz, 1982).

6.11.5 Consider the number of and the degree in which velocities are corrected because of oblique current angles. Keep in mind the assumption that the angle observed at the surface prevails through the entire vertical may be incorrect. Numerous angles of flow approaching the cross section from various directions may indicate turbulence resulting in non representative velocity samples.

6.11.6 Take notice of the condition of the equipment. Rate the measurement accordingly if problems with equipment occurred during the measurement.

6.11.7 Look at the role of the weather in the measurement. Wind over the water surface can obscure the direction of flow. Cold weather may cause ice or slush to form in the cross section. Ice or slush can adversely affect the operation of mechanical current meters and the ability to measure depth accurately.

6.11.8 Water level bounce and velocity pile up on wading rods and stadia rods adds uncertainty to depth determinations. Take into account these factors when rating a measurement.

6.11.9 Not all of the variables affecting the quality of a discharge measurement can be described here. Field staff must consider all the conditions and use professional judgment in rating a measurement.

6.11.10 The principle investigator or basin lead thoroughly reviews all of the components of the field rating and renders the final decision in all quality code assignments. It is extremely important field notes are thorough and complete.

6.11.11 Measurements are rated as excellent, good, fair, or poor on the discharge note form. An excellent measurement indicates the measured discharge value is within 2 percent of the actual flow value. A rating of good means the measured value is within 5 percent. A fair rating of a measurement indicates the measured value is within 8 percent. Poor means the error in the measured flow is within 13 percent of the actual discharge.

6.11.12 Field staff do not consider the level of difficulty in obtaining gage height observations in the measurement rating. Base the rating solely on measurement conditions. However, field staff should focus particular attention in field notes on the circumstances and degree of difficulty in obtaining an accurate gage height observation. Consider water level bounce and velocity pile up on staff gages or stadia rods. Wind can cause wave action that may make it difficult to locate the water surface with sounding weights. The principle investigator considers carefully these factors in formulating the final rating.

6.11.13 Principle investigators should keep in mind that in situations when stage changes rapidly the measurement is purposefully accelerated and some accuracy is lost. Stage determinations in these circumstances are often calculated rather than directly observed. Rate the measurement appropriately in these circumstances.

6.12 Assessing the Control

6.12.1 The physical features of a stream that regulates the relationship between stage and discharge at a gauge site is the control.

6.12.2 There are three basic types of controls; section control, channel control, and flood plain control.

6.12.3 The following discussion focuses on section and channel controls.

6.12.4 *Section Control*

6.12.4.1 A section control governs the stage and discharge relationship in a localized reach of the stream, downstream from the gage. The section control can be a natural or artificial structure, a channel constriction, or a downward break in slope in the stream bed.

6.12.4.2 Common section control structures include the buildup of rocks and boulders, or woody debris stretched across the channel. Manmade dams, weirs, or water diversion structures often serve as section controls. Channel constrictions can include rock outcrops or gravel bars. Bridges where the waterway opening is narrower than the natural channel is a common section control caused by channel constriction. Downward breaks in the slope of a streambed include heads of riffles, cascades or the brink of a falls.

Figure 7 This section control is defined by the downward break in slope at the head of the riffle. (Photo by Washington Dept. of Ecology)

6.12.4.3 At some stations more than one section control may be present. In these instances an upstream section control is in effect at lower stages. At higher stages another downstream section control becomes effective.

6.12.5 *Channel Control*

6.12.5.1 Channel control exists when the physical attributes of a long reach of the channel controls the relationship between stage and discharge. These physical attributes include shape, length, width, slope, sinuosity, and roughness of the channel. The length of the channel as an effective control increases as discharge increases (Rantz, 1982).

Figure 8 An example of channel control at the same location as figure 7. (Photo by Washington Dept. of Ecology)

6.12.6 *Control Dynamics*

6.12.6.1 Typically, section control is effective at lower stages and channel control effective at higher stages. An intermediate range of flows are subject to partial control. Partial control occurs when the relationship between stage and discharge is governed by both section and channel controls or by two separate section controls. As flows increase, section control is progressively drowned out by channel control. Partial control occurs between two section controls when stage increases such that the downstream control becomes increasingly effective.

6.12.6.2 The propensity of the channel to change over time determines the stability of the station controls. If high flow events frequently cause scour or filling of a channel, the controls are unstable. If a control changes frequently, the stage and discharge relation changes frequently. If controls are stable, the stage and discharge relationship remains stable.

6.12.6.3 Ideally all stations would have stable controls. Unfortunately this is not the case in the natural environment. Stations with unstable controls require frequent adjustment of rating curves. Additional measurements are required to establish new ratings rather than further define and reaffirm existing ones. More time is needed to manage records when ratings need frequent adjustment. This increases the costs to operate a station and can reduce the timeliness, and reliability of discharge records.

6.12.7 *Documenting the Condition of Controls*

6.12.7.1 Because controls may change over time, field staff must monitor and document the condition of controls at each discharge measurement. The station's principle investigator will need detailed information about the control if a change in rating occurs.

6.12.7.2 Field staff should identify the effective control or if there is a partial control situation. If section control is in effect, note the location of the control relative to the gage. Note the type of section control i.e., the control is a structure in the stream, or the control constricts flow like a gravel bar or outcrop. Note the materials making up the section control. Have copies of past notes or photos on hand to document changes in the control relative to previous visits.

6.12.7.3 It is important to note any forms of seasonal vegetal growth or ice on or around the control, and on the bed and banks. Aquatic vegetation and ice on the control or in the channel alters the stage and discharge relationship by reducing velocity and the effective waterway area (Rantz, 1982).

6.12.7.4 In autumn the accumulation of leaves can temporarily change the stage and discharge relationship. Note the extent of accumulation of leaves on the control or in the channel where the stage and discharge relation may be impacted.

6.13 <u>Measuring the Point of Zero Flow</u>

6.13.1 The point of zero flow (PZF) is the lowest point on a control and the stage at which flow ceases.

6.13.2 A PZF should be identified and measured whenever possible. A determination of the PZF is important because it helps define the lower end of a rating and serves as a first estimate of the off-set of a rating.

6.13.3 A change in PZF elevation or location indicates alteration of the control. A measurable alteration in the control can signify a shift in the rating.

6.13.4 Attempt to measure a PZF only when it is safe to do so. Follow these steps to identify and determine a PZF.

6.13.4.1 Locate the section control.

6.13.4.2 Take depth soundings across the control with a wading rod.

6.13.4.3 Locate and measure the depth of the PZF, the deepest point of the control.

6.13.4.4 Include in the depth reading the pile up of water on the wading rod.

6.13.4.5 Note the depth of the PZF to the nearest one-tenth of a foot.

6.13.4.6 Note the locations of the control and the PZF.

6.13.4.7 Take photographs of the control if the control is altered.

6.13.5 A record of PZF soundings and locations are kept in station notes.

6.14 <u>Calculating Stream Flow Using the Midsection Method</u>

6.14.1 Ecology's Freshwater Monitoring Unit uses the midsection method to calculate stream flows measured with mechanical current meters or acoustic velocimeters.

6.14.2 The equations comprising the midsection method are written into a variety of computer programs. The Freshwater Monitoring Unit uses Ecology's proprietary discharge calculation program, QWin to compute discharge. The SonTek® FlowTracker® contains a built in midsection calculation program to calculate discharge (Burks, 2009).

6.14.3 Although computation of discharge by hand is virtually unnecessary, an understanding of the midsection calculation method can influence strategies and decisions while conducting discharge measurements.

6.14.4 The midsection calculation method involves summing the discharges of the individual cells comprising the cross section. This is expressed in the following equation, $Q = \sum(a\ v)$ where
Q = total measured discharge
a = area
v = velocity.

6.14.5 The discharge of an individual cell is the product of its area and velocity normal to the cross section.

6.14.6 The area of the cell extends laterally half the distance from the previous vertical to half the distance to the next. The area also extends vertically from the water surface to the sounded depth. Evenly spaced verticals are not necessary. The mean velocity sampled at the vertical represents the velocity through the entire cell.

6.14.7 The discharge of any cell at vertical x is represented in the equation,

$$q_x = v_x \left[\frac{(b_x - b_{(x-1)})}{2} + \frac{(b_{(x+1)} - b_x)}{2} \right] d_x$$

$$= v_x \left[\frac{b_{(x+1)} - b_{(x-1)}}{2} \right] d_x$$

where
q_x = discharge through cell x,
v_x = mean velocity at cell x,
b_x = distance from initial point to vertical x,
$b_{(x-1)}$ = distance from initial point to preceding vertical,

$b_{(x+1)}$ = distance from initial point to next vertical, and
d_x = depth of water at vertical x.

6.14.8 For example, the discharge in highlighted cell 4 in figure 9 is expressed as $q_4 = v_4 \left[\frac{b_5 - b_3}{2} \right] d_4$.

6.14.9 The total discharge of the stream is equal to the sum of the discharges of each cell.

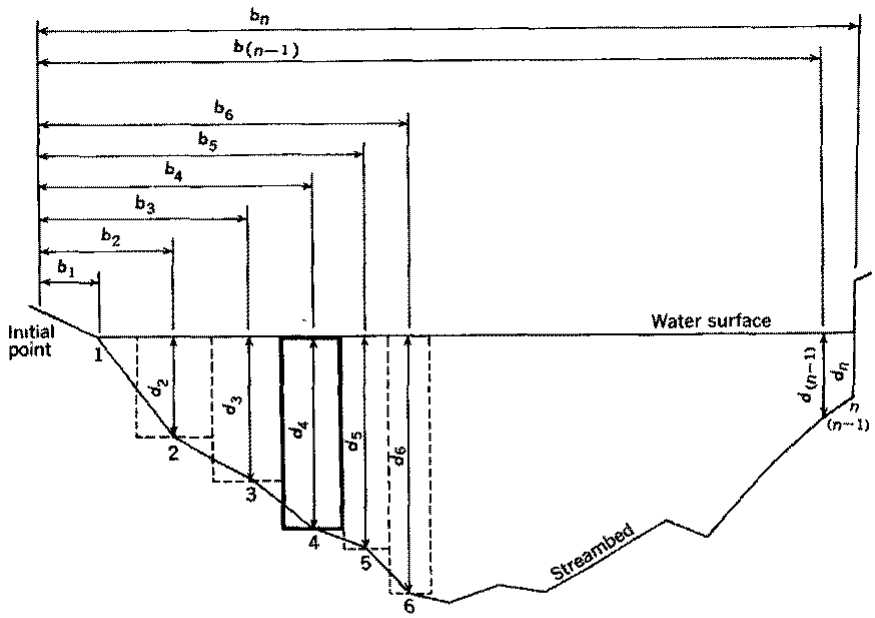

Figure 9 Cross section sketch illustrates depth and distance between verticals that define the area of measurement cells. Velocities measured at these verticals represent the velocity of the cell. (Illustration from USGS Water Supply Paper 2175, pg. 81.)

6.14.10 Observe in figure 9 the discharge of q_1 is zero. This is because the depth of observation point 1 is zero. There may or may not be actual discharge in this half cell on the edge of the cross section. Typically the amount of actual flow here is inconsequential. However the first and last vertical should be placed close to the edge when the depth at the edge is zero. This insures any residual, uncounted flow remains insignificant.

6.14.11 Now observe vertical *n* at right edge of figure 9. Here the edge is a vertical boundary where the depth is not zero and the velocity may or may not be zero.

6.14.12 Because velocity cannot be measured accurately at a vertical boundary, the velocity at the boundary can be estimated by measuring the mean velocity at a distance from the boundary equal to the depth at the boundary (Rantz, 1982).

6.15 Use of a Wading Rod

6.15.1 Ecology's Fresh Water Monitoring Unit uses a standard four foot top setting wading rod in graduated English units. Top setting wading rods have a 1/2-inch hexagonal rod to measure depth. The hexagonal rod is marked by single lines scored in one-tenth foot graduations up the length of the rod. Every one half foot is marked with double lines. At every one foot increment a group of three lines are scored. The top of the hexagonal rod at the base of the rod handle is 4 feet from the base of the rod.

6.15.2 The wading rod is placed in the stream such that the round base sits on the streambed. The depth of the water is read on the hexagonal rod. Care should be taken to make sure the depth is read consistent with the incremental marks on the rod as these markings are not numbered. Make sure the correct foot, half-foot, and tenth-foot marks are referenced.

6.15.3 Velocity head or pile up will occur to varying degrees depending on the velocity. If the pile up on the rod is low a depth reading is somewhat easy. In higher velocities pile-up and surface bounce will make the depth reading more difficult. Attempt to read the surface by eyeballing the water surface around the rod and estimate where it intersects the rod. To account for bounce try to estimate the average between the top and bottom of the bounce. Depending on the severity of these conditions the depth should be read to the nearest 0.1 or 0.05 depth.

6.15.4 A 3/8-inch diameter round rod is used for setting the position of the current meter to the desired measurement depth of two tenths, six tenths, or eight tenths of total depth at the measurement vertical.

6.15.5 The round rod is engraved with numbered lines extending around the circumference of the rod. The numbered lines correspond to the whole foot increment of the measured depth, for example 1.7 feet.

6.15.6 On the wading rod handle, the numbers 0 through 10 are pressed with corresponding hash marks. These numbers correspond to the tenths of feet measured in addition to the whole feet, for example 1.7 feet. The current meter is set by aligning the numbered lines corresponding to whole feet of the round rod with the appropriate tenths number pressed into the rod handle.

6.15.7 For example if a depth of 1.7 feet was observed, set the meter at six tenths of depth by aligning the 1 foot line on the round rod with the seven tenths mark on the rod handle. If the depth can be read to increments less than one tenth of a foot, set the round rod appropriately.

Figure 10 Wading rod with meter set at six tenths of a depth of 1.7 feet. (Photo by Washington Dept. of Ecology)

6.15.8 Meter settings at two tenths, eight tenths, and six tenths of depth means the meter is positioned in the water column at the respective depth setting measured from the surface of the water. The top setting rod is designed to automatically set the meter at six tenths of depth from the water surface.

6.15.9 Described alternatively, when the meter is set at six tenths from the water surface it is also set at four tenths of depth from the streambed. This point is important in understanding how to set the rod to measure velocities at two tenths and eight tenths of depth. By dividing the depth by 2 and setting the meter at that corresponding value, the meter is now set at two tenths depth from the bottom or eight tenths from the water surface. The current meter set at this depth is referred to as an eight tenths velocity sample because the meter is set at eight tenths of the depth from the water surface.

6.15.10 Conversely, if the observed depth is multiplied times 2, the meter is set at eight tenths from the bottom and two tenths from the water surface. This current meter setting is referred to as a two tenths velocity sample because the meter is set at two tenths of the depth from the water surface.

7.0 Records Management

7.1 Field Note Forms Archives

7.1.1 All original field discharge measurement notes are stored in central locations at Ecology Headquarters, Regional, and Field Offices.

7.2 Discharge Records in Hydstra Database

7.2.1 All discharge measurement details are recorded and stored electronically to the Hydstra Gauging and Sections databases.

7.2.2 Measurement details stored in the Gaugings database include the stage, discharge, date and time of the measurement. Other details stored in the Gaugings database include average velocity, area, maximum depth and velocity, width, and wetted perimeter.

7.2.3 Cross section details such as vertical locations and bed elevations are stored in the Sections database.

8.0 Safety

8.1 All EAP safety policies are followed and safety is always the top priority when conducting stream discharge measurements. Refer to the EAP Safety Manual, pages 36-37 and 54-59 (EAP, 2010) for further information about working in and around streams.

8.2 In all measurement situations unsafe deployments that may result in injury to staff, loss or damage to equipment are not attempted.

8.3 Always consider the safety and traffic situations when measuring from a bridge and take appropriate actions including suspending the measurement if unsafe conditions exist. Consult the EAP Safety Manual, page 59-60 (EAP, 2010) for further guidance regarding bridge measurement safety.

8.4 Crossing the stream is done safely and in accordance with the guidelines for working in and around streams established in the EAP Safety Manual, pages 36-37 and 54-56 (EAP, 2010).

9.0 References

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9.1 Burks, T.W., 2009. Standard Operating Procedure (SOP) for Operation of the Sontek® FlowTracker® Handheld ADV®, Version 1.0. Washington State Department of Ecology, Olympia, WA. SOP Number EAP058. www.ecy.wa.gov/programs/eap/quality.html

9.3 Environmental Assessment Program, 2010. Environmental Assessment Program Safety Manual, February, 2010. Washington State Department of Ecology, Olympia, WA. http://aww.ecology/programs/eap/Safety/Safety1.html

9.4 Holt, Z., 2009. Standard Operating Procedure (SOP) for Operation of Mechanical Velocity Indicators, Version 1.0. Washington State Department of Ecology, Olympia, WA. SOP Number EAP 059. www.ecy.wa.gov/programs/eap/quality.html

9.5 Rantz, S.E., et al, 1982. Measurement and Computation of Streamflow: Volume 1. Measurement of Stage and Discharge. United States Geological Survey Water-Supply Paper 2175.

9.6 Shedd, J.R., Springer, C., Clishe, C.J., 2008. Standard Operating Procedure (SOP) for Operation of the Teledyne RD Instruments® Stream-Pro Acoustic Doppler Current Profiler®, Version 1.0. Washington State Department of Ecology, Olympia, WA. SOP Number EAP055. www.ecy.wa.gov/programs/eap/quality.html

9.7 Teledyne RD Instruments®. WinRiver Quick Start Guide. 2007. P/N 957-6230-00.

Appendix A (Discharge Measurement Form Number 040-56)

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY

Meas.No. .....

Comp. by.....

DISCHARGE MEASUREMENT NOTES

Check by .....

Sta. No. ..... Prop. No. .....

Name: .....

Date ....., 20 ..... Party .....

Width ..... Area ..... Vel. ..... G.H. ..... Disch .....

Method ..... No. secs. ..... G.H. change ..... in ..... hrs.

Max Depth. ..... Hor. angle coef. ..... Wetted Perim. ..... Meter No. .....

GAGE READINGS

Time	TD/WT	Logger	Staff	+/-
REW				
LEW				
WW				
Cell Width				

Type of meter .....

Calibration Pre: ..... Post: .....

QA form attached Y/N

Vel Unc .....

Depth Unc .....

Overall Unc .....

Wading, cable, ice, boat, upstr., downstr., side bridge .... feet, mile, above, below gage, and ....

Check-bar, found ....
changed to ..... at .....

Measurement rated excellent (2%), good (5%), fair (8%), poor (over 8%), based on following conditions:

Cross section .....

Flow .....

Control .....

..... Photo taken Y / N

Gage .....

Weather .....

Other .....

Remarks .....

.....

.....

.....

.....

Zero flow = GH _____ -depth at control _____ = _____ ft.

ECY 040-56 (Rev. 12/07)

River at –

Dist. from initial point	VELOCITY							
	Depth	.6	.6	.8	.8	.2	.2	
1	REW @							
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								

.0 .10 .20 .30 .40 .50 .60 .70 .75

.80 .85 .90 .92 .94 .96 .97 .98 .99 1.00 .99 .98 .97 .96 .94 .92 .90 .85 .80

Appendix B (Equipment Checklist)

Vehicle and Equipment Checklist

Standard Vehicle Equipment:

This equipment should be present anytime the vehicle is used.

- Cell Phone and Charger

Vehicle Folder containing

- Mileage Logs
- Emergency Information
- Fuel Card
- Maps

Safety Equipment

- First Aid Kit
- MUTCD compliant Safety Vests (2)
- CG Approved PFD (1 per person)
- PFD CO_2 Refill
- Road Cones
- Signs
- Hard Hats (2)
- Orange Strobe

Tools / Other

- Mechanic's Toolbox
- Shovel
- Loppers/Clippers/Machete
- Tire Chains
- 2- 150 ft. Ropes
- Spare Key
- Jack, jack handle, adequate spare
- Flashlight
- Lighter
- Electrical Tool Box
- Pens
- Pencils
- Note Paper
- Flagging Tape
- Orange Spray Paint
- Spare Bucket

Standard Flow Gear

Flow Box:

- Weighted Tape for Tape Down
- Tag Line
- 300 ft. Transect Tape
- Line Clamps
- Swoffer Kit w/ Cables and Fans
- Swoffer Meter
- Bridge Depth Sounding Correction Sheets (2/10, 6/10, 8/10's)
- Survey Pins and Hammer
- Flow Tracker
- Wading rod
- Laser Level
- Stadia Rod
- Thermistor
- Spare Batteries for All Devices
- Battery Chargers
- Discharge Measurement Sheets

Station Visit

- Station Visit Data Flash Card
- Multi-meter
- Logger Menu Flow Chart
- Desiccant
- Station Key
- USGS key
- Other Keys as needed
- Appropriate DCP Batteries

ADCP Gear

- ADCP Unit
- PDA (CHECK BATTERY STATUS)
- SD card for PDA
- Tow Ropes and Carabiners
- ADCP Data Sheet

Bridge Gear (If Needed)

- Lead Flow Weights, all sizes
- Bridge Board
- T-bar
- Reel w/ Swoffer Cable

3-Wheel Crane

- Reel
- Crane Assembly

4-Wheel Crane

- HS Meter Box
- Props
- Meter Body w/Fiber-Fin
- Cleaning Soln.
- Lubricant
- Reel
- Crane Assembly/Boom
- Counterweights
- Wheel Chocks

Personal Equipment

- Water
- Food
- Dry Clothes
- Rain Gear
- Sunscreen
- Gloves
- Waders/Hip Boots
- Up to Date Ratings Sheets
- Maps/Station Directions
- Notebook w/ Extra Data Sheets

Washington State Department of Ecology

Environmental Assessment Program

Directed Studies Unit

Standard Operating Procedure for Estimating Streamflow

Version 2.0

Author - James Kardouni, Directed Studies Unit
Date -

Reviewer – George Onwumere, Directed Studies Unit Supervisor
Date -

Reviewer – Paul Pickett, Modeling and Information Support Unit
Date -

Reviewer – Jim Shedd, Freshwater Monitoring Unit Lead Hydrogeologist
Date -

QA Approval - William R. Kammin, Ecology Quality Assurance Officer
Date -

EAP024

APPROVED: 02/11/2013

Three-year Review – 08/26/2012

Signatures on File

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
2/28/2011	2	Updated several sections, added 6.3.3 measuring culvert discharge, added USGS reference.	all	James Kardouni
3/2/2011	2			George Onwumere
3/17/2011	2		1,5,6,9,10	Paul Pickett
3/24/2011	2		6	Jim Shedd
02/11/2013	V2.0	Three-year review		James Kardouni

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Standard Operating Procedure (SOP) for Estimating Streamflow for wade able streams, culverts, and other volumetric measurements such as pipes, drain tiles, or seeps. Additional stream discharge measurement techniques may be found in the following SOPs: *Measuring and Calculating Stream Discharge* (EAP056) or *Measuring Stream Discharge from a Bridge* (EAP060). Consider requesting Ecology's Freshwater Monitoring Unit assistance for measuring streamflow where conditions are beyond the techniques presented in this SOP.

2.0 Applicability

2.1 This document should be used for wade able streams, culverts, and other volumetric measurements such as pipes, drain tiles, or seeps.

3.0 Definitions

3.1 Fixed Point Averaging (FPA) - An average of velocities over a fixed period of time.

3.2 Reference Point (RP) - A fixed point or datum on the bridge or other structure from which a measurement can be made to the surface of the water under for all flow conditions.

3.3 Staff Gage - A graduated measuring device securely fixed to a permanent structure in the streambed from which river stage height can be read directly to the 100th of a foot.

4.0 Personnel Qualifications/Responsibilities

4.1 Trained in safety procedures for work in streams.

5.0 Equipment, Reagents, and Supplies

5.1 Flow/current meter. Currently the Directed Studies Unit uses two types of flow meters, the Marsh-McBirney® and the Swoffer®. Also of interest is the Acoustic Doppler current profiler (ADCP) if available.

5.2 Wading Rod designed to measure water depth and position the current meter at 0.2, 0.6, and 0.8 of the total stream depth

5.3 Field notebook or other recording device

5.4 Measuring Tape (100 ft or long enough to span the width of the stream)

5.5 2 Stakes

5.6 Clamps with attached springs (for clamping the measuring tape to the stakes)

5.7 Life Vest/Personal floatation device (PFD)

5.8 Hip or Chest Waders

5.9 Staff gage (if used) and equipment for installation

5.10 Tape down (if used)

5.11 Container (such as a bucket) and stop watch (both used for volumetric measurements)

6.0 Summary of Procedure

6.1 Selecting a Representative Cross Section to Measure Discharge

6.1.1 Selecting a suitable stream cross section for measuring discharge is very important and cannot be over emphasized. Site selection is, in most cases, the most important factor in developing accurate flow information. The limitations of a poor cross section can not be overcome by the ability of the individual taking the measurement. It is often not possible to find the ideal site, but try to find a reasonably accessible site that meets criteria to the maximum extent possible. Use the following criteria for deciding which cross section to use:

6.1.1.1 The stream reach should be relatively straight and uniform for a long enough distance to provide uniform flow through the measuring section (preferably 200-300 feet upstream and downstream of the measurement site). The site should be free of large eddies, slack water, and excessive turbulence.

6.1.1.2 The stream channel should have minimal vegetative growth and be relatively stable (free from major seasonal scouring or deposition of bed material).

6.1.1.3 The stream bed should be relatively uniform with only minor irregularities (few or no large cobble or boulders).

6.1.1.4 The stream channel should be confined to a single course, or multiple channels should have optimal characteristics. Braided channels tend to develop during low flow conditions (typically August-October).

6.1.1.5 The stream bank should be stable and able to contain the maximum stream discharge (floods) when the site will be accessed during the study period.

6.1.2 If these criteria are met, the cross section should be relatively stable under most conditions and the streamflow should be smooth (non-turbulent) and parallel (perpendicular to the cross-section). It is, however, unrealistic to assume all stream cross sections will meet all of these criteria. Therefore, complete and accurate field notes describing the cross section and noting how well the site meets these criteria are vital when determining the relative accuracy of the discharge measurement.

6.2 Site preparation

6.2.1 Ideally, the cross section to be measured will meet all of the selection criteria, however, some will not. In general work with what is available and do not make large scale channel modifications if any. If the cross section selected is compromised by excessive aquatic plants, the presence of woody debris, or has minor irregularities in the stream bed (rocks and manageable boulders), an attempt should be made to minimize their

impact on flow measurements. This may require physical removal of interference and minor alterations of the streambed. However be mindful not to disturb fish and wildlife habitat keeping impacts on the stream bed and riparian area to a minimum. After the cross section has been cleared, inspect the stream banks to ensure they are confining enough to provide a distinct edge. Do not modify the cross section while velocity measurements are being conducted. Modifying the cross section after velocity measurements have begun may alter the flow characteristics and therefore compromise the accuracy of your measurement.

6.3 Dividing the stream channel into segments

6.3.1 Channel geometry, substrate, and other stream features cause horizontal variability across the stream. Because most stream velocity and bottom contours vary as you proceed across the stream channel, the cross section is divided into manageable segments.

6.3.2 At the cross section stretch a measuring tape (tagline) across the stream. The tagline should be perpendicular to the stream flow.

6.3.3 Anchor the tape with stakes or to surrounding vegetation. The clamps may be used to anchor the tape to the stakes.

6.3.4 Note width of the stream channel and divide into conveniently measurable segments. Segments in the thalweg should have shorter distances between measuring point than segments where less discharge is apparent. The total number of segments should be large enough to ensure no more than 10% of the total flow is contained in any one segment (preferably 5%). In general 20 or more velocity measuring points will achieve 5% or less of discharge in any given segment. A minimum of 0.3 feet is required between measuring points.

6.4 Measuring stream velocity of the stream segments

6.4.1 Stream velocities not only vary horizontally across the stream transect, but vertically as well. Currently two methods are used to address vertical variability within a segment; one applies with stream depths less than 2.0 feet and the other for streams over 2.0 feet (Ecology's Freshwater Monitoring Unit cutoff is 1.5 feet instead of 2.0 feet). For stream segments under 2.0 feet in depth, the velocity is measured at sixth-tenths below the water's surface (six-tenths method). For streams with depths greater than 2.0 feet, the velocity is measured at two-tenth and eight-tenths of the depth and the results are averaged (two-tenths/eight-tenths method). In situations where stage is changing significantly during the course of the measurement, the six-tenth method should be applied at all depths. The idea is to get the measurement finished quickly and limit the loss of accuracy caused by rapidly changing stage.

6.5 Measuring Water Depths and Velocities

6.5.1 Record site information in the field notes.

6.5.2 Measure and record stage gage readings or tape down (if available).

6.5.3 Select a suitable stream cross section for measurement.

6.5.4 Determine which safety requirements are warranted based on in-stream conditions. Do not proceed if the conditions are not deemed safe such as deep swift moving water, soft muddy bottom, or steep slippery banks.

6.5.5 Prepare cross section as necessary.

6.5.6 Stretch measuring tape (tagline) across the stream channel perpendicular to streamflow and note total stream wetted width.

6.5.7 Divide stream width into approximately 20 segments with no more than 5 to 10 % estimated flow in any one segment. Consider reducing the number of segments under rapidly changing stage conditions. Visually determine where segments should be reduced or expanded based on anticipated streamflow velocities and depths. For example shorten the segment where high velocities occur and lengthen the segment where low velocities may occur. Also decrease segment size when measuring close to boundaries (i.e., boulders, logs, near bridge pilings etc). The two-tenth and eight-tenths methods may also be use near or behind stream obstructions.

6.5.8 Turn current meter on and make sure settings are correct (See Appendix A for current meter instructions).

6.5.9 Note at what bank the measurements begin (either left bank or right bank). Left and right bank is determined when facing downstream. Begin the flow measurement by recording the number on the measuring tape that corresponds with the wetted edge of the stream. Record a zero value for the depth if the wetted edge is not a vertical boundary. If the wetted edge is a vertical boundary with depth and velocity then the velocity at the wetted edge should be estimated. Because velocity cannot be measured accurately at a vertical boundary, the velocity can be estimated by measuring the mean velocity at a distance from the boundary equal to the depth at the boundary (Rantz, 1982).

6.5.10 Measure the depth and length of the first vertical by reading the water level (stream depth) on the wading rod and number on the tape. The “vertical” refers to the point in the cross-section where depth and velocity are measured. To get a representative water depth reading, take into account the water mounding on the upstream side of the wading rod and any transient variation in the water level. Look for a reading of the central tendency that represents conditions around the rod for the segment you selected. Record the stream depth and number on the measuring tape. Always stand downstream and to the side of the wading rod so your presence in the water doesn’t create a local backwater that affects the velocity reading.

6.5.11 Adjust the wading rod to the proper depth and reset/clear the meter to begin averaging stream velocities.

6.5.12 For stream depths < 2.0 feet, use the scale on the wading rod to place the meter sensor at six-tenths (0.6) depth. Basically the stream depth measurement value will correspond with the same number on flow rod. For example the flow rod should be set 1.2 for a stream depth of 1.2 feet.

6.5.13 For stream depths > 2.0 feet, adjust the wading rod so that the meter sensor is at half the total depth for the eight-tenths (0.8) depth and double the total depth for two-tenths (0.2) depth. For example if the stream depth is 2.5 feet, the current meter should be positioned at 1.25 and 5.0 on the wading rod in order to measure eight-tenths and two-tenths depths respectively.

6.5.14 After the velocity meter has completed its averaging interval, record the velocity in the proper column in field notes.

6.5.15 If the velocity measurement is a negative number, turn the flow meter 180° to face downstream to check if here too is a negative number. If both directions are negative record a zero velocity value. If the downstream measurement is positive, then record this velocity as a negative number. This will capture any back eddies that can cause significant error when calculating streamflow.

6.5.16 Proceed across the stream, repeating steps 10-15 at each vertical segment until the entire wetted width is accounted for.

6.5.17 Measure and record the stage height (if available).

6.6 Measuring Water Depths and Velocities from a Bridge

6.6.1 Refer to the EAP Freshwater Monitoring Unit's SOP for Measuring Discharge from a Bridge (EAP060).

6.7 Measuring Water Depths and Velocities from a Boat.

6.7.1 Refer to the EAP Freshwater Monitoring Unit's SOP for Measuring Discharge from a Boat (EAP056).

6.8 Calculating Stream Discharge

6.8.1 The midsection method is used to calculate stream flow based on United States Geological Survey (USGS) technique (Rantz, 1982). The equations comprising the midsection method are written into a variety of computer programs. Ecology's Directed Studies Unit uses an Excel® spreadsheet to calculate stream discharge.

6.8.2 Although computation of discharge by hand is virtually unnecessary, an understanding of the midsection calculation method can influence strategies and decisions while conducting discharge measurements. The midsection calculation method involves summing the discharges of the individual cells comprising the cross section using the following equation:

$$q_x = v_x ((b_{(x+1)} - b_{(x-1)})/2)\ d_x$$

where for segment 'x':

$b_{(x+1)}$ = distance from initial point to next vertical (feet),
$b_{(x-1)}$ = distance from initial point to preceding vertical (feet),
d_x = depth of water at vertical x (feet)
v_x = mean velocity at cell x (feet per second)
q_x = discharge through cell x (cfs)

6.9 Float Method to Estimate Velocity

6.9.1 When usual flow measurement methods cannot be used, a neutrally buoyant object can be used to estimate velocity. The object can be an orange, a plastic sample bottle partially filled with water, or any other practical semi-buoyant object. This method is not recommended for estimating discharge used to calculate loads in a Total Maximum Daily Load study. However this method is more intended for remote streams where over night backpacking is necessary where hauling all the necessary discharge equipment is too difficult.

6.9.2 Locate a straight stretch of stream

6.9.2.1 Select two cross-sections within the stretch, measure (or estimate) their cross-section area and distance between them. Sites should be far enough apart that float movement between sites exceeds 20 seconds.

6.9.2.2 Release the float at the upstream site and record the time it takes to reach the downstream site. Repeat at least twice (more if the times are variable) and average the measurements. To increase accuracy, release the float at different places across the width of the stream.

6.9.2.3 Calculate the velocity as distance traveled divided by average travel time.

6.9.2.4 Calculate the adjusted (true mid-depth) mean velocity of the water by multiplying the surface velocity by 0.85.

6.9.2.5 Calculate discharge by multiplying velocity by the average cross-sectional area.

6.10 Measuring Flow from Pipes or Culverts

6.10.1 Volumetric Measurements

6.10.1.1 In this method, discharge is calculated by observing the time required to fill a container such as a bucket or 1 liter bottle of a known volume (also known as a "bucket flow"). Note the container does not have to be filled completely as long as a known volume is captured at a known time this will give a discharge volume over time. A limiting factor of this technique is that it can only be used with small discharges (i.e., where all of the flow can be caught in one container). This technique can also be used to estimate discharge over a weir or at any place where flow is concentrated into a narrow stream.

6.10.1.2 Place bucket or other container below the discharge.

6.10.1.3 Time how long it takes to fill the container. Repeat three times (or more if there are large differences between results). Record the volume and time.

6.10.1.4 Another volumetric measurement technique is to measure the volume of discharge over a fixed time. This will require using a graduated container to measure discharge volume. Mark the side of a bucket at graduated volume intervals. For example add one liter water to a bucket and mark the water level and volume in liters. Next add an additional liter, mark the water level and volume repeating these steps until the entire bucket is marked. The bucket can now be used as a graduated container.

6.10.1.5 Fill the bucket over a fixed amount of time and record the volume. Repeat three times (or more if there are large differences between results). Record the volume and time.

6.10.1.6 Calculate discharge as the volume of the container divided by the time to fill it. Average either the time or volume depending on the technique used when performing repeat measurements.

6.10.2 <u>Discharge of a Jet of Water</u>

6.10.2.1 This technique can be used on any discharge regardless of size. The limitations are that the pipe must be horizontal and it must be running completely full.

6.10.2.2 Measure or estimate the diameter of the pipe.

6.10.2.3 Measure the distance from the end of the pipe to the spot where the stream of water hits the ground ("x").

6.10.2.4 Measure the vertical distance from ("x") to the midpoint of the pipe orifice ("y").

6.10.2.5 Calculate the velocity as: $V = 4.01(x)/\sqrt{y}$

6.10.2.6 Calculate the area ("A") of the pipe as: $A = \pi r^2$

6.10.2.7 Calculate the volume by multiplying the area by velocity. Units of measurement must be the same.

6.10.3 <u>Measuring Discharge from a Culvert</u>

6.10.3.1 Culvert discharge can be calculated by measuring the culverts circular diameter, the average velocity, and the depth of water at the end of the culvert. These measurements can then be entered into a spreadsheet used to calculate discharge.

6.10.3.2 The three necessary measurements needed to calculate culvert discharge include (1) culvert diameter, (2) water depth, and (3) average velocity. The average velocity should be measure using a velocity meter. Three or more average velocity measurements may be necessary to find the central tendency.

6.10.3.3 The spreadsheet used to calculate culvert discharge uses the Manning equation for circular culvert geometry and can be obtained from Directed Studies Unit personnel.

6.11 Use of Staff Gage for Estimating Flow

6.11.1 A staff gaging station may be set up at a sample site (preferably at the mouth of the watershed). The purpose of a staff gaging station is to develop a relationship between stream height (stage) and flow. Once this relationship is established, stream discharge may be estimated based on gage heights.

6.11.2 Site Selection

6.11.2.1 The stream course should be relatively straight and free flowing for 200-300 feet upstream and downstream of the measurement site. The site, however, should be free from excessive turbulence.

6.11.2.2 The stream channel should be free from vegetative growth and be relatively stable (free from major seasonal scouring or deposition of bed material).

6.11.2.3 The steam bed should be relatively uniform with only minor irregularities (no large cobble or boulders).

6.11.2.4 During low flow conditions, the stream channel should be confined to a single course.

6.11.2.5 The stream bank should be stable and able to contain the maximum stream discharge (floods).

6.11.2.6 Gaging stations should be located a sufficient distance upstream of tributaries and tidal action to prevent the distortion of stage/discharge measurements.

6.11.2.7 Discharge should be measured within the reach. It is not necessary to measure low and high flows in exactly the same place.

6.11.3 Staff Gage Installation

6.11.3.1 Attach staff gage vertically on a permanent structure (concrete piling, revetment, etc.) or install in the stream by driving an appropriate post into the substrate and then attaching the staff gage to the post.

6.11.3.2 At locations where the stage range is high, it may be necessary to set staff gages in series to accommodate a variety of stream levels. Sometimes two or more staff gages are necessary for low and high flows.

6.11.3.3 Set the zero point of the staff gage below the lowest level of possible streamflow to prevent negative values of the gage height. Staff gages with higher ranges reduce the possibility of negative values such as 6-9 ft or 9-12 ft ranges rather than a 0-3 ft range. Place in a location where de-watering is not likely.

6.11.3.4 Establish a datum point on the gage, and make two or three reference points (RP's) at the same level on nearby permanent features. (Use a point on the gage that is above the highest expected gage height to prevent flow-related erosion of the marks.) It is recommended to install and survey in at least 3 reference points at locations that will not be disturbed during the study period. With three RP's one can determine with good certainty any staff gage movement or replace staff gage (reestablish or reconfirm datum) if necessary during study period. Three RP's also gives ability to check if any of the RP's themselves have moved. The datum may also be referenced to an official surveyor's benchmark. Establishing reference elevations allows data to be recovered if the staff gage is destroyed.

6.11.4 Reference Point Measurement

6.11.4.1 The distance from a reference point (RP) to the water surface is measured with a weighted fiberglass measuring tape (tape-down). The weighted tape is lowered to the water surface just to the point were the wake from the water passing by the weight forms a slight distinctive "V" shape. The distance from the RP to the water surface is recorded to the nearest 100th of a foot. If possible use tape-downs as a secondary/back up gage to a staff gage rather than the primary or sole gage at a site. Keep in mind tape-downs are subject to inaccuracies from windy conditions (under-measures stage) and fiberglass tapes tend to stretch over time (also resulting in under-measurement of stage).

6.11.4.2 Establish a RP on the bridge by locating or creating a permanent mark over an area of the stream not likely to dewater during low flow.

6.11.4.3 Find the RP mark on the bridge.

6.11.4.4 Lower the weighted tape until it just touches the water (a distinctive "V" should appear downstream of the weight). Raise the weight to make sure you are just touching the water.

6.11.4.5 Read the tape at the edge of the RP to the hundredth of a foot.

6.11.4.6 Record the time, RP measurement, and the correction factor for the tape (written on the side of the tape) in the field notes.

6.11.5 Establish a Rating Curve

6.11.5.1 The development of rating curves is complex. The USGS has detailed information about rating curve development (Sauer, 2002). Rating curve regressions work well over a relatively narrow range of discharge. Most stage/discharge relationships are logarithmic and usually regression correlations breakdown as range of measured flows increases.

6.11.5.2 Take streamflow measurements over a wide range of gage heights and/or RP measurements. It is very important that measurements are not just made in average flow conditions, but also at high and low flows to develop a rating cure for a wide range of flow conditions. Make sure to note the gage height/RP measurement before and after the flow measurement.

6.11.5.3 Develop a rating curve using regression analysis of instantaneous flow measurement and stage height. Rating curves should be based on sufficient number of measurements to allow a smooth curve to be drawn through the points (usually 8 to 10 measurements).

6.11.5.4 Periodically check the discharge curve, especially after high flows, to ensure the stream bed has been unaltered by sediment deposition or erosion, and that a reasonably accurate rating curve still exists. Smaller streams are subject to regular channel/control changes that may result in frequent rating adjustments. This can occur several times a year in some cases.

6.12 Other Flow Measurement Methods

6.12.1 A good reference for a variety of ways to measure flows in different situations is the *Water Measurement Manual* published by the U.S. Bureau of Reclamation (http://www.usbr.gov/pmts/hydraulics_lab/pubs/wmm/).

7.0 Records Management

(Not Applicable)

8.0 Quality Control and Quality Assurance Section

8.1 QA/QC procedures will be addressed thoroughly on a project-by-project basis in the Quality Assurance Project Plan (QAPP) for the project.

9.0 Safety

9.1 Wading streams is one of the most dangerous activities undertaken by field staff especially during higher flows. Two people are required at all times when streams are to be waded. Life jackets are to be worn if there is any chance of being pushed downstream or being submerged after falling into the water. Life jackets should also be worn when new sites are being established and when-stream conditions to be encountered are unknown.

9.2 If there is any chance of the streamflow being strong enough to potentially cause injury (by being swept downstream into rocks or other dangerous settings, drowning, hypothermia, etc.), do not consider taking a flow cross section. If a safety harness or safety rope is warranted, do not take velocity measurements. Consider requesting Ecology's Freshwater Monitoring Unit assistance for measuring streamflow where conditions are beyond the techniques presented in this SOP. When in doubt, err on the side of safety.

9.3 For further field health and safety measures refer to the: http://aww.ecology/programs/eap/Safety/safety.html

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Appendix A: Current Meter Instructions

1.0 Current Meter Instructions

1.1 Marsh-McBirney® Model 2000

The Marsh-McBirney® 2000 consists of a transducer probe cable, and a signal processor. The probe emits an electromagnetic frequency used measure velocity. The Marsh-McBirney® wading rod has an adapter capable of holding the transducer probe.

Marsh-McBirney® model 2000 current meters should be zeroed periodically to ensure accurate measurements. The meters should be zeroed at least once a week during use; however, it is preferable that they are zeroed at the beginning and end of each day of use. They should also be sent to the factory for calibration at least once a year. First clean the sensor because a thin film of oil on the electrodes can cause noisy readings. Then place the sensor in a five gallon plastic bucket of water. Keep it at least three inches away from the sides and bottom of the bucket. To make sure the water is not moving, wait 10 or 15 minutes after you have positioned the sensor before taking any zero readings. Use a filter value of 5 seconds. Zero stability is ± 0.05 ft/sec.

Marsh-McBirney® Model 2000 Zero Adjust

- Position the sensor as described in the zero check procedure.
- To initiate the zero start sequence, press the **STO** and **RCL** keys at the same time. You will see the number 3 on the display.
- Decrement to zero with the 6 key.
- The number 32 will be displayed.
- The unit will decrement itself to zero and turn off. The unit is now zeroed.

Measuring Velocity

- Attach transducer to the probe to wading rod.
- Turn the meter on.
- Set selector to desired measuring unit (e.g., FT/S). You can toggle between M/S and FT/S by pressing the **On** and **Off** keys simultaneously. Set the meter to Fixed Point averaging (FPA) and set the interval between 20 and 40 seconds. Press the ↑ and ↓ keys simultaneously to alternate between the FPA and rC displays. The display will show the letters FPA when you first switch to the FPA display. Except for the first period, the display is updated at the end of each averaging period. For example, if the FPA is set to 10 seconds, the display is updated once every ten seconds. The FPA display will have a horizontal time bar under the velocity output. The time bar provides an indication as to the amount of time left until the display is updated. The FPA time is specified in seconds. The ↑ key increments time and the ↓ key decrements time. The display will show the FPA length in seconds. After you have reached the desired setting, wait and the display will automatically switch to velocity.

- Place the probe in the stream with the round end of the sensor facing into the stream.
- The wading rod should be set to the appropriate depth (see Measuring Stream Velocity-Section 6.4).
- Wait for the appropriate time delay and then record the stream velocity.

1.2 Marsh-McBirney® Model 201

The Marsh-McBirney® 201 consists of a transducer probe cable, and a signal processor. The probe emits an electromagnetic frequency used measure velocity. The Marsh-McBirney® wading rod has an adapter capable of holding the transducer probe.

Measuring Velocity

- Attach transducer to the probe to wading rod.
- Set selector switch to **Cal** and the time constant switch to **2**. After approximately
  10 seconds, the readout should be on or between 9.8 and 10.2. If not, change the batteries and recheck.
- Set selector to desired measuring unit (e.g., FT/SEC).
- Set time constant switch. The purpose of the time constant is to help stabilize flow readings. This produces a delay between when the unit is first turned on and the time the first full scale reading is reached. This delay can be calculated as seconds by multiplying the switch setting by five. Start with the smallest time constant
  2 (10 second delay). If after the calculated time delay the output has not stabilized, move to the next highest number.
- Place the probe in the stream with the round end of the sensor facing into the stream.
- The wading rod should be set to the appropriate depth (see Measuring Stream Velocity-Section 6.5).
- Wait for the appropriate time delay and then+ record the stream velocity.

1.3 Swoffer®, Inc. Model 2100®

The Swoffer® is composed of a rotor assembly, sensor body, and cable which are attached to a wading rod (assembly is called the Sensor Wand), an indicator panel then attaches to the Sensor Wand to form the whole unit.

Quality Assurance Project Plan
for
Drayton Harbor, Microbial Source Tracking Pilot Study, Cain Creek Update
EPA Region 10, Washington Operations Office (WOO)

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1.0 Project Management

1.1 Distribution List

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1.2 Project Organization

(EPA QA/R-5 A4)

The following individuals are responsible for design and implementation of this project, and/or will be the primary data users and decision makers:

- **Stephanie Harris**, (360) 871-8710, EPA Region 10 Environmental Laboratory investigator, is responsible for assisting with the preparation of the quality assurance project plan (QAPP), analysis of samples, and preparation of the final report.

- **Suzanne Skadowski**, (360) 753-9469, EPA project manager, will serve as the primary point of contact for the project.

- **Debby Sargeant**, (360) 236-3320,

- **Julie Hirsch,** (360) 758-4046, Hirsch Consulting Services. Project Manager, Sample Analysis Lead, Data Analysis Lead and Monitoring Technician Lead. Will provide

- **Stephanie Bailey**, (360) 871-8739, EPA microbiologist, will assist with project planning and analysis.

- **Geoff Menzies,** (360) 384-9135. Puget Sound Restoration Fund, Project Coordinator. Will….

- **Roy Araki**, (206) 553-6395, EPA Quality Assurance (QA) Officer, is responsible for assisting the project manager and investigator in the development of the QAPP, subsequent revisions, and amendments.

- **Brandon Perkins,** (206) 553-6396, EPA Regional Sample Control Coordinator (RSCC) for the EPA Region 10 Technical Support Unit, will coordinate sample analyses performed by the Region 10 Laboratory and will provide sample numbers for samples that will be analyzed at the Region 10 Laboratory.

1.3 Problem Definition/Background

This QAPP is updated to distinguish human fecal input to Cain Creek, a tributary to Semiahmoo Bay which in 2010 was found likely to influence Drayton Harbor that is currently in the TMDL implementation process.

Cain Creek is an urban watershed that drains a large portion of the city of Blaine to Semiahmoo Bay. Since 1999, water quality monitoring data have shown that fecal bacteria levels exceed water quality standards and may impact shellfish harvest along Semiahmoo Bay shorelines in the US and Canada and inside Drayton Harbor (HCS, 2010). Impaired water quality affects tribal, commercial and

recreational shellfish harvest in Drayton Harbor and in Semiahmoo and Boundary bays. The Federal Clean Water Act requires that a Total Maximum Daily Load is developed for impaired water bodies that calculates the amount of a pollutant that a waterbody can receive and still safely meet water quality standards. In 2008, the Washington State Department of Ecology began the bacteria Total Maximum Daily Load Study (TMDL) of the Drayton Harbor watershed (including Cain Creek) to improve water quality for safe primary contact and shellfish harvest. The TMDL study (Ecology, May 2010) found that reductions of fecal bacteria up to 95% at Cain Creek are required to meet the water quality standard. Data collected by Ecology and by H.C.S indicate that bacteria levels were relatively low at the headwaters of Cain Creek but increased sharply downstream where the subsequent sampling station was located. Data were collected by Hirsch Consulting Services in 2009 for the Nooksack Indian Tribe (NIT). This TMDL support study found that upper watershed sites met water quality standards but downstream sites exceeded both fecal coliform thresholds with geometric means up to 15 times the allowable level of 50 CFU/100 mL (599 – 762 CFU/100 mL). DNA microbial source tracking data that were collected during the study identified human fecal markers more often in water samples collect from downstream sites that correlated with extremely high fecal coliform densities.
This current study establishes four sampling sites that segment this portion of Cain Creek and characterizes water quality to assist in identifying source(s) of fecal bacteria. Because Blaine Public Works has partnered to provide fecal coliform analysis for this study for the express purpose of further resolving human *Bacteroides* markers identified during the 2010 study, additional MST data will assist them in determining whether high FC could originate due to potential problems with sewage collection lines in proximity of sampling locations. Through a grant funded by the Whatcom Community Foundation and partnership with the City of Blaine, the Puget Sound Restoration Fund is collecting data that will assist the City of Blaine in reducing fecal bacteria discharged to Cain Creek while involving high school students in the process. An understanding of water quality issues in their community will help these students to present to their City Council, data and their recommendations for improving water quality and making Cain Creek an asset for the community.

(EPA QA/R-5 A5)

1.3.1. Background

The health of Drayton Harbor has been a concern for nearly 20 years. Poor water quality and concerns about failing on-site sewage systems and livestock waste in the California and Dakota Creek subwatershed led to a downgrade of 500 acres of shellfish growing areas at the south end of the harbor in 1988. Further shellfish downgrades occurred in 1995 and 1999, due to continually degrading water quality throughout the harbor. Due to progress made addressing sources of pollution, by 2004 DOH was able to reclassify portions of Drayton Harbor to “Conditionally Approved” with closures occurring after large rainfall events. In spite of recent successes, harvesting is still Prohibited on several hundred acres of important tribal, commercial, and recreational shellfish beds. In addition, trends of high fecal coliform bacteria (FC) levels in Blaine Harbor marina continue.

In 2005, the Puget Sound Restoration Fund (PSRF) funded a literature review and case study analysis of microbial source tracking (MST) techniques appropriate for application to Drayton Harbor (Hirsch Consulting services [H.C.S.] 2005). This review was shared with local agencies to build support for an MST study in the Drayton Harbor watershed and to establish an approach for implementing corrective actions based upon MST pilot study results. The review recommended a phased approach to MST

beginning with a small- scale pilot study followed by a watershed-wide study based upon results of the pilot work. The recommended MST methods were *Bacteroides* host specific polymerase chain reaction (PCR) and ribotyping.

Whatcom County has begun a sampling project in tributaries of California Creek that lends itself to augmentation with MST techniques. The EPA Region 10 laboratory has proposed utilizing Drayton Harbor for a laboratory pilot study using the *Bacteroides* host specific (PCR) method. In addition, Whatcom County is in the process of initiating a pilot study to identify septic system input to California Creek sampling stations using sampling for optical brighteners

The PSRF has directed H.C.S. to prepare and implement a monitoring plan that incorporates the projects described above with DOH and PSRF marine sampling in Drayton Harbor, into an MST pilot study. The study is proposed to begin in the fall of 2006 to be completed in 2007.

Drayton Harbor Watershed and the California Creek Sub-basin

The Drayton Harbor watershed is located in the northwest corner of Whatcom County, Washington and straddles the international border with Canada. The watershed encompasses 35,102 acres including 256 acres in British Columbia. There are 129 miles of tributary streams contained within the Drayton Harbor Watershed. California and Dakota Creeks constitute the primary freshwater inputs to the Harbor draining over 90% of the watershed area (Peterson, 1995). The remaining portion of the watershed drains directly into Drayton Harbor. The mouth of Drayton Harbor lies just south of Semiahmoo Bay that is bisected by the international border with Canada. Figure 1 shows the boundaries of the Drayton Harbor watershed. Total annual rainfall recorded at Blaine averaged 41 inches per year between 1948-1998 with about 68% in the fall and winter ranging from 24.8 inches in 1952 to 52.7 inches recorded in 1997. The highest daily rainfall value was 3.4 inches in November 1955 (Determan, 1999).

Drayton Harbor is an enclosed marine embayment with a rich history as a shellfish resource (Figure 1). Shellfish harvest and growing has been restricted intermittently due to fecal pollution since 1952. In 1988 the Drayton Harbor watershed was designated the priority watershed in Whatcom County by the Puget Sound Water Quality Authority. Classification by the Washington State Department of Health (DOH) resulted in closure and restriction of portions of Drayton Harbor in January 1995. In September 1999, the remainder of the shellfish growing area was downgraded to Prohibited status.

In response to the January 1995 closure, Whatcom County established the Drayton Harbor Shellfish Protection District in July of 1995 and the Drayton Harbor Initial Closure Response Strategy was developed. In 2001 the Puget Sound Restoration Fund (PSRF) gained approval from DOH and Washington State Department of Natural Resources (DNR) to seed two acres in the central portion of Drayton Harbor with Pacific oysters as the Drayton Harbor Community Oyster Farm. The goal of the project was to involve community members in growing Drayton Harbor oysters for harvest in 2004. The oyster farm inspired efforts by various local agencies to control fecal pollution sources and water quality improvements were realized. In June 2004, DOH reopened 575 acres in the central portion of Drayton Harbor for conditional harvest based upon rainfall. Shellfish harvest was closed for five days immediately following more than ½ inch of rainfall in a 24-hour period (Lennartson, 2004). In February 2006, the thresholds for the conditional approval were adjusted resulting in a six-day closure following a rainfall event of ¾ inches in a 24-hour period. Since the summer of 2004, 2000 bushels of oysters have been harvested most of which have been exported to China. Drayton Harbor is included on the state

303(d) list for fecal coliform bacteria and has been selected by the Washington State Department of Ecology (Ecology) for a Total Maximum Daily Load (TMDL) beginning in 2006 (Hood, 2006).

California Creek is one of two major streams in the Drayton Harbor watershed emptying into the harbor from the SE about ¾ mile south of the Dakota Creek entrance. The California Creek sub-basin is 13,762 acres which is about 40% of the total Drayton Harbor watershed area. Four stream systems and 5 major ditches drain into California Creek (Peterson 1995). The average mainstem flow estimated by the Northwest Indian College during bi-monthly sampling events from 2002 through spring 2005 was approximately 15.5 cubic feet per second (cfs) with a minimum of 3.52 cfs in August 2003, and a maximum of 43 cfs in November 2003 (Cochrane, 2005). The upper mainstem has been extensively channelized and impacted by agricultural activities and lacking vegetative cover. In 1991, a watershed characterization by the Puget Sound Cooperative River Basin Team found the lower mainstem was not as heavily impacted as were upper reaches, however, tributaries to the lower mainstem were heavily impacted (PSCRBT, 1991). In 2003 a wetland analysis estimated impervious surface at 5% in the California Creek sub-basin (Stanley, 2003). According to Stanley (2003), process changes to the hydrology and to the delivery of nutrients in the California Creek watershed have resulted in significant impacts to the water quality and habitat within Drayton Harbor.

California Creek soils are silt and clay estuarine deposits of the Sumas Stade glacial outwash yielding slow to moderate infiltration rates with fair bank stability (Matthews et. al, 1997). The high percentage of silt an clay particles in these soils results in low permeability with high run-off potential and pollutant transport. The California Creek terrace soils have a higher degree of saturation throughout the soil profile for a longer period of time than in the Dakota Creek sub-basin due to the presence of impermeable lacustrine and glaciomarine drift deposits (Stanley, 2003). A large peat deposit east of the town of Custer drains to California Creek and has been largely altered for pasture and cropland (PSCRBT, 1991).

Land Use

Land use in the Drayton Harbor watershed is diverse. Urban, residential, agriculture (commercial and hobby farms), surface mining, forestry, and marinas including industry are all identified as significant land uses in the watershed by the 1995 Drayton Harbor Management Plan (Peterson, 1995). The incorporated city of Blaine flanks the eastern shore of Drayton Harbor just south of the Canadian border. The Resort Semiahmoo and residential development borders the western shore of the harbor. California and Dakota Creeks discharge into the harbor from the southeast and east respectively. Custer is an unincorporated town located in the upper California Creek drainage. In 1991, the PSCRBT found that grazed pasture occupied 59% of land adjacent to stream corridors, with 30% forested and 8% in low density residential or recreational land use, with septic systems for sewage treatment. One hundred-thirty-four acres of Blaine's 3280 acre urban growth area encompasses 134 acres of wetland in the California Creek sub-basin. Figure 2 shows a land use map developed by Stanley (2003).

In 1998 Whatcom County passed the Agricultural Nutrient Management ordinance (WCC 16.28) regulating ground application of liquid manure. There are 18 commercial dairies in the Drayton Harbor Watershed and a high concentration of hobby farms. The Whatcom Conservation District's 2005 Livestock Survey map is shown in Figure 3. The windshield survey identified 111 farms and a total of

4,276 animals with 4022 cattle (94%), 212 horses, 24 sheep, and a total of 18 swine, goat and alpaca (WCD, 2005). Locations of certified dairies compiled by the Whatcom Conservation District are shown in Figure 4.

On-site septic systems serve the sewage disposal needs for most of the California Creek basin. According to the Whatcom County Health Department there are 1,040 known OSS location within watershed boundaries as shown in Figure 5 (Whatcom County, 2006).

Beneficial Uses

Drayton Harbor was classified by the Department of Ecology as a Class A waterbody (WAC-173-201A-140) (State of Washington, 1997). Class A waters are considered excellent where water quality shall meet or exceed the requirements for all or substantially all uses. Under the 2003 standards revision Drayton Harbor is classified as excellent for aquatic uses, classified for shellfish harvest, primary contact recreation, wildlife habitat, harvesting, commerce/navigation, and aesthetics (WAC-173-201A-612, 2003). Tributaries of Drayton Harbor are given the same classification as the waterbody into which they merge unless they have their own designation, as Dakota Creek does. Characteristic uses include:

- Water supply (domestic, industrial and agricultural)
- Stock watering
- Fish (including salmonid) and shellfish spawning, rearing, migrating, and harvesting
- Wildlife habitat
- Recreation including primary contact (swimming), sport fishing, boating, and aesthetic enjoyment
- Commerce and navigation

Fecal coliform standards for Class A waters:

Freshwater	Geometric mean shall not exceed 100 colonies/100mL *and* not more than 10% of all samples shall exceed 200 colonies/100mL
Marine	Geometric mean shall not exceed 14 colonies/100mL *and* not more than 10% of all samples shall exceed 43 colonies/100mL. This is also the National Shellfish Sanitation Program (NSSP) criteria for approved shellfish growing waters except that NSSP uses the estimated 90th percentile calculation (NSSP, 1997).

Historically, Drayton Harbor has been a significant shellfish resource for several user groups. Environmental conditions were ideal for the harvest and farming of several species of clams and oysters. The Lummi Nation and the Nooksack Tribe hold ceremonial and subsistence rights to shellfish harvesting in the Harbor. Commercial shellfish growing began in 1905 when the Drayton Harbor Oyster Company seeded 500 acres in the southern portion of Drayton Harbor. Neptune Aquafarms grew shellfish for commercial markets on 400 acres in the 1970s and 1980s until their assets were purchased

by the Drayton Harbor Oyster Company in 1992 (Menzies, 1999). Commercial shellfish harvest by Drayton Harbor Oyster Company continued until 1997 when the shellfish growing area was downgraded. In past years, Semiahmoo Spit and the west side of Drayton harbor have produced 10-20% of the Lummi Nation shellfish harvest (Cochrane, 1999). Recreational harvest of shellfish has been popular at public and private beaches. Recreational harvest includes hard-shell clams, butter clams, and Dungeness crab.

Drayton Harbor watershed provides groundwater for the City of Blaine's public drinking water supply, small community water supplies, and individual wells. The watershed provides habitat for a variety of marine and freshwater fishes. Drayton Harbor and its tributaries, including ditches, support migration, rearing, and spawning of anadramous salmonids. Drayton Harbor watershed also supports spawning and rearing of native resident salmonids. The predominant species are coho and chum salmon, steelhead, and cutthroat trout. Chinook salmon also utilize the Drayton Harbor watershed (Peterson, 1995). The native spring chinook is listed as threatened under the federal Endangered Species Act. Forage fish (e.g. Pacific herring, sand lance, and surf smelt provide an important food source for salmonids and many marine birds and mammals in Drayton Harbor. The intertidal shoreline of Drayton Harbor and subtidal areas support extensive eelgrass beds that are sensitive spawning habitat for forage fish. Out migrating juvenile salmon and Dungeness crab also utilize these nearshore areas (PSCRBT, 1991). While California Creek is important from the fisheries standpoint, production is limited by stream channelization and ditching (Peterson, 1995).

Drayton Harbor is a popular destination for contact recreation, sport fishing, boating, and shellfish harvest. The Resort Semiahmoo and housing development is a destination location on Semiahmoo Spit offering proximity to a county park, beaches, recreational shellfish beds, and a 300-slip marina for pleasure craft. The Port of Bellingham operates a 681slip marina for commercial and pleasure craft at the northeastern side of the mouth of Drayton Harbor.

Potential Pollution Sources

Pollution sources identified by DOH in the 2004 sanitary survey (Lennartson, 2004) with the potential to impact affected shellfish growing areas include:

- Agricultural discharges: deficient manure management practices on farms located in the Dakota and California Creek watersheds;
- Port of Bellingham Marina: boat wastes from commercial and recreational watercraft; also, feces from large bird populations (gulls, cormorants, pigeons, ducks, etc.) on the rip-rap breakwater and the various building roofs and docks;
- Semiahmoo Marina: boat wastes from recreational watercraft and bird populations within the marina; also, feces from concentrations of seals which haul out on the floating breakwater surrounding the marina;
- Blaine sewage collection system: cracks or breaks in pipes, manholes, or lift stations located along Marina Drive; and, the jointed submarine force main which transports untreated sewage under the harbor mouth;
- Blaine sewage treatment plant (STP): discharges of partially treated effluent from the STP into Semiahmoo Bay;
- Seafood processors: discharge of process wastewater from these facilities into the area around sampling station 15;

- Stormwater discharges: several discharge points along the shoreline potentially transport pollution to the harbor from a variety of sources;
- Individual on-site septic systems (OSS): failing OSS situated along the creeks and the eastern and southern shoreline of Drayton Harbor.

The primary sources for potential fecal pollution in the California Creek sub-basin include agricultural, hobby farm manure management, stormwater runoff, failing OSS, and wildlife.

Existing Water Quality Monitoring Data

The DOH has been the primary source of fecal coliform data for shellfish growing areas in Drayton Harbor. The DOH has monitored shellfish growing operations in Drayton Harbor since 1952 for the purpose of classifying shellfish beds for commercial harvest. Currently, the DOH samples Drayton Harbor six times each year as part of their systematic random sampling (SRS) program. These data were supplemented by bi-monthly sample collection by the PSRF in 2004/2005. Since June 2004, PSRF has conducted wet weather marine sampling to provide additional data for use in supporting an increase in the rainfall threshold that triggers growing area closure under the DOH conditional classification. Current DOH classification is shown in Figure 9 along with recent data. Of 12 sampling stations, five meet the NSSP standards for harvest under low rainfall conditions but not under high rainfall conditions (4 of these stations are classified for conditional harvest), 3 stations do not meet standards under either rainfall condition, and 3 stations meet standards under both conditions (Whatcom County, 2006).

The Northwest Indian College collects water quality data for freshwater tributaries to Drayton Harbor including 3 stations along the mainstem of California Creek that have been sampled since 1999 (Cochrane, 2005). NWIC station locations are shown in Figure 5. Trends for moving geometric means at all three stations from 1999 through 2005 show decreases in FC levels until 2003 when they began to rise to current levels as shown in Figure 7. Geometric means for the past two years range from 56 to 65 FC/100 mL meeting the Washington State Water Quality standard for freshwater FC geometric means of 100 FC/100mL. However all three stations exceeded the 10% of samples greater than 100 FC/100 mL allowed by the standard, from 31% at C1 to 37% at C3 (Table 2).

Table 1. NWIC California Creek FC data summary; 2003 through 2005.

Station	Geomean	%>100	Min	Max	n
C1	65	31	10	310	26
C2	58	32	10	890	25
C3	56	37	10	570	30

Past studies have documented violations of fecal coliform water quality criteria in freshwater tributaries and nearshore marine waters in Drayton Harbor (Cook, 1987; Dickes, 1992; Saban and Matthews, 1992; Cykler, *et al.,* 1995; Vasey Engineering, 1995 and Matthews *et al.*, 1997, PSRF 2002). The Saban and Matthews (1992) study sampled tributary sites throughout the California Creek sub-basin on two occasions. Matthews et. al. (1997) selected 3 sites identified as priority stations from Saban and Matthews (1992) for a 1 year monthly sampling project. Cycler *et al.,* (1995) sampled one site on the mainstem of California Creek. In 2003, a watershed analysis of California and Dakota Creek sub-basins sampled California Creek and its tributaries at 13 locations for nitrate, dissolved oxygen, and conductivity to identify areas altered by land uses (Stanley, 2003).

In April 2006 the California Creek Tributary Monitoring Project (CCTMP) began sampling for fecal coliform bacteria at 10 sites representing distinct sub-basins of California Creek. The project includes a total of 10 sampling events with 4 quarterly random sampling events and 6 rainfall related events (H.C.S., 2006). Data for the 5 completed sampling events are presented in Table 2. Data reported for 5 sampling events indicates that 6 of the 10 stations failed the former Class A state freshwater fecal coliform geometric mean standard and they all failed the second part of the standard. (The standard was a geometric mean of no more than 100FC/100mL and no more than 10% of samples above 200 FC/100mL). Fecal coliform bacteria loading generally followed discharge patterns. Maximum FC counts of 1000 FC/100mL or above occurred at 6 out of 10 stations. The FC peaks occurred primarily after storm events in June and September (H.C.S. 2006a).

Table 2. California Creek fecal coliform summary data ranked in descending order by loading. (Bold indicates violation of former Class A geometric mean standard of 100FC/100mL.)

Station	Mean load FC/day (using FC log 10)	Mean Discharge (cfs)	Geometric Mean	Geometric Mean*	%>43	%>100	Min (FC/100 mL)	Max (FC/100 mL)	n
CA6	1.63E+08	3.86	**108**		80	60	23.0	440.0	5
CA15	4.50E+07	1.33	53	56	60	60	9	150	5**
CA3a	2.55E+07	1.10	46	92	80	60	3	540	5**
CA3	2.48E+07	0.72	53		33	33	6	1100	3
CA16	1.42E+07	0.26	**413**		100	80	380	1200	5
CA14	1.23E+07	0.20	**566**	**494**	100	100	140	1700	4**
CA4	1.00E+07	0.41	**141**		75	50	30	1000	4
CA8	3.90E+06	0.05	**230**	**165**	80	60	13	2200	5***
CA2	4.06E+06	0.09	77		100	33	48	160	3
CA13a	4.05E+06	0.12	**136**	**301**	80	80	20	1900	4**

* Geometric mean includes standing water or low flow samples (insufficient for discharge measurements) in July and September.
** Includes standing water/low flow sample in July and September no discharge or loading values.

1.3.2 Objectives and Goals

The health of Drayton Harbor has been a concern for nearly 20 years. Poor water quality and concerns about failing on-site sewage systems and livestock waste in the California and Dakota Creek subwatersheds led to a downgrade of 500 acres of shellfish growing areas at the south end of the harbor in 1988. Further shellfish downgrades occurred in 1995 and 1999, due to continually degrading water quality throughout the harbor. Due to progress made addressing sources of pollution, by 2004 DOH was able to reclassify portions of Drayton Harbor to "Conditionally Approved" with closures occurring after rainfall events of 0.5 inches in a 24-hour period. The conditional approval was further adjusted in 2006 to require closures after rainfall events of 0.75 inches in a 24-hours. In spite of recent successes, harvesting is still Prohibited on several hundred acres of important tribal, commercial, and recreational shellfish beds. Throughout the watershed, bacteria are still carried into the harbor during major rain events. The frequency of temporary closures due to rainfall events makes commercial harvest of shellfish currently impractical. The community is now turning its attention to the upper watershed, where on-site sewage systems and livestock waste from hobby farms are likely sources of bacteria. A particular emphasis has been placed on the California Creek subwatershed, where patterns of deteriorating water quality have been observed and the locations and densities of on-site sewage systems and hobby farms have been characterized. In addition, trends of high FC levels in Blaine Harbor marina continue.

In 2005, PSRF funded a literature review and case study analysis of MST techniques appropriate for application to Drayton Harbor (H.C.S., 2005). The review recommended a phased approach to MST in the Drayton Harbor watershed beginning with a small- scale pilot study followed by a watershed-wide study based upon results of the pilot work. A microbial source tracking project that can identify the presence of human, bovine, or equine contributions will assist in targeting solutions to improve water quality in the creeks and harbor.

This project serves to implement recommendations made in the PSRF MST review to use *Bacteroides*

host specific polymerase chain reaction (PCR) and ribotyping to accomplish the following objectives at selected California Creek sampling stations and at selected stations in Drayton Harbor and Blaine Harbor:

1) The primary objective of the study is identification of human sources at all selected sites during a wet season.

2) The secondary objective is to distinguish bovine and equine fecal pollutions sources at California Creek sites during a wet season.

1.4 Project/Task Description and Schedule

(EPA QA/R-5 A6)

This QAPP provides the supportive information used in developing the plan for collection and analysis of water samples from Drayton Harbor and California Creek and associated tributaries. The basic field and analytical tasks required to achieve the objectives of this project are listed below:

1) Collect grab samples of water from designated sites within these three waterbodies,
2) Analyze the samples for the presence/enumeration of fecal coliform, *E. coli* and *enterococci*;
3) Analyze the samples for the presence of species-specific *Bacteriodes* markers using polymerase chain reaction (PCR) technology.

The quality assurance (QA) requirements described in this document are critical to the success of this project and are derived from EPA QA/R-5 EPA Requirements for Quality Assurance Project Plans (March 2001).

Table 3 includes a schedule for conducting tasks related to this project. It is a guideline only as it is possible that unforeseen circumstances and conditions will require adjustment to some or all of the following proposed dates.

Table 3 – Schedule of Tasks

Project Schedule
This study will begin in November 2006 with completion before December 2007. The project timeline is shown in Table 3.

Table 3. Timeline for the California Creek/Drayton Harbor MST Pilot Study

Task	2006			2007											
	O	N	D	J	F	M	A	My	Jn	Jly	Ag	S	O	N	D
Monitoring Plan	x	x													
Freshwater sampling		x	x	x	x	x	x								
Marine Sampling		x	x	x	x	x	x	x							
Lab analysis									x	x					
Reporting											x	x	x		
Outreach														x	x

1.5 Data Quality Objectives and Criteria

Data from this phase of the project will be used as background to develop further data quality objectives for this project.

1.5.1 Objectives and Project Decisions

The primary data quality objective for the Drayton Harbor microbial source tracking (MST) study is to characterize the source(s) of contamination within the targeted water bodies to the following level of differentiation: human source, ruminant source, and/or non-human/non-ruminant source.

1.6 Special Training and Certification

The Region 10 Environmental Laboratory staff has completed the required annual 8-hour health and safety training. The Region 10 Laboratory is accredited by the National Environmental Laboratory Accreditation Council. The Region 10 Environmental Laboratory analysts have the appropriate training to conduct both bacteriological and molecular analyses. The fecal coliform analyses will be performed by an FDA-approved State Laboratory, under FDA's shellfish program. The DOE, DOH and Squaxin Island Tribe currently employ sample collectors that have the training necessary to appropriately sample ambient water.

1.7 Documents and Records

1.7.1 QAPP Distribution

It will be the responsibility of the project manager to ensure that appropriate project personnel have the most current, approved version of the QAPP, including updates (see Section 1.1 Distribution List). The final version of the QAPP and any updates will be distributed in portable document file format.

1.7.2 Determination Levels

Only those water samples determined to contain more than 14 colony-forming units of *fecal coliform* per 100 mL (MPN) will undergo further analysis for *Bacteriodes* species. This level may be adjusted later in the project based on sample results. The filters from sites below the cut off point will be retained for future testing.

1.7.3 Measurement Performance Criteria/Acceptance Criteria

The measurement performance criteria/acceptance criteria for this project are discussed in Section 2.4, Quality Control. In general, if a sample, or associated controls, fall outside of the acceptance criteria, they are invalidated and either re-sampled or re-analyzed, as appropriate.

1.7.4 Field and Laboratory Documentation and Records

Laboratory documentation will include but is not limited to raw data, sample preparation and analysis logs, and results of calibration and quality control (QC) checks.

Field documentation will include but is not limited to field notes, photographs, and sample data and chain-of-custody forms.

1.7.5 Quarterly and/or Final Reports

Field and laboratory documentation will be kept in a case file and submitted to the State (DOE and DOH), PSRF and EPA Region 10 Office of Water and Watersheds with the final report. The EPA Region 10 Laboratory will archive the following documents: (1) signed hard copies of sampling and chain-of-custody records; and (2) electronic and hard copies of analytical data. The Laboratory will store all sample receipt, sample log-in, and Laboratory instrument documentation for a minimum of ten years.

2.0 Data Generation and Acquisition

The elements in Sections 2.1-2.10 ensure that appropriate methods for sampling, measurement and analysis, data collection, data handling, and quality control (QC) activities are employed and documented.

2.1 Sampling Design (Experimental Design)

Samplers will collect grab samples of approximately 500 ml of water at each designated site. The sample will be split in the field and half will be sent to the EPA Region 10 Laboratory, the other half to the Institute for Environmental Health (IEH) Laboratories. The measurement parameter of interest is microbial. The proposed sampling locations include the following (these station numbers have been assigned by the DOH, the PSRF, Whatcom County Public Works Department and Hirsch Consulting Services for purposes of water quality results associated with shellfish growing areas and to track potential sources of fecal coliform bacteria):

2.1.1 Sampling Locations

California Creek

Table 4. Freshwater MST sites (5 sites)

Sample Site Number	Lat (48.)/long(122.)		Ribotype	Rationale for Site Selection	Location and Description	Land Use
CA3a	92575	68805		Third highest loading of 10 sites in CCMP	Ham Rd. approximately 1800 ft S of intersection w/Birch Bay Lynden Rd. downstream (E.) side	hobby farm, dairy
CA4	92583	66031		Geometric mean (GM) 4th highest of 10 CCMP sites, 141 FC/100 ML, flow throughout dry season	ValleyView Rd, 400 m N of Arnie Rd., upstream (N.) side.	dairy, hobby farms
CA6	92082	65221	X	Highest loading of 10 sites in CCMP, flow throughout dry season	Arnie Rd. where stream crosses rd. on N side, upstream from staff gauge	hobby farm, forested, urban(high OSS)
CA15	90072	61526	X	Second highest loading of 10 sites in CCMP	Portal Way near Farris across rd. from Dbl. L Mobile Ranch, 20 ft from concrete pipe	dairy. Hobby, OSS
CA16	91727	64944		GM 2nd highest of 10 CCMP sites, 413 FC/100 ML,flow throughout dry season	Bruce Rd.spur near Arnie (dead end of Main St, Custer, 3112 Main)	high OSS (>10)

Table 5: Marine Sites (Drayton Harbor, Semiahmoo Bay, 8 sites)

Sample station	Coordinates		Ribotype	Rationale for Site Selection	Description
	Lat. º N	Long. º W			
12	48.98296	122.78213	X	Conditionally approved recreational and tribal shellfish growing area.	Drayton Harbor west side, near shore inside Semiahmoo Spit. At west end and in line with row of pilings.
4	48.98067	122.75789		Prime commercial shellfish growing area, conditionally approved	Drayton Harbor, south end of the commercial growing area.
6	48.98544	122.75964	X	Prime commercial shellfish growing area, conditionally approved	Drayton Harbor, north end of the commercial growing area.
8	48.99001	122.76305		Highest FC concentrations in Drayton Harbor, prohibited status	Drayton Harbor, 20 ft. W of Blaine Marina breakwater S.W. corner.
15	48.99185	122.76677	X	Highest FC concentrations in Drayton Harbor, prohibited status	Semiahmoo Bay, at Eastern entrance to Drayton Harbor, 10 ft N. of Marine Drive dock
313	48.9784	122.7886		Likely to be affected by California Creek outflow	Offshore of concrete slab of burned out house.
314	48.9701	122.7806		Likely to be affected by California Creek outflow	Offshore of road up hill to posh homes
315	48.9655	122.7678	X	Likely to be affected by California Creek outflow	Offshore of last house at start of spit.

able 5. Marine MST sites.

Field Procedures

Sample Custody and Documentation

Samples will be placed into ice chests upon collection. Samples will be shipped to EIH and to EPA Region 10 Laboratory delivered for analysis within 24 hours of sample collection. A chain-of-custody (COC) form will accompany all samples to the laboratory. A temperature control sample will be submitted to the laboratory with each cooler of samples. Samples will be accepted for analysis only if the temperature control is measured below 10°C at the time of sample submittal.

Laboratory Procedures

Analytical methods, expected range of results, and required detection limits are summarized in Table 5.

Table 6. Analytical Methods Summary

Parameter	Description	Method	Lab	Sample Container	Preservation	Holding Time	Precision/ Quantitation Limits
Fecal coliform bacteria	Membrane filtration	APHA, 9222D	IEH	PE, 125 mL, sterile	10 °C, dark	(max)24-hours	20% RSD*/ 1 cfu/100 mL
HSPCR	PCR-2 marker	MEL SOPs	EPA	PE, 250 mL, sterile	10 °C, dark	(max)24-hours	**/1 DNA strand
	PCR-3 marker marker	IEH SOPs	IEH	PE, 500 mL, sterile	10 °C, dark	(max)24-hours	place holder
Ribotyping	2-enzyme	IEH SOPs	IEH	PE, 100 mL, sterile	10 °C, dark	(max)24-hours	place holder

* RSD-Relative standard deviation, standard deviation divided by the mean

** Standard Accuracy and Precision for analysis by PCR is unknown at this time. Identification is not quantitative.

Fecal coliform bacteria

Analysis of water samples for fecal coliform bacteria will be utilized by the EPA Region 10 Laboratory to screen water samples for PCR analysis. Freshwater samples will be analyzed by Avocet Environmental Testing in Bellingham Washington as part of the CCTMP, using the membrane filtration method, Standard Methods 9222 (APHA, 1998). The laboratory is accredited by Ecology for fecal coliform bacteria analysis. Marine samples will be analyzed at the DOH laboratory in Seattle using the most probable number (MPN), five-tube fermentation (Standard Methods 9221 E(a) using A-1 Medium) technique. Samples will be analyzed in conformance with National Shellfish Sanitation Program (NSSP) guidelines (NSSP, 2003). Analysis of water samples for fecal coliform bacteria will be utilized by EIH for screening and colony isolation for ribotyping. Water and sewage grab samples will be processed for fecal coliforms using the membrane filtration method, Standard Methods 9222 (APHA, 1998).

PCR and Ribotyping

Bacteroides HSPCR is the primary DNA microbial source tracking method that will be utilized at each selected sation. Ribotyping will be used for a subset of samples to corroborate PCR results and to

further distinguish fecal sources. Water samples from the CCTP sites will be split for analysis by HSPCR at the IEH laboratory utilizing human, bovine, and equine primers and at the EPA Region 10 laboratory using human vs. ruminant primers. Marine samples will be analyzed by host specific PCR at the EPA's MEL where only human and ruminant primers are available. Methods at both laboratories were derived form the PCR method developed by Kate Field at Oregon State University.

The EPA Region 10 Laboratory will follow the method developed by Office of Research and Development the DNA extraction, polymerase chain reaction and gel electrophoresis methods utilized in this study as Standard Operating Procedures as referenced in the EPA QAPP for this study (MEL, 2006).(Quality Assurance Project Plan for Drayton Harbor Microbial Source Tracking Pilot Study EPA Region 10, Washington Operations Office (WOO). including:

- Sample Preparation
- Sample Filtration
- DNA Extraction
- PCR and Electrophoresis
- Gel Visualization

2.1.2 Sampling Schedule

4) Samples will be collected eight times at each site. The November 2006 through April 200 period has six sampling events scheduled. Proposed sampling dates for the project correspond with The Region 10 Laboratory must receive all samples no later than Wednesday of the sampling week, to allow for reading of the samples without staff overtime/compensatory time charges. Oakland Bay is less than a one hour drive from the EPA Region 10 Laboratory. For routine fresh water quality monitoring, DOE and Squaxin Island Tribe staff can typically collect samples in the morning and deliver them to the Region 10 Laboratory by noon the same day. Samples will be transported on ice within 30 hours of sampling to the DOH Laboratory in Seattle for fecal coliform enumeration.

2.2 Sampling Methods

2.2.1 Sample Collection and Handling

The samples will be collected following guidelines as cited in EPA-600/8-78-017, 1978, Microbiological Methods for Monitoring the Environment: Water and Wastes. Sampling methods may vary slightly between standard operating procedures used by samplers and different conditions encountered in the field. The following is general guidance for the samplers.

If samples are collected manually, rubber gloves should be worn and the use of safety glasses should be considered.

When the collection site is identified, the sampler should collect the sample as follows:

When dip samples are taken for analysis, the sampler should carefully remove the foil cap, ensuring that neither the inside of the sample bottle or the cap are touched. Hold the foil covered cap, do not

set it down.

When possible, take the sample by holding the bottle near its base and plunge it neck downward, below the surface. Use an extension pole, if needed, to keep from walking into the stream and stirring up the sampling area. Turn the bottle until the neck points slightly upward and mouth is directed towards the current. If there is no current, create a current by pushing the bottle forward horizontally in a direction away from the hand or pole. For surface waters, samples should be collected far enough below the surface to avoid contamination with any surface scum or floating detritus, such as leaves or other debris.

After collection, carefully recap the sample bottle securely, leaving the foil cap in place. There should be a 1 inch head space in the neck of the bottle, to allow adequate mixing by the analyst. If, however, the sample container is overfilled, DO NOT pour out any excess sample. Place the cap securely on the sample bottle and return it to the analyst overfilled and a notation will be made in the analyst's report.

Water samples will be collected using either a sampling wand or hand dipping in midstream. Water sampling will be conducted in accordance with the water sampling protocol for coliform bacteria detailed in Standard methods 9060A and 9060B (APHA, 1998) and in the Washington State Department of Health Procedure #003 (DOH, 1996). All sample containers will be pre-sterilized and provided by IEH or EPA Region 10 laboratory. Samples for HSPCR will be collected in a 500ml sterile plastic bottles provided by EPA Region 10. Samples will be immediately split into 250 mL bottles provided by MEL. Both sets of samples will immediately be placed on ice in separate coolers. Samples for fecal coliform and subsequent ribotyping analysis at IEH will be collected in 120 mL sterile bottle provided by IEH. Standard sampling procedure for ribotype samples includes collection of 5 field replicate samples per sampling station per sampling event. Fecal coliform analysis is performed upon each of the 5 replicated samples. At least 2 *E. coli* isolates are utilized from each of the 5 samples for ribotyping analysis. The results for these fecal coliform analyses will be provided to the EPA Region 10 Laboratory within 72 hours.

Samples will be delivered to the IEH and MEL within 24 hours of sampling. Each bottle will be labeled with a site number prior to sampling. Site numbers will be recorded on write-in-the-rain field data sheets prior to sampling. Site numbers, date, and time sampled for each sample will be transcribed to the chain-of-custody sheet prior to submitting samples to the laboratory. The following information will be recorded on field data sheets.

- Sampler(s) name(s)
- Site numbers
- Field Replicate numbers
- Laboratory replicate numbers
- Date
- Time sample collected
- Additional observations
- Weather conditions
- Temperature control °C (measured upon arrival at laboratory)

A transfer blank will be created each day that samples are collected. The transfer blank is a bottle containing sterile water that is prepared at the laboratory. The purpose of the transfer blank is to check for potential contamination of samples during collection and transport. Halfway through the sampling for the day, the sampler will transfer the contents of the bottle containing sterile water into a sterile sample bottle, being careful not to contaminate the inside of the bottle or cap during transfer. The sampler will label this bottle with the date and time of transfer, name of collector, sample number and label the bottle as a 'Transfer Blank'. This transfer blank will serve as a blank for microbial and DNA extraction testing.

2.2.3 Sample Custody and Transport

A unique sample number assigned by the RSCC will identify each sample. Transporters will use EPA Region 10 chain-of-custody procedures and forms. Samples will be hand-delivered to the EPA Region 10 Laboratory. Samples will also be transported to the Avocet Environmental Testing, Inc. for fecal coliform enumeration.

2.2.4 Health and Safety

When working with potentially hazardous materials, investigators are to follow USEPA, Occupational Safety and Health Administration and site-specific health and safety procedures. Since these waterbodies may be contaminated with fresh fecal material, it is advised that samplers wear gloves and wash their hands prior to handling food or food products.

2.3 Analytical Methods

The EPA Office of Research and Development has developed the DNA extraction, polymerase chain reaction and gel electrophoresis methods utilized in this study as Standard Operating Procedures. The SOPs are in Appendix C-2 of this document.

2.4 Quality Control (QC)

The following QC activities will be performed by the laboratories performing analytical services in support of this project.

2.4.1 Samples Analyzed by the EPA Region 10 Laboratory

Polymerase Chain Reaction (PCR) Samples:

Filtration Controls: The purpose of filtration controls for PCR is to ensure that proper aseptic technique is used during sampling and laboratory filtration to avoid contamination.

Negative Controls: For each sampling batch (1-20 samples processed on the same day), one filtration negative control will be prepared using the transfer blank for that day. For each sampling batch, a second filtration negative control will also be prepared using 100 mL of ultra-pure water. Field blanks monitor for the introduction of extraneous material into the samples during field sample handling, transport and storage. A positive result indicates the presence of contamination most likely due to poor aseptic technique in the field, contact with other samples or damaged storage containers.

The method blank (filtration blank) is designed to screen for contamination throughout the entire MST process. False positive results indicate contamination at some point in the process. False positive results may not disqualify the data if a cause can be determined and if the cause is determined not to impact the remainder of the samples processed in that batch.

PCR Controls: The purpose of the PCR amplification controls is to ensure that the amplification system (the polymerase, master mix, templates etc) is functioning properly, cross contamination is absent and that a negative result in an unknown (sample) is actually negative for that particular primer. The following controls are assessed in each analysis (i.e. for each primer, which is the species specific DNA strand used for DNA replication).

1) One positive control, consisting of plasmid DNA containing the target sequence or the appropriate fecal DNA. This will serve as the reference sample.
2) One extraction negative control, made by following the extraction protocol with ultra pure water rather than a sample.
3) One PCR negative control, made by setting up PCRs with water instead of template.
4) A actual sample will not be considered negative until it has been subjected to replicate analyses using at least 5 different concentrations of the sample containing the purified DNA.

Results that are out of specification will be reviewed by the Technical Director for Microbiology and the Project Officer for a decision on whether the data should be included in the report. Method experts in EPA's Office of Research and Development (ORD) may also be consulted for advice on the quality of the data in these situations.

Replicate Analysis (Analyst and Method Precision):

Identical replicate analyses: Identical replicates (i.e. aliquots of the same sample) are assayed simultaneously using the same sample preparation, method and the same reagents. These replicates can serve two functions. They can preserve data (in case of replicate failure) and they can be used to monitor variability in the method and/or analyst's performance of the method. While replicate filters will be prepared for 100% of the field samples, at least 10 % of these filter replicates will be subjected to the remainder of the analyses (extraction, gene amplification, and gel electrophoresis) to access precision. EPA ORD's Microbial Source Tracking Guide Document (June 2005) does not contain a numerical standard to apply to precision data resulting from identical replicate analyses, however, the results for these identical replicates should be in agreement. If they are not in agreement, the overall results for that batch of samples will be evaluated for validity in discussions between the Technical Director for Microbiology, Project Officer and method experts from ORD.

Method Accuracy: Blind Sample Control:

PSRF or its designees will submit one water sample containing horse, cow, or human fecal material (on a rotating or intermittent schedule) for a 'blind' PCR analysis by the Region 10 Laboratory. The blind sample will be clearly identified as such (without identifying the source of the fecal material) and will be submitted with each sample event batch or intermittently. The blind sample will be submitted for PCR analysis only. The results of these blind samples will be used to establish a base line expectation for the Laboratory. Currently, there is no established guideline for method accuracy using blind samples. If the method fails to correctly identify the type of fecal material in a blind

sample, the overall results for that batch of samples will be evaluated for validity in discussions between the Technical Director for Microbiology, Project Officer and method experts from ORD.

Specificity:

Specificity is the ability of a given MST method to discriminate between various animal sources. Known animal sources are used to ensure that the primers work in a given geographic region. Availability of useful and applicable scat samples from the Drayton Harbor watershed is problematic, so regional samples (collected from the Puget Sound area) will be used to evaluate the specificity of the primers. Specificity testing can be used to provide a specific pattern-detectable percentage as follows:

$$\text{Specificity} = \frac{\text{Test Negatives}}{\text{Test Negatives} + \text{False Positives}} \times 100$$

Although there is currently no consensus, specificity values below 80 percent reflect questionable discriminatory power.

The following table summarizes the quality control for the methods to be carried out at the Region 10 Laboratory.

	Duplicates[1]; *Frequency* (corrective action/consequence of failure)	**Positive Control[2]; *Frequency* (corrective action/ consequence of failure)**	**Negative Control[2]; *Frequency* (corrective action/consequence of failure)**	**Blind Control[2]; *Frequency* (corrective action/consequence of failure)**
Polymerase Chain Reaction	Identical replicate analysis using the same sample preparation, method and reagents. *Replicate filters will be prepared for 100% of the field samples; at least 10 % of these filter replicates will be subjected to extraction, gene amplification, and gel electrophoresis* (may or may not disqualify data depending on consequences of cause)	Reference sample/positive control of plasmid DNA containing target sequence or the appropriate fecal DNA; *for each analysis/primer* (may or may not disqualify data depending on consequences of cause)	a) method/filtration control using transfer blank assessing entire process; *one/1-20 samples/day* (may or may not disqualify data depending on consequences of cause) b) filtration control using ultra-pure water; *one/1-20 samples/day* (may or may not disqualify data depending on consequences of cause) c) one extraction negative control using extraction protocol with ultra pure water; *for each analysis/prime* (may or may not disqualify data depending on consequences of cause) d) one PCR negative controls - PCRs with water instead of template; *for each analysis/primer*(may or may not disqualify data depending on consequences of cause)	PSRF water sample containing horse, cow, or human fecal material for a 'blind' PCR analysis *with each sample event batch or intermittently* (may or may not disqualify data depending on consequences of cause)

1 To measure precision
2 To measure accuracy
3 Per the SOP

2.4.2 Samples Analyzed by Avocet Environmental Testing Laboratory, Inc

Fecal Coliform Enumeration:

Fecal coliform will be analyzed at the Avocet Environmental Testing, Inc. laboratory in Bellingham using the most probable number (MPN), five-tube fermentation (Standard Methods 9221 E(a) using A-1 Medium) technique. Samples will be analyzed in conformance with National Shellfish Sanitation Program (NSSP) guidelines (NSSP, 2003).

Microbial Identification and Enumeration: The purpose of these controls is to ensure that the quality system associated with the filtration process for bacteria is within the criteria established below.

Comment [SH1]: What kind of QC and media will be used by Avocet??

Negative Controls: For each batch of prepared media, the appropriate negative culture control organism will be tested to ensure that the media demonstrates the correct reaction. These reactions are media-specific and are detailed in the specific method SOP. Media that does not meet these criteria will not be used in the analyses, and will be discarded. For each sampling batch (1 -20 samples processed on the same day), a filtration negative control will be prepared using sterile rinse buffer. If a positive result occurs, the samples for that batch will be invalidated and samples recollected. Each day of analysis, one negative culture control will be seeded and incubated in each operating water bath. Inappropriate results for the negative control will result in invalidation of all samples with which that negative control was incubated.

Positive Controls: For each batch of prepared media, the appropriate positive culture control organism will be tested to ensure that the media demonstrates the correct reaction. Media that does not meet these criteria will not be used in the analyses, and will be discarded. Each day of analysis, one positive culture control will be seeded and incubated in each operating water bath. Inappropriate results for the positive control will result in invalidation of all samples with which that positive control was incubated.

Confirmatory results: Tubes with turbidity and no gas will be subjected to a confirmatory step by transferring a small amount of the culture into EC broth with MUG (4 methyl-umbelliferyl-ß-D-glucuronide), incubating and observing for proper reaction.

2.5 Instrument/Equipment Testing, Inspection, and Maintenance

2.5.1 Field Measurement Instruments/Equipment

This phase of the project requires no field instruments.

2.5.2 Laboratory Analysis Instruments/Equipment

Laboratory instruments such as waterbaths, incubators, thermal cyclers and other equipment required by the applicable analytical methods will be maintained according to the manufacturers' instructions and the Laboratory SOPs. Records for equipment service shall be maintained by the Laboratory.

2.6 Instrument/Equipment Calibration and Frequency

2.6.1 Field Measurement Instruments/Equipment

No field instruments will be used during this phase of the project.

2.6.2 Laboratory Analysis Instruments/Equipment

Laboratory equipment (e.g. pH meter, waterbath, etc.) will be calibrated using the method and frequency specified in the Laboratory's SOPs. Records on calibration of laboratory equipment shall be maintained by the Laboratory.

2.7 Inspection/Acceptance Requirements for Supplies and Consumables

2.7.1 Field Sampling Supplies and Consumables

Consumable supplies used in the field will consist primarily of sterile plastic sample containers. The quality of consumable supplies such as plastic sample bottles used for this project should be documented by the supplier and certificates should be available on request. Bottles provided by the Region 10 Laboratory will be batch tested for sterility prior to sending them to the field for sample collection, as per SOP Mi_G004A. Bottles other than those prepared at the Region 10 Laboratory will not be acceptable for use during this project.

2.7.2 Laboratory Analyses Supplies and Consumables

The quality of chemicals, media and other supplies and consumables used in the Laboratory is dictated by the sensitivity and specificity of the analytical techniques being used. In the Region 10 Laboratory, chemicals, media, and other supplies and consumables are marked with the date received and the receiver's initials. In the event an expiration date has not been assigned by the manufacturer, the expiration date will be assigned by the receiver according to the Region 10 Laboratory's work instruction, "General Guidelines for Assigning Standard Expiration Dates." The quality of all laboratory supplies is documented by the supplier and the Region 10 Laboratory requests and keeps the vendor certificates on record per NELAC requirements.

2.8 Data Acquisition Requirements (Non-Direct Measurements)

Not applicable to this project**.**

2.9 Data Management

A field notebook, photos, and the Field Sample and Chain-of-Custody Data Sheets will be used to document the sampling activities. The Field Sample and Chain-of-Custody Data Sheets will have the following information: site name, sample number, date and time of each sample collection, and the sampler's name or initials and if possible, notes regarding human or animal activity in the area at the time of the sampling. In addition, the Laboratory will maintain a logbook that includes the above information provided in the Field Sample and Chain-of-Custody Data Sheets, as well as time of analysis and analyst initials. Quality control results will be recorded on bench sheets. All data generated by

Region 10 will be subject to a peer review then signed-off by the Microbiology Team Technical Director. Data entry staff will process and distribute all information mentioned above in accordance with the Laboratory's SOP. Logbooks, bench sheets and final reports will be stored on-site. All data generated during this project will be processed, stored, and distributed according to Laboratory SOPs.

3.0 Assessment and Oversight

3.1 Assessments/Oversight and Response Actions

Laboratories routinely perform performance checks using method-specific positive and negative controls, blind samples, etc. The positive controls, consisting of plasmid DNA containing the target sequence or the appropriate fecal DNA, will be provided by EPA's Office of Research and Development. Blind samples will be provided by the Squaxin Island Tribe. An internal assessment of the data and results is also routinely conducted by the supervisor and the Laboratory QA Coordinator.

A quality assurance assessment beyond routine QA procedures will be conducted during the course of this project. Given the short time frame, only one assessment is planned before completion of the project. The quality assurance assessment performed during this project will include the following:

1) Oversight of field sampling activities.
2) Oversight of sample handling and chain-of-custody procedures.
3) Laboratory inspection.

The Quality assurance assessment will be conducted by the EPA Region 10 Quality Assurance Officer or QA staff delegated by the Officer to conduct the assessment.

Corrective actions will be implemented in response to any QA results or detection of unacceptable data. These corrective actions will be developed in consultation with the Office of Research and Development, keeping the data user informed of any impacts on the data. If required, corrective actions will be documented in Appendix A-3.2, Corrective Action Form.

3.2 Reports to Management

If, for any reason, the schedules or procedures above cannot be followed, the project manager shall work with the appropriate parties to complete the Sample Alteration Form (SAF) found in Appendix A-3.1. The SAF will be reviewed and approved by the QA Officer and the Laboratory(ies) and/or samplers will be given a copy of the QA Officer-approved SAF for reference and for the project file.

A final report will be generated at the completion of the project. This report will include a discussion of the findings, interpretation of data and an executive summary. The report will be provided to all individuals listed in section 1.1 (Distribution List). In addition, short summary reports will be provided during the time of the study to keep those on the distribution list informed of the progress of the project. Difficulties that may result in delayed data and reporting will be communicated to those parties on the Distribution List.

4.0 Data Review and Usability

4.1 Data Review, Verification, and Validation Requirements

4.1.1 Data Verification/Peer Review

Region 10 data verification and peer review will be accomplished following the Laboratory's SOP Mi_D001A (Data Review). Data will be qualified as necessary to convey to the user any important information that needs to be considered in its use.

4.1.2 Data Validation

Data validation is an evaluation of the technical usability of the verified data with respect to the planned objectives of the project. This is accomplished by applying a defined set of performance criteria to the body of data in the evaluation process. Data validation for this project will be performed by the project manager.

4.2 Verification and Validation Methods

4.2.1 Data Verification

Data verification will include a review of the findings of all QA assessment activities including:

1) Field collection procedures
2) Sample labeling methods.
3) Chain-of-custody procedures.
4) Assessments of analytical data collection, recording, and reporting.

Verification of field collection procedures, sample labeling methods and chain of custody procedures are the responsibility of the Project Manager. Verification of the Region 10 analytical results is the responsibility of the Microbiology Technical Director, as required by the Laboratory's QA Manual. If any deviations are identified, the potential impact of those deviations on the reliability of the data will be assessed, and the information will be provided to the project manager through the QA Memo and appropriate flagging of the data.

4.2.2 Data Validation

Data validation will evaluate all individual samples collected and analyzed to determine if the results are within acceptable limits. Quantitative or qualitative limits of acceptability are defined for precision, accuracy, representativeness, comparability, and completeness.

1) Precision is defined as the agreement between a set of replicate measurements without assumption and knowledge of the true value. Agreement is expressed as either the relative percent difference (RPD) for duplicate measurements or the range and standard deviation for larger numbers of replicates. Data on precision are obtained by analyzing duplicate and replicate samples. RPD is not applicable to qualitative data.

2) Accuracy is a measure of the closeness of a sample analysis result to the "true" value. Accuracy will be determined primarily by an evaluation of the agreement between repeat analyses within the laboratory.

3) Representativeness is defined as the degree to which data accurately and precisely represents characteristics of a population, parameter variations at a sampling point, or an environmental condition. For this project, representativeness will be ensured by selection of sampling locations in accordance with the sampling design requirements in this QAPP.

4) Data are comparable if collection techniques, measurement procedures, methods, and reporting units are equivalent for the samples within a sample set. Comparable data for this project will be obtained by specifying standard units for physical measurements and standard procedures for sample collection, processing and analysis.

5) Data are complete when a prescribed percentage of the total intended measurements and samples are obtained. Analytical completeness is defined as the percentage of valid analytical results requested. For this project, acceptable completeness is > 90%.

4.3 Reconciliation with User Requirements

All data and related information obtained during the course of this project will be included in a data report. Presentations of data and data analysis may be made to relevant user groups upon request.

APPENDICES

Appendix A. Sampling Design and Data Quality Procedures

- A-1. Figures
- A-2. Tables
- A-3. Data Quality Forms

Appendix B. Field Documentation

- B-1. Equipment/Instrument Manual
- B-2. Standard Operating Procedures
- B-3. Field Data Forms

Appendix C. Laboratory Documentation

- C-1. QA Manual
- C-2. Standard Operating Procedures
- C-3. Data Report Forms

Appendix D. Data Evaluation

APPENDIX A. SAMPLING DESIGN AND DATA QUALITY PROCEDURES

Appendix A-1. FIGURES

Figure 1-1. Organization

Project Organization: Refer to section **1.2** for project participant roles and responsibilities.

Figure 2-1. Site Map with MST Sampling Locations

Comment [SH2]: Julie, can those maps that you had before be added to this section with the appropriate headers??

Figure 2-2. 2006 Drayton Harbor Shellfish Growing Area Classifications and DOH Sample Sites.

Appendix A-2. TABLES

Table A-1. Data Quality Objectives Summary

Analytical Group	Number of Samples	# of QA Samples: Reference Samples	Matrix	Method	Method Detection Limits	Accuracy	Precision (RPD)	Completeness	Volume, Container	Holding Time (days)
				Laboratory Measurements						
Fecal coliform	12 per sampling event	1 each batch	Water	Standard Methods 9221E(a)	1 CFU	+/- 10%	+/- 10%	> 90%	250 ml PP, sterile	6 hours
Filtration	12	1 each batch	Water	ORD prep by filtration	1 strand DNA	See Section 2.5.1	See Section 2.5.1	> 90 %	500 ml PP, sterile	30 hours
PCR	Varying depending on fecal coliform levels	1 each batch	Frozen prepared filter	*Bacteriodes* identification	1 strand DNA	See Section 2.5.1 [1]	See Section 2.5.1 [1]	> 90 %	500 ml PP, sterile	none

1 – Standard Accuracy and Precision for analysis by PCR is unknown at this time. Identification is not quantitative.
CFU: Colony Forming Unit
PP: Polypropylene

Table A-2. Analytical Parameters and Target Limits

Matrix/Media:

Analytical Parameter	Project Action Limit/Level (applicable units)	Laboratory Limits[1] (applicable units)	
		Quantitation Limits	Detection Limits (if appropriate)
Fecal coliform	1 cfu/100 mL	1 cfu/100 mL	1 cfu/100 mL
Bacteriodes species/DNA	Detect	Detect	Detect

[1] Laboratory quantitation limits and detection limits are those that an individual laboratory or organization is able to achieve for a given analysis on a routine basis.

- Quantitation limits are the minimum concentrations that can be identified and quantified above the detection limit within some known limits of precision and accuracy/bias. It is recommended that the quantitation limit is supported by the analysis of a standard of equivalent concentration (typically, the lowest calibration standard).
- Detection limits are the minimum concentration that can be detected above background or baseline/signal noise of an instrument.

cfu: colony forming unit

Table A-3. Sampling Design and Rationale

Sampling Location/ID Number	Matrix/ Media	Depth (Appropriate Units)	Analytical Parameter1	Rationale for Sampling Design 2
614 North Oakland	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station not meeting NSSP standards for shellfish harvest
NE North Oakland	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station not meeting NSSP standards for shellfish harvest. Station at northern end of clam beds.
615 Chapman	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station threatened to not meeting NSSP standards for shellfish harvest. Determining if site is influenced by pollution from human sources.
639 Chapman	Marine Water	6" below surface	Enterococcus, E. coli, bacteroides (MST)	Marine station threatened to not meet NSSP shellfish harvesting standard.
UNC 3 Chapman	Fresh Water	Midpoint of stream depth at thalweg3	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
UNC 2 Chapman	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
UNC T Chapman	Fresh Water	Midpoint of stream depth at thalweg[3]	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
UNC 1 Chapman	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
CAM 1 Chapman	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
DEE O North Oakland	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
CRA 0 North Oakland	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station
TR 03 North Oakland	Fresh Water	Midpoint of stream depth at thalweg	Enterococcus, E. coli, bacteroides (MST)	Freshwater station upstream of threatened marine station

[1] Analytical parameters include all planned field measurements (e.g., dissolved oxygen, turbidity, pH, etc.), field screening analysis (e.g., PCBs by immunoassay test kit, selected metals by XRF), and laboratory analyses.

[2] Rationale supports the selection of sampling locations and associated analytical parameters.

3 Thalweg - the line of deepest water in a stream channel as seen from above, normally associated with the zone of greatest velocity in the stream channel.

Comment [SH3]: Can you draft the rationale for each of the sample sites and add to this??

Table A-4. Summary of Field and QC Samples To Be Collected

Matrix/ Media	Analytical Parameter[1]	No. of Sampling Locations	Depth[2] (surface, mid, or deep)	No. of Field Duplicates	Organic Analyses[3] No. of:		Inorganic Analyses[3] No. of:		No. of Transfer Blanks	No. of Equipment Blanks	No. of PE Samples[4]	Total No. of Samples
					MS	MSD	Dup	MS				
LABORATORY ANALYSES: See Section 2.4												
FIELD ANALYSES:												
FIELD MEASUREMENTS:												

[1] Analytical parameters include all laboratory analyses, field analyses (e.g., nutrients by various field test kits, PCBs by immunoassay test kit, select metals by XRF, etc.), and field measurements (e.g., dissolved oxygen, turbidity, pH, etc.).
[2] When samples are collected at different depths at the same location, information for each depth category (e.g., surface, mid, or deep/bottom) is provided on a separate line.
[3] Information includes the number of associated analytical QC samples, if collection of additional sample volume and/or bottles is necessary. If the QC samples listed are part of the analysis and don't require the collection of additional sample volume and/or bottles, ANAS@ (for Ano additional sample@) is included in the column. (Note: MS=matrix spike, MSD=matrix spike duplicate, Dup=laboratory duplicate/replicate.)
[4] PE or Performance test will be submitted for laboratory analysis along with the associated field sampled where noted.

Table A-5. Analytical Method, Containers, Preservation, and Holding Times Requirements

Matrix/Media: Ambient Water/Microbial

Analytical Parameter[1] and/or Field Measurements[2]	Analytical Method Number	Containers (number, size/volume, type)	Preservation Requirements (chemical, temperature, light protection)	Maximum Holding Times[3]
ANALYTICAL PARAMETER:				
Fecal coliform	Standard Methods 9221E(a)	250 ml	< 10 º C	24 hours
PCR	Appendix C	500 ml PP	< 10 º C	6 (2) hours
FIELD MEASUREMENTS:				

[1] Analytical parameter includes both field and laboratory analyses.
[2] Field measurement parameters include those parameters measured directly in the field (e.g., dissolved oxygen, turbidity, pH, etc.). Single 500 ml PP bottle provides adequate sample for all analyses conducted at the MEL.
[3] Maximum holding times include all pertinent holding times for each analytical parameter (e.g., from sample collection to sample preparation, from sample preparation to analysis, from sample collection to analysis, etc.) and field measurement (e.g., from sample collection to measurement).

Table A-6.2 Quality Control Requirements for Analyses

Analytical Method/SOP: PCR qualitative[2]

QC Sample:	Data Quality Indicator (DQI)	Frequency/ Number	Method/SOP QC Acceptance Limits	Acceptance Criteria/ Measurement Performance Criteria[1]	Corrective Action
LABORATORY ANALYSIS:					
Filtration control		1 per day	Negative, no DNA	Negative, No DNA	Data reviewed, decision made based on cause
Transfer blank		1 per day	Negative, no DNA	Negative, no DNA	Data reviewed, decision made based on cause
Positive control		1 per day	Positive reaction	Appropriate DNA Amplification	Data reviewed, decision made based on cause
Negative control		1 per day	Negative reaction	Appropriate DNA Amplification	Data reviewed, decision made based on cause
Replicate analysis		10 % of samples	Same result	Appropriate DNA amplification	Data reviewed, decision made based on cause
FIELD ANALYSIS:					
Not applicable					

2- See section 2.5.1 for additional QC requirements associated with the qualitative PCR analyses.

Table A-7. Quality Control Requirements for Field Measurements

Field Parameter:

QC Sample:	Data Quality Indicator (DQI)	Frequency/ Number	Method/SOP QC Acceptance Limits	Acceptance Criteria/ Measurement Performance Criteria[1]	Corrective Action
<<PARAMETER 1 – Instrument Name (Manufacturer, Model)>>					
Not applicable					
<<PARAMETER 2 – Instrument Name (Manufacturer, Model)>>					
Not applicable					
<<PARAMETER 3 – Instrument Name (Manufacturer, Model)>>					
Not applicable					

[1] Information supports the acceptance criteria/measurement performance criteria introduced in section 1.7.3.

Table A-8. Field Equipment/Instrument Calibration, Maintenance, Testing, and Inspection

Analytical Parameter	Field Equipment/ Instrument	Calibration Activity	Maintenance Activity	Testing/ Inspection Activity	Frequency	Acceptance Criteria	Corrective Action
Not applicable							

Appendix A-3. Data Quality Forms.

A-3.1. Attachment 1 – Sample Alteration Form

Project Name and Number: _____

Material to be Sampled:_____

Measurement Parameter:

_____

Standard Procedure for Field Collection & Laboratory Analysis (cite reference):

_____

Reason for Change in Field Procedure or Analysis Variation:

_____

Variation from Field or Analytical Procedure:

_____

Special Equipment, Materials or Personnel Required:

_____

Initiators Name:_____Date: _____

Project Officer: _____Date: _____

QA Officer: _____Date: _____

A-3.2. Attachment 2 – Corrective Action Form

Project Name and Number: _____

Sample Dates Involved:_____

Measurement Parameter:

_____

Acceptable Data Range:

_____

Problem Areas Requiring Corrective Action:

_____

Measures Required to Correct Problem:

_____

Means of Detecting Problems and Verifying Correction:

_____

Initiators Name:_____Date: _____

Project Officer: _____Date: _____

QA Officer:

APPENDIX B. FIELD DOCUMENTATION

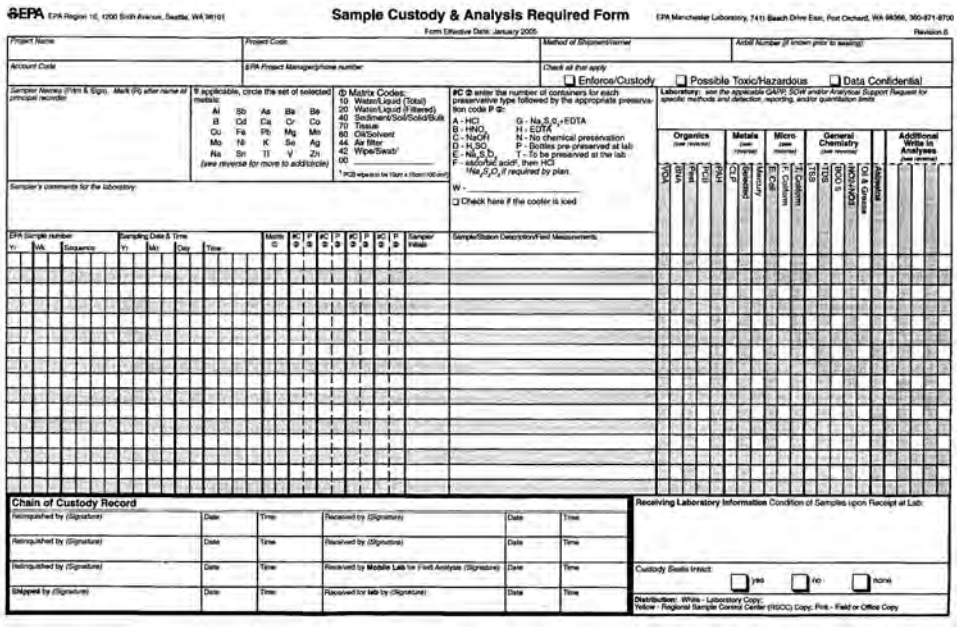
Appendix B-1. Equipment/Instrument Manual

Not applicable to this project.

Appendix B-2. Standard Operating Procedures

Not applicable to this project.

Appendix B-3. Field Data Forms and Chain-of-Custody Documentation



EPA Region 10, 1200 Sixth Avenue, Seattle, WA 98101

Sample Custody & Analysis Required Form

Form Effective Date: January 2005

EPA Manchester Laboratory, 7411 Beach Drive East, Port Orchard, WA 98366, 360-871-8700

Revision 0

Project Name | Project Code | Method of Shipment/Carrier | Airbill Number (if known prior to sealing)

Account Code | EPA Project Manager/phone number | Check all that apply: ☐ Enforce/Custody ☐ Possible Toxic/Hazardous ☐ Data Confidential

Sampler Name(s) (Print & Sign). Mark (R) after name of principal recorder

If applicable, circle the set of selected metals:
Al Sb As Ba Be
B Cd Ca Cr Co
Cu Fe Pb Mg Mn
Mo Ni K Se Ag
Na Sr Tl V Zn
(see reverse for more to add/circle)

Matrix Codes:
10 Water/Liquid (Total)
20 Water/Liquid (Filtered)
40 Sediment/Soil/Solid/Bulk
70 Tissue
80 Oil/Solvent
44 Air filter
42 Wipe/Swab/
00 _____

PCB wipe size to be 10cm x 10cm (100 cm²)

#C ② enter the number of containers for each preservative type followed by the appropriate preservation code P ③:
A - HCl
B - HNO_3
C - NaOH
D - H_2SO_4
E - $Na_2S_2O_3$
F - ascorbic acid*, then HCl
G - $Na_2S_2O_3$+EDTA
H - EDTA
N - No chemical preservation
P - Bottles pre-preserved at lab
T - To be preserved at the lab
*$Na_2S_2O_3$ if required by plan.
W - _____
☐ Check here if the cooler is iced

Laboratory: see the applicable QAPP, SOW and/or Analytical Support Request for specific methods and detection, reporting, and/or quantitation limits

Organics (see reverse): VOA, SVOA, Pest, PCB, PAH
Metals (see reverse): CLP, Dissolved, Mercury
Micro (see reverse): E. Coli, Fecal Coliform
General Chemistry (see reverse): TSS, TDS, RCC 5, INORGANICS
Oil & Grease
Additional Write in Analyses (see reverse)

Sampler's comments for the laboratory:

EPA Sample number: Yr. WA Sequence | Sampling Date & Time: Yr. Mo. Day Time | Matrix ① | #C ② | P ③ | #C ② | P ③ | #C ② | P ③ | #C ② | P ③ | Sampler Initials | Sample/Station Description/Field Measurements

Chain of Custody Record

Relinquished by (Signature)	Date	Time	Received by (Signature)	Date	Time
Relinquished by (Signature)	Date	Time	Received by (Signature)	Date	Time
Relinquished by (Signature)	Date	Time	Received by Mobile Lab for Field Analysis (Signature)	Date	Time
Shipped by (Signature)	Date	Time	Received for lab by (Signature)	Date	Time

Receiving Laboratory Information Condition of Samples upon Receipt at Lab:

Custody Seals Intact: ☐ yes ☐ no ☐ none

Distribution: White - Laboratory Copy; Yellow - Regional Sample Control Center (RSCC) Copy; Pink - Field or Office Copy

Additional Matrix Codes: 30 Leachate 50 Sludge 60 Air

Matrix codes: these are the codes in use at the EPA Region 10 Laboratory. Pick the matrix code that best matches the sample matrix. If in the opinion of the sampler, the sample matrix needs to be specially described, select 00 and write in a matrix description. Remember, tissue can be animal or vegetable in nature.

If the write in area becomes filled, cross out one of the pre-printed analyses and write in what is needed. Try to use the bolded analyte symbol/abbreviation (some analyses are not abbreviated).

Organics pre-printed on the form:

PAH Polynuclear Aromatic Hydrocarbons (these are a subset of the compounds reported from GC-MS analyses for BNA - PAH by HPLC or SIM-GC/MS methods are usually requested in order to get low reporting limits). **Pest** Organochlorine Pesticides **PCB** Polychlorinated Biphenyls aka Aroclors **VOA** (aka VOC) - volatile organic compounds **BNA** (aka SVOC or SVOA) - semivolatile organic compounds

Organics that can be written in:

AED scan (detects chlorinated or brominated hydrocarbons) **Butyltins** Butyltins (mono, di, tri, tetra substituted) **CB Cong** - Chlorinated Biphenyl Congener analysis **Chlor Hyd** Chlorinated Hydrocarbons **Chlorophenols** **Gua/Cat** Guaiacols/Catechols scan **Herb** Herbicides **OP Pest** Organophosphorous Pesticides **PBDE** Polybrominated diphenylethers **Resin Acids** **TPH-Dx** Total Petroleum Hydrocarbons, diesel range **TPH-Dx-ext** Total Petroleum Hydrocarbons, diesel range extended to motor oil **TPH-Gx** Total Petroleum Hydrocarbons, gasoline range **TPH-HCID** Total Petroleum Hydrocarbons, identification **THMs** Trihalomethanes

Metals pre-printed on the form *(underlined = 'CLP metals' - mercury must be separately requested)*:

Al aluminum **Sb** antimony **As** arsenic **Ba** barium **Be** beryllium **B** boron **Cd** cadmium **Ca** calcium **Cr** chromium **Co** cobalt **Cu** copper **Fe** iron **Pb** lead **Mg** magnesium **Mn** manganese **Hg** mercury **Ni** nickel **K** potassium **Se** selenium **Ag** silver **Na** sodium **Sn** tin **Tl** thallium **V** vanadium **Zn** zinc

Metals that can be written in and then circled under the box used for designating selected metals:

Au gold **Cr+6** hexavalent chromium **Mo** molybdenum **Sr** strontium **Ti** titanium **W** tungsten **Zr** zirconium

Note: some metals may not be analyzed for on matrices other than solids or water.

Microbiology Analyses pre-printed on the form:

E. Coli Escherichia coli **F. Coliform** Fecal Coliform **T. Coliform** Total Coliform

Microbiology Analyses that can be written in:

Enterococci **MPA** Microscopic Particulate Analysis for Determining GWUDI
G/C Giardia/Cryptosporidium **Coliphage** **Staph a** Staphylococcus aureus

Toxicity Characteristic Leaching Procedure (TCLP) write in analyses[a]:

TCLP BNA **TCLP Herb** TCLP Herbicides **TCLP met+Hg** TCLP metals including mercury **TCLP met** TCLP metals not including mercury **TCLP Hg** TCLP mercury **TCLP Pest** TCLP Pesticides **TCLP VOA**

[a] *Analyses are normally only conducted for analytes with a TCLP regulatory criteria.*

General analyses pre-printed on the form:

BOD Biochemical Oxygen Demand **NO_2+NO_3** Nitrite plus Nitrate **Oil & Grease** **TDS** Total Dissolved Solids **TSS** Total Suspended Solids

General analyses that can be written in:

Acidity **Alk** Alkalinity **TNH3** Ammonia **HCO_3** Bicarbonate **Br** Bromide **CO_3** Carbonate **COD** Chemical Oxygen Demand **Cl** Chloride **Color** Color **Cond** Conductivity **CN** Cyanide **CN-W&D** Cyanide, weak & dissociable **Flash** Flash Point **F** Fluoride **Grn Siz** Grain Size **Hard** Hardness **NO_2** Nitrite **NO_3** Nitrate **TNVS** Non-Volatile Solids **NVSS** Non-Volatile Suspended Solids **CLO_4** Perchlorate **pH** **Phenol** Phenolics **SiO_2** Silica - dissolved **SO_4** Sulfate **S** Sulfide **TOC** Total Organic Carbon **TS** Total Solids **% V Sids** % Volatile Solids **TVS** Volatile Solids **TVSS** Volatile Suspended Solids **SetSlds** Settleable Solids **% Tot** % Total Solids **TKN** Total Kjeldahl Nitrogen **T-Phos** Total Phosphorous **D-Phos** Dissolved Phosphorous **O-Phos** Ortho Phosphorous **D-O-Phos** Dissolved Ortho Phosphorous **Turb** Turbidity

Container guidance.

Note: this is general information only - consult the QA Project Plan on appropriate containers and preservatives for each project. Modifying methods may require modifying the number/type of containers. Freezing samples for one or more analysis may require collection of individual containers. Contact the laboratory for minimum sample volumes in situations where sample material is limited. Minimum volumes required for analysis will depend on the analysis and required reporting limits.

Containers for soil/sediment:

Metals/cyanide/mercury: 1, wide mouth 8 ounce glass or HDPE.
Extractable organics: 1, 8 ounce wide mouth amber glass, for one or two analyte groups
Inorganics and organics: 1, sixteen ounce wide mouth amber glass.
VOAs/purgeables: Contact the laboratory for the proper number/type of special Closed-System sample containers.

Containers/chemical preservatives for water[a]:

Metals/regular mercury: 1, one liter HDPE, HNO3 to pH<2
Mercury by method 1631: HCl and 250 mL containers provided by MEL
Cyanide: 1, 250 mL or larger HDPE, remove sulfides and/or residual chlorine then add NaOH to pH>12
Extractable organics (BNA, Pest, PCP, PAH etc.): two, one liter amber glass containers **for each analysis** - if more than one liter will be extracted for the project, it is advisable that the container size match (but not exceed) the volume to be extracted. Two separate volumes are usually collected for each analysis to allow for re-extraction if needed.
VOAs/purgeables: 3, zero headspace 40 mL amber glass vials with Teflon Septa, remove residual chlorine then add HCl to pH<2
Alkalinity: 1, 250 mL or larger HDPE, no extra volume for lab QC
Ammonia: 1, 250 mL or larger HDPE, H2SO4 to pH<2, no extra volume for lab QC
BOD 5: 1, one gallon HDPE, no extra volume for lab QC
TSS: 1, one liter or larger HDPE, no extra volume for lab QC
TDS: 1, 250 mL or larger HDPE, no extra volume for lab QC
Oil & Grease: 1, one liter clear glass, HCl to pH<2, submit **4 separate** containers for the lab QC sample
NO2+NO3: 1, 250 mL or larger HDPE, H2SO4 to pH<2, no extra volume for lab QC
Br, Cl, F, SO4, CLO4: for analysis by ion chromatography, 1, 100 mL or larger HDPE, no extra volume for lab QC

[a] *Water samples to be designated for lab QC should have double volume submitted for metals, triple volume for organics. In general, extra volume is usually not required for lab QC for soil/sediment.*

APPENDIX C. LABORATORY DOCUMENTATION

Appendix C-1. QA Manual

Laboratory analysis and procedures will comply with the guidelines described in the document entitled, *Quality Assurance Manual for the U.S. EPA Region 10 Manchester Environmental Laboratory (October 2005).* The QA Manual is available at the following website on EPA's Intranet (G:\Sops\NELAC 2005 QAM\NELACTable.htmL). If you are unable to access this document and would like to obtain a copy, please contact Stephanie Harris.

Appendix C-2. Standard Operating Procedures.

C-2.1. SOP for recreational and waste water DNA purification

NRMRL-CI-SOP- _____
DATE _____January 25, 2006_____
REVISION # __0___DATE_____
BRANCH/DIV_____MCCB/WSWRD ___

Standard Operating Procedure (SOP):

Recreational Water and Wastewater Filtration for DNA Extractions

SCOPE AND APPLICATION

This procedure instructs user in how to filter recreational and wastewater samples for microbial DNA extraction.

APPROVALS

Originator/User:	Orin C. Shanks
Branch Chief:	Mark Rodgers
Division Director:	Jim Goodrich
Quality Assurance Officer:	Steve Harmon

Equipment and Supplies:

1. Sterilized gloves.
2. Disposable Filter unit: Analytical Test Filter Funnel (Catalog #: 09-740-30G; Fisher Scientific).
3. Vacuum manifold that can accommodate a 47 mm diameter filter.
4. #8 silicone stopper with 0.365 inch hole (Catalog #: XX2004718; www.millipore.com)
5. Vacuum pump or other vacuum source.
6. Filter forceps.
7. Sterilization source for forceps (10% bleach with Ultrapure water rinse or immerse in 95% ethanol and flame).
8. Ultrapure water (Catalog #: TMS-006-A; Specialty Media, Phillipsburg, NJ).
9. Supor-200 membrane filters, 0.2µm pore size, 47mm diameter (PALL, Ann Harbor, MI).
10. Polycarbonate filters, 0.4 µm pore size, 47mm diameter (Catalog #: K04CP04700; Osmonics Inc.)
11. GITC buffer [5M Guanidine isothiocyanate, 100 mM EDTA (pH8), 0.5% Sarkosyl].

Recreational Water and Wastewater Handling:

Recreational water or wastewater samples should be chilled immediately following collection, and stored at a temperature ranging from 0-10°C until time of filtration.

Duplicates and Controls:

1. Two aliquots from each sample will be filtered and assayed individually (sample duplicate).
2. Two filtration negative controls for batch of samples (batch = 1-20 samples).

Disposal:

After filtration, recreational water and wastewater can be discarded in the sink.

Safety Measures:

1. Wear gloves. Always treat unfiltered samples as if they are contaminated with pathogens. Keep them contained and clean up with bleach solution.

2. GITC buffer is toxic; avoid contact with skin. Standard good lab practice should always be followed. Consult the Material Safety Data Sheet for information on hazards

associated with undiluted powdered GITC. Most safety precautions for guanidine isothiocyanate apply to undiluted powdered GITC; workers mixing the stock solutions should use a mask and skin protection and work under a hood with spill protection.

Store 5M GITC at room temperature in the dark. Aqueous 5 M GITC buffer has negligible vapor pressure and so does not present a hazard from fumes, and presents negligible hazard from splashing. Workers should wear nitrile gloves and skin protection. If there is a spill, it should be mopped up with paper towels that should then be placed in a plastic bag. Broken or leaking tubes should also be bagged and disposed of according to laboratory regulations. Wipe the work area with a wet paper towel after a spill to minimize dust.

Protocol:

1. Remove filter funnel from bag. Attach funnel adapter by pressing firmly in place and securely seat assembled unit in stopper. Then insert entire filter apparatus into a manifold or vacuum flask. To remove the stock filter membrane, firmly grip blue collar with thumb and forefinger. Grip funnel top with other hand. With fingers, gently squeeze funnel top into an oval to break seal. Peel funnel top away in one motion to expose the stock membrane. DO NOT TWIST! Aseptically remove the stock membrane. For recreational water samples, aseptically install a 0.2 µm pore size 47mm diameter Supor-200 membrane (shiny side down). For wastewater samples, aseptically install a 0.4 µm pore size 47mm diameter polycarbonate membrane. Be sure to leave behind the filter pad underneath the membrane. Reassemble filtration apparatus. Connect vacuum source to designated area on vacuum manifold or vacuum flask.

2. Pour 25 ml of mixed wastewater sample, 100 ml of recreational water, or 100 ml filtration control into the filtration unit and apply a vacuum until water has completely passed through the filter. Rinse the sides of the filtration apparatus with 10 ml of Ultrapure water to capture any remaining residues. Note: Perform filtration controls using the same filter type used for wastewater or recreational samples (see Step 1.).

3. Turn off the vacuum and dismantle the filtration unit.

4. Carefully roll up the contaminated filter using sterile filter forceps and/or a sterile toothpick. Do not touch the contaminated surface to the base of the filtration unit.

5. Place the rolled filter into a 15 ml tube containing 0.5 ml GITC buffer. Ensure that the filter goes to the bottom.

6. Gently invert the tube several times to ensure that the entire filter is wet with buffer.

7. Label the tube and place the tube into a freezer (-20°C) for storage. Label information should include origin of sample (ie. name of wastewater facility), date, and technician initials.

8. Sterilize the forceps using 10% bleach with Ultrapure water rinse or immersion in 95% ethanol and flame. To filter a duplicate sample, place a fresh filter into the filtration unit using sterilized forceps and reassemble Analytical Test Filter Funnel unit. Please note that the same filter type should be used for duplicate samples (see step 1.).

9. Repeat process (steps 2-8) with duplicate sample. After all duplicate samples are filtered, sterilize forceps (10% bleach with Ultrapure water rinse or immerse in 95% ethanol and flame) and discard filtration unit.

10. For each sampling batch (1-20 samples processed on the same day), prepare 2 filtration negative controls (single filter unit with duplicate filters) by following the above procedure, but substituting 100 ml Ultrapure water for a recreational water or wastewater sample.

C-2.2. Scat Fecal Sample DNA Extraction

Fecal DNA Extraction

Equipment:
-Pipetmen pipettors (p200 and p1000) with barrier tips
-Vortex mixer
-Bead Beater Instrument
-Heating block or hybridization oven (60oC)
-Microcentrifuge (14,000 x g)
-2.0ml and 0.65ml low-adhesion microtubes
-1ml disposable transfer pipets
-15ml Falcon tubes

Reagents:
-FastDNA Kit (Q-BIOgene # 6540-400)
-GITC Buffer (5M Guanidine isothiocynate, 100mM EDTA pH 8.0, 0.5% Sarkosyl)
-AE Buffer (Qiagen)

Protocol:

1. Place 2g feces (wet weight) into 15ml falcon tube.
2. Add 5ml GITC buffer, vortex, and incubate at room temperature for five minutes [up to 5 hours is acceptable].
3. Add 800ul CLS-VF and 200ul PPS into a bead beating microtube.
4. Add 200ul of GITC buffer/fecal slurry into bead beating microtube [use disposable transfer pipet].
5. Agitate with bead beater for 30 seconds and centrifuge for 5 minutes at 14,000 g.
6. Pipet 600ul of extract supernatent from bead beating microtube and 600ul binding matrix into 2.0ml low-adhesion microtube, incubate at room temperature for 5 minutes [agitate gently], and centrifuge for 1 minute at 14,000 x g.
7. Discard supernatent and wash pellet with 500ul SEWS-M [make sure to add ethanol], resuspend pellet, and centrifuge for 1 minute at 14,000 x g.
8. Repeat step 7.
9. Dry silica pellet with 60oC incubation for 5 minutes.
10. Elute DNA with 100ul AE buffer [Qiagen Dneasy Kit], mix gently, incubate at 60oC for 5 minutes, centrifuge for 5 minutes at 14,000 x g, remove supernatent, and store in 0.65ml low-adhesion microtube at -20oC.

C-2.3. Laboratory Reagent Methods

Lab Reagents/Recipes

Orange G
15.0 g sucrose
0.175 g Orange G – Sigma 3756
MilliQ water to 50 mL

25% Acteamide
2.5 g acetamide
10 mL MilliQ water

Primer Dilutions
(dilute to be 25 pmol/µl since that is the amount we use in a PCR reaction)

Note concentration of primer (from primer vial; usually nmol concentration)

nmol = 10^{-9}
pmol = 10^{-12}

conc.of primer in nmol X 1000 pmol/nmol ÷ 25 pmol/µl

will give you the amount of distilled water to add to the primer to give a final concentration of 25 pmol/µl

LB (Luria-Bertani) Medium and Plates

In a 1L bottle dissolve 7.75g LB Broth Media in 500 mL deionized water
Autoclave on liquid cycle for 15 minutes at 15 psi.
Allow solution to cool to 55ºC.
Add 50mg ampicillin (add the ampicillin to ~5ml of water and use a 0.22µm syringe filter to add to media). Mix.

LB Agar Plates
LB medium as above, but add 7.5g agar before autoclaving (boiling is recommended to help agar go into solution). After autoclaving, cool to ~55ºC, add ampicillin and pour into petri plates. Let harden, invert and store at 4ºC, in the dark.

C-2.4. Off-Site Collaborator Fecal Sample Collection Protocol

Off-Site Collaborator Fecal Sample Collection Protocol

Project Overview:
The goal of my project is to study the fecal microorganism communities from different animals. Once the fecal material passes out of the animal, the microbial community starts to change in response to its new environment. Therefore, it is important to collect fresh fecal samples when possible. It is also important to make sure that each fecal sample represents one individual.

Fecal Sampling Protocol:
Fecal sample amounts should range from approximately 5-10 grams (wet weight). Do not spend time massing samples just take your best guess. Wear fresh sterilized latex gloves for each individual sample collected. Place the fecal material into a sterilized 50 ml conical tube using a disposable spatula, label the tube (animal species, date, and estimate time on the ground), store on ice in the field, and freeze at -20°C as soon as possible. Use a fresh spatula for each fecal sample. Some fecal samples will be too large to fit in the 50 ml conical tube. For larger samples, collect a manageable amount, seal in a ziplock bag, and label as described above. Once in the laboratory, collect a small portion of the fecal material and place in 50 ml conical tube using a sterilized disposable spatula. Otherwise, you can mail the large samples in the ziplock bags. What ever is the easiest will work for me. Please keep in mind that the samples will need to be shipped in dry ice and that small tubes are easier to handle and ship.

Equipment Supplied by MCCB:
I will arrange to have a minimum of 100 50 ml conical tubes, a small cooler, and disposable spatulas sent to your lab. You will need to provide sterile gloves and ziplock bags.

Shipment of Samples:
Samples should be shipped in an insulated cooler (no Styrofoam). Each sample must be stored in a leak-proof primary container (50 ml conical tube) and sealed with tape or Parafilm®. Each sealed primary container must be individually wrapped in an absorbent material such as a paper towel. This step is required for shipment of diagnostic samples and helps to prevent mixing of the samples in case of leaks. Primary containers should be stored inside a secondary container (ziplock bag). Please pack samples with several frozen ice packs and overnight FedEx immediately. The FedEx package does not require a special label or other markings noting the contents of the package. The FedEx account number is 293966389. Do not use the FedEx number for any other purposes (I have to say this). Please ship samples on a Monday or Tuesday and mail the samples to:

Orin Shanks
US EPA
26 West Martin Luther King Drive
MS 387
Cincinnati, OH 45268

Orin C. Shanks
U.S. Environmental Protection Agency
Last Updated: January 23, 2006

C-2.5. PCR and Gel Electrophoresis Methods

TABLE 1. Primers

Primer	Sequence (5'-3')	Target
Bac32F	AACGCTAGCTACAGGCTT	*Bacteroides-Prevotella*
Bac708R	CAATCGGAGTTCTTCGTG	*Bacteroides-Prevotella*
CF128F	CCAACYTTCCCGWTACTC	ruminants
CF193F	TATGAAAGCTCCGGCC	ruminants
HF134F	GCCGTCTACTCTTGGCC	human
HF183F	ATCATGAGTTCACATGTCCG	human

HF, human-specific; CF, ruminant-specific. Numbers correspond to the numbers of the *E. coli* 16S rRNA gene. All forward primers are paired with Bac708R.

***Bacteroides* PCR**

Cycler Conditions for General *Bacteroides* Assay

Step 1. Incubate at 94°C for 2 minutes
Step 2. Incubate at 94°C for 1 minute
Step 3. Incubate at 53°C for 1 minute
Step 4. Incubate at 72°C for 1 minute
Step 5. Go to step 2 (34 more times)
Step 6. Incubate at 72°C for 5 minutes
Step 7. Incubate at 4°C forever

Cycler Conditions for Ruminant *Bacteroides* Assay

Step 1. Incubate at 94°C for 2 minutes
Step 2. Incubate at 94°C for 1 minute
Step 3. Incubate at 62°C for 1 minute
Step 4. Incubate at 72°C for 1 minute
Step 5. Go to step 2 (34 more times)
Step 6. Incubate at 72°C for 5 minutes
Step 7. Incubate at 4°C forever

Alpha Innotech Alpha quant 6
Cat 60-13069-00
www.Alphainnotech.com

orange G.

Cycler Conditions for Human *Bacteroides* Assay

Step 1. Incubate at 94°C for 2 minutes
Step 2. Incubate at 94°C for 1 minute
Step 3. Incubate at 63°C for 1 minute
Step 4. Incubate at 72°C for 1 minute
Step 5. Go to step 2 (34 more times)
Step 6. Incubate at 72°C for 5 minutes
Step 7. Incubate at 4°C forever

4µL/200mL agarose

Agarose Gel

Prepare a 1% agarose gel using 0.5X of TBE. Agarose can be dissolved by heating the solution on a hot plate or a microwave. Do not allow the solution to spill over, otherwise you are changing the concentration of agarose. Cool agarose to 50-55°C in an incubator and add adequate amount of GelStar (3-4 µl per 100ml of agarose; www.cambrex.com/). This is our DNA stain of choice as it is more sensitive than conventional stains like ethidium bromide. Since GelStar contains a nuclei acid stain, treat it as potentially carcinogenic, thus wear gloves while handling the gels. We run ours gels for one hour at 75-100 V. The running time will depend on the percentage of agarose, buffer, and type of gel box used. The expected size of the PCR products is approximately 700 bp for the general *Bacteroides* assay and between 515 and 580 bp for the host specific assays.

References

Bernhard AE and Katharine G. Field. 2000. A PCR Assay To Discriminate Human and Ruminant Feces on the Basis of Host Differences in *Bacteroides-Prevotella* Genes Encoding 16S rRNA. Appl. Envir. Microbiol. 66: 4571-4574.

Simpson, J. M., J. W. Santo Domingo, and D. J. Reasoner. 2002. Microbial Source Tracking – State of Science. Environ. Sci. & Tech. 36:5279-5288.

Appendix C-3. Data Report Forms.

C-3.1. Bacteriodes PCR Analysis Report Form

PCR Setup Sheet _____ Cycle Method: _____ - _____ - _____ - _____ - _____

Exp. # _____
Date: _____

	Time	Temp
Hold:	_____	_____
Denat:	_____	_____
Anneal:	_____	_____
Extend:	_____	_____
Extend Hold:	_____	_____
Final Extend:	_____	_____
No. Cycles	_____	

Reagents	Name	Lot. No.	ul	1	2	3	4	5	6	7	8	9	10
Dist. Water		_____	_____										
10X Buffer II		_____	_____										
dNTP mix		_____	_____										
25 mm MgCI		_____	_____										
Primer 1	_____	_____	_____										
Primer 2	_____	_____	_____										
Template	_____	_____	_____										
DNA Pol.		_____	_____										
Total volume			_____										

				11	12	13	14	15	16	17	18	19	20
Dist. Water		_____	_____										
10X Buffer II		_____	_____										
dNTP mix		_____	_____										
25 mm MgCI		_____	_____										
Primer 1	_____	_____	_____										
Primer 2	_____	_____	_____										
Template	_____	_____	_____										
DNA Pol.			_____										
Total volume			_____										

				21	22	23	24	25	26	27	28	29	30
Dist. Water		_____	_____										
10X Buffer II		_____	_____										
dNTP mix		_____	_____										
25 mm MgCI		_____	_____										
Primer 1	_____	_____	_____										
Primer 2	_____	_____	_____										
Template	_____	_____	_____										
DNA Pol.			_____										
Total volume			_____										

Data to Input

_____ Collection Site
_____ Date/Time Collected
_____ Species
_____ Approx. Age of Sample
_____ Sample # Assigned

_____ Date Purified
_____ Date Analyzed/PCR
_____ PCR Reaction #
_____ Confirmation Status

APPENDIX D. DATA EVALUATION

Appendix D-1. Data Evaluation/Documentation Form.

D-1.1. Microbiology Laboratory Data Review/Release Form

Project: Project Code:_____
Sample Numbers
Peer Reviewed by:
Date:_____

Raw Data/Quality Control Check

____ Verify positive and negative culture controls associated with media are satisfactory.
____ Verify media sterility was checked.
____ Check for sample carryover/contamination if membrane filtration method used. Note any deficiencies.
____ Check duplicate analyst counts are within 20 %, when applicable.
____ Verify that media was prepared within method specifications.
____ Verify that samples were received and analyzed within the holding time.

Bench Sheet Check

____ Is the data package properly labeled?
- __ Analyst name
- __ Sample numbers and project name
- __ Analytical method used
- __ Date and time of collection/analysis

____ Verify that there is a bench sheet for each sample listed on the Analysis Required forms.
____ Verify that there is a Data Review Memo written for the project -forwarded to ESAT Data Entry Technician
____ Verify that there is a Data Release Memo for this project - forwarded to ESAT Data Entry Technician

Results

____ Verify that the reported results:
- __ have appropriate qualifiers assigned
- __ reflect the correct units
- __ reflect dilution factors used in the analysis
- __ were transferred correctly from the bench sheets
- __ were calculated correctly

Measuring Velocity

- Turn knob on Swoffer® meter to **Calibration**. It should read 185-186. If it is does not, change the nine-volt battery. Record the Calibration # in the proper space in the field notes. Install the propeller on the wading rod and tighten the Allen screw.
- Turn the knob on the Swoffer® meter to **Ave. Velocity**.
- Press the **start** button on the Swoffer meter.
- Place the propeller in the stream with the propeller facing into the streamflow.
- Record the velocity in the proper column in the field notes.

Upon completion of the flow measurement, turn the knob on the Swoffer® meter to **Calibration** and record the number in the proper space in the field notes.

Lighthouse Point Water Reclamation Facility
Quality Control Laboratory

QUALITY ASSURANCE STATEMENT

Updated September 17, 2014 By Christina Ness

1. ORGANIZATION/RESPONSIBILITIES

The Lighthouse Point Water Reclamation Facility (LPWRF) is operated by a Lead Operator, and two Operator/Samplers. The Lead Operator is responsible for establishing quality assurance and quality control (QA/QC) policies and ensuring those policies are followed. The Lead Operator is also the primary laboratory analyst responsible for performing analyses on wastewater and quality control samples and recording results. In the absence of, or under the supervision of the Lead Operator, the operators may perform analyses on wastewater and QC samples and record results. The Lead Operator will verify such results. The Lead Operator or his designated alternate is also the sample custodian.

2. POLICY FOR QUALITY ASSURANCE/QUALITY CONTROL

a. The principal objective for operating the LPWRF laboratory is to consistently produce complete analytical data, which accurately represents the waste stream from which samples are taken.

b. All analytical procedures will be completed according to approved methods to include all QA/QC measures required by those methods. The initial data quality objective (DQO, which is a target for precision and accuracy) for each method is to achieve better precision and accuracy levels than those cited for each method in the latest edition of *Standard Methods for the Examination of Water and Wastewater.* Once control charts have been established for a given method, the statistics used for the chart (the mean and standard deviation) become the DQOs for the specific test, provided they are better than the initial DQOs.

c. While general QC/QA principles will be applied to all analyses performed in the LPWRF Quality Control lab, special QA/QC analyses will be performed on those samples taken to verify plant performance (as opposed to process control). Those analyses, as identified in Table 1, are BOD, TSS, pH, Temperature, Nitrogen Ammonia, Nitrates, Nitrites, Total Chlorine Residual, Total and fecal coliform.

d. Sample data will be recorded including results for any analyses of QC samples associated with the data.

e. Raw data will be entered on printed bench sheets (lab worksheets) and kept on a clipboard in the lab. All data will be reviewed and validated prior to entry into our computer programs. Again, all data will be reviewed and validated prior to the computerized monthly release of data from the LPWRF Quality Control laboratory. The bench sheets will be transferred to the file cabinet monthly after completion of monthly reports.

f. Initial training for new staff on analytical methods and QA/QC requirements and procedures will be conducted on a priority basis. Additional training will be conducted periodically (not less frequently than twice per year) as required to maintain competence in analytical skills.

g. Located in the Control Room will be copies of the LP WRF discharge permit, the current edition of *Standard Methods for the Examination of Water and Wastewater*, applicable methods, *Discharge Monitoring Report (DMR) Instruction Manual for Municipal Sewage Plant Operators,* this QA manual and, in a readily available binder, Safety Data Sheets (MSDS) for all potentially hazardous chemicals used in the lab.

3. SAMPLE MANAGEMENT

a. Samples will be taken according to the schedule in table 1. See the current edition of *Standard Methods for the Examination of Water and Wastewater,* for sample handling requirements (container type and size, preservation techniques, and maximum holding times).

b. Daily samples, including collection of composite samples, will be taken between 06:00 and 18:00 hours.
Weekly composite samples are taken at the beginning of each week. This allows time for follow-up sampling if needed.
Due to the small staff (frequently just one operator), the operator collecting the sample performs the analysis.
The Lead Operator, as sample custodian assures:

(1) Samples are stored properly and handled by a minimum number of people.
(2) The laboratory is secured at all times when not in use.
(3) Only authorized personnel are allowed in lab.
(4) Samples are logged on a lab benchsheet, or chain-of-custody record as a permanent lab record (see Appendix E).
(5) Samples are distributed to the proper analysts.

4. METHODS

Methods used in the LPWRF Quality Control lab are those prescribed without modification in the latest edition of *Standard Methods for the Examination of Water and Wastewater*. Methods used by this lab are described in full in the lab procedure sections of this manual.

5. CALIBRATION AND QUALITY CONTROL PROCEDURES

a. Lab Facility. The lab is kept clean and orderly at all times. Specific issues related to the facility are addressed in Appendix C.

b. Instrument Calibration. Instruments are calibrated on a daily basis just before use and every two hours during use over prolonged periods during any given day. Those requiring calibration are the pH meter and probe, the Ammonia meter and probe, the

spectrophotometer, and the D.O. meter (if used). The D.O. meter is normally air calibrated. Records of calibration are maintained in the plant log for each piece of equipment.

TABLE 1

Table 1			
TESTS	**SAMPLE POINT**	**SAMPLING FREQUENCY**	**SAMPLE TYPE**
COMPLIANCE:			
Flow	Influent	7/7	Continuous recording
Flow	Effluent	7/7	Continuous recording
BOD_5	Influent	2/7	24-composite
	Effluent	2/7	24-composite
Total Suspended Solids	Influent	2/7	24-composite
	Effluent	2/7	24-composite
Fecal Coliform	Effluent	4/7	Grab
Total Available [Residual] Chlorine	Outfall	7/7	Grab
pH	Effluent	7/7	Grab
Temperature	Effluent	7/7	Grab
PROCESS CONTROL:			
Nitrogen ammonia	Influent	7/7	Grab
Nitrogen Ammonia	Effluent	7/7	Grab
Nitrate	Effluent	7/7	Grab
Nitrite	Effluent	7/7	Grab
Temperature	Influent	7/7	Grab
pH	Influent	7/7	Grab
pH	Influent	7/7	Grab
Dissolved oxygen	Influent	7/7	Grab
Dissolved oxygen	Aeration basin in	7/7	Grab
Dissolved oxygen	Aeration basin out	7/7	Grab
Dissolved oxygen	Anoxic basin	7/7	Grab
Dissolved oxygen	Effluent	7/7	Grab
Total Suspended Solids	Mixed liquor return	7/7	Grab
Table 1-r			
RECLAIMED WATER	**TESTING IN**	**SEASON ONLY**	
TESTS	**SAMPLE POINT**	**SAMPLING FREQUENCY**	**SAMPLE TYPE**
Flow	LS 11	7/7	Continuous recording
Flow	LPWRF Reclaimed	7/7	Continuous recording
BOD_5	Reclaimed sampler	1/7	24-composite
Total Suspended Solids	Reclaimed Sampler	1/7	24-composite

Table 1-r continued			
TESTS	**SAMPLE POINT**	**SAMPLING FREQUENCY**	**SAMPLE TYPE**
Dissolved Oxygen	Reclaim site (basement LPWRF)	7/7	Grab
Total Coliform	Reclaim site (basement LPWRF)	7/7	Grab
Free Chlorine measured as Total Available Chlorine	Reclaim site (basement LPWRF)	7/7	Grab
Temperature	Reclaim site (basement LPWRF)	7/7	Grab
pH	Reclaim site (basement LPWRF)	7/7	Grab

c. Equipment maintenance. All lab equipment is maintained so as to keep it in peak working order at all times. Lab personnel may make simple repairs. Qualified service representatives will perform more extensive repairs. Breakdowns and repair procedures will be noted in the plant logbook on each piece of equipment. Daily temperature checks by a certified thermometer of the drying oven, water baths and/or incubator temperatures are performed and recorded. The analytical balance is checked at least daily or more often using standard weights and serviced by professional service representatives as required, or annually, as a minimum requirement. The dissolved oxygen membrane and the nitrogen ammonia membranes are replaced whenever readings become erratic or unable to standardize. Backup meters and probes are kept on hand in case of probe or meter failures. Records of all routine maintenance and repairs are kept in the plant logbook. The sample refrigerator is maintained at a temperature between 1and 4 degrees Celsius.

d. Analytical Reagents. Only certified analytical grade reagents are used. Labels on all chemical reagents are marked with the date received, date opened, and if applicable, the expiration date. Chemicals are stored out of direct sunlight. Those requiring cold storage are kept in the refrigerator, separate from sample storage. Acids and bases are stored separately in specially designated storage areas (secondary containment). Care is taken to prevent cross contamination of reagents and samples. Contaminated reagents and outdated chemical solutions are disposed of in accordance with accepted practices. For reagents mixed in the lab, shelf life recommendations provided in the analytical method are followed and bottles are marked with date prepared. Distilled water is stored in plastic carboys (water purchased when necessary). Care is taken not to contaminate distilled water Water suspected of being contaminated is discarded. Standard solutions are stored separately and safeguarded to prevent contamination.

d. Labware Cleaning. After each use, glassware is washed in out Laboratory dishwasher, allowed to dry, and stored in a cabinet. Sample bottles and equipment for microbiological evaluations are sterilized in accordance with *Standard Methods* (SM # 9020 B- table 9020:3).

f. Quality Control Analysis. Quality control measurements are made for all analyses related to "plant performance" samples. It is very important that these tests be performed *exactly* as written in published methods. Routine analyses of blanks, duplicates, and standard solutions are preformed according to the frequency shown in Table 2. Results from blank analyses are treated in the manner specified by the method.

Data from results of duplicate analyses are treated in the manner specified in the following sections on evaluating accuracy. Records of analyses of standard solutions (i.e., the glucose/glutamic acid test for BOD, and the cellulose standard for TSS) are kept on appropriate plant sample bench sheets. The results are also logged into our computer along with the plant sample results. QC considerations for specific tests follow:

(1) **BOD.** The traditional method for calibration of the dissolved oxygen probe for BOD determination is the Winkler titration, which does not require instrument calibration. It does require standardization of the titrant with either potassium biniodate or potassium dichromate. This may be performed if the dissolved oxygen probe readings are suspect. The D O probe is air calibrated each day during which analyses are normally run or once every two hours during prolonged runs. Samples are incubated at 20 degrees C (±1 degree

C), as measured by a certified thermometer [[i.e., one that has been checked against the National Institute of Standards and Technology (NIST) thermometer]. Glucose/Glutamic Acid standards at varying concentrations are run during every sample set. Triplicate blanks are run on unseeded and seeded dilution water with each set of samples. Depletion (the DO drop) on the blanks should not exceed 0.2 mg/L. Depletions of > 0.2 mg/L to 0.5 mg/L on all the blanks need an explanation on the DOE Cause and Remedy letter. A depletion of >0.5 mg/L on all the blanks may void the test as will an increase in DO on all the blanks. The DO drop for samples should be at least 2 mg/L with a residual DO of at least 1.0 mg/L at the end of the incubation period. If these targets are not met, corrective action is taken.

(2) **Total Suspended Solids (TSS).** The principal calibration item in the TSS determination is the analytical balance, which is checked by lab personnel daily with class 1 weights and by an authorized service representative annually. Duplicate blanks are run on distilled water. If the TSS value for the blank varies by more than .2 mg from zero, the cause is sought and, if necessary, corrective action is initiated. Cellulose check standards are run with each batch.

(3) **pH.** The manufacturer instructions are followed for storage and preparation for use of the pH meter and sensor. Three buffer solutions are used to calibrate the meter, other bracketing the pH expected for most samples and within 3 pH units of the first. Our meter requires pH 7 and a buffer below 7 for acidic samples, and above 7 for basic samples. Calibration is checked just prior to use and once every two hours during prolonged runs, with three buffer solutions.

Chlorine Residual. Chlorine residuals are measured by a HACH spectrophotometer. We have two spectrophotometers and occasionally cross check our results on both machines. We use HACH chlorine check standard on for calibration checks.

(4) **Fecal Coliform.** There are no instruments requiring calibration for fecal coliform determinations, nor are there requirements for analyzing check standards. A blank is furnished with each sample set. The filter in the blank determination is rinsed with buffer solution to assure that the buffer is not contaminated. In house duplicates are run ocassionally.

(5) In addition, the lab splits a sample for analysis with a commercial laboratory as an interlab check on the accuracy of the fecal Coliform test results.

The normal method for fecal coliform determination is the membrane filter (MF) technique.

g. **<u>Evaluating Accuracy.</u>** The ability of the lab to perform accurate analyses is evaluated by analyzing quality control samples.

(1) Check standards (samples with concentration known to the analyst) are used primary to check on bias and precision. Check standards are analyzed infrequently for pH and residual chlorine and frequently for TSS and BOD. There is no check standard for total or fecal coliform. By preparing a control chart using the results of several analyses of the same standard, the lab can monitor overall accuracy by comparing the mean or average value of all results to the true or expected value. The lab also monitors precision by plotting results on the control chart.

(2) PE samples (of concentrations unknown to anyone at the LPWRF lab, but known to the supplier of the sample) are used as a check of overall accuracy. PE samples for all plant performance parameters listed in table 1, are analyzed twice per year, except total and fecal coliforms, which are analyzed annually, using samples provided by our contract PE sample provider.

(3) To evaluate precision of analysis for pH, BOD and TSS, control charts will be maintained for appropriate determinations. The control charts will be based on analyses of the standard solution for BOD and the standard suspension for TSS. The pH control will be based on the pH 5.0 buffer solution.

(4) Duplicate control charts Will be based on effluent samples for total and Fecal Coliform, TSS, and BOD. A control chart will also be used for Fecal duplicates run with an outside lab.

(5) **Control Charts Based on Standard Solutions**. After a minimum of ten (but preferably 20) replicates of the standard solution and/or duplicate sample results for a given parameter have been completed, the standard deviation for the analysis will be calculated using the worksheet at Appendix B. Appendix B also includes a completed computerized sample worksheet. Using the calculated standard deviation (s), a control chart is constructed as shown below. Concentration of the standard solution is indicated on the chart as X.0 mg/L. Where the chart indicates +3s, for example, the actual control chart would indicate a value of the standard solution concentration (or mean of 10-20 replicate results) *plus* three times the calculated standard deviation. Likewise the standard solution concentration *minus* three standard deviations. Page 15 (Appendix B) shows a control chart based on the data presented on page 14.

Once the control chart has been constructed, all subsequent determinations of the same standard will be plotted on the control chart. As long as the values are within the action limits, values for samples analyzed in conjunction with the standard may be reported. If values exceed either action limit, if more than three consecutive values exceed one warning limit, or if more than seven consecutive values are on one side or the other of the central line, the lab will be considered out of control for that analysis. Analysis for that parameter must cease until the situation is investigated, the cause found and corrective actions taken. When a chart becomes cluttered or whenever there is a significant change in procedure (e.g., a new analyst takes over the test) new control charts will be constructed based on standard analyses for the past 20 results. The points on the sample control chart on Page B-3 represent analyses of the standard solution taken after constructing the basic chart.

Table 2

QUALITY CONTROL PROCEDURES FREQUENCY CHART

Parameter	Calibration	Check Standards	Blanks	Duplicates	Performance Evaluation Samples
DO (for BOD Procedure)	N/A for Winkler titration. If using DO probe, air calibrate each day[1]	Each day[1]	Each day[1]	Min 1/month	1 each 6 months
TSS	Balance check with class "1" weights each day[1] & annually by service representative	Each day[1]	Each day[1]	Min 1/week	1 each 6 months
pH	Each day[1]	First of the week	N/A	1/week	1 each 6 months
Chlorine residual	N/A	Every 6 months	Each day[1]	1/quarter	1 each 6 months
Nitrogen Ammonia	Each day[1]	Weekly	N/A	1/quarter	1 each 6 months
Total Coliforms	N/A		With every set of samples	Min 1/month Monthly split with outside lab	Annually
Fecal Coliforms	N/A	N/A	With every set of samples	Min 1/month Monthly split with outside lab	Annually

Notes: 1. "Each day" above means each day that compliance analyses are run.

6. DATA MANAGEMENT

All records mentioned in the preceding and subsequent paragraphs and required in standard methods are retained at the LPWRF office in file cabinets for a period of at least three years. Before any result is reported, all raw data and calculations are reviewed for accuracy and signed by the senior operator acting as the quality assurance officer. If data contained on any record is transcribed to facilitate brevity or neatness, the original record is also kept on file.
All data is recorded waterproof high contrast ink and corrections, if made are by one line through the erroneous entry and are initialed. A list of initials identifying the person to whom they belong is maintained as a permanent lab record.

2. AUDITS

Two types of audits are used to determine status of the LPWRF Quality Control lab operations. A *system audit* is used to assess personnel, equipment, facilities, and analytical procedures. The system audit is conducted periodically by the Department of Ecology and at least every six months by the senior operator. *Performance audits* are conducted at least once every six months for each plant performance parameter except fecal coliform as part of the EPA Water Pollution Study.

3. REPORTS

A QA/QC summary is prepared by the Lead Operator semi-annually and reported to the Division Manager. The Lead Operator may provide the report in writing (verbatim or in summary) or verbally. The checklist at Appendix C is used to assist in drafting these reports and otherwise assessing lab capability and performance.

APPENDICIES

A- Glossary of common QA terms
B- Control Charting Standards
C- Laboratory Quality Assurance Checklist
D- Summary of Analytical Methods
E- Chain of Custody Procedures

APPROVED;

_____

Lead Operator initials

_____

Operator/Sampler initials

_____

Operator/Sampler initials

APPENDIX A

GLOSSARY OF QUALITY ASSURANCE/QUALITY CONTROL TERMS

Accuracy	The degree of agreement of an analytical result with the true value. The accuracy of a result is affected by both systematic errors (i.e., bias) and random errors (i.e., imprecision). Some writers improperly use accuracy to denote only systematic error. (see "bias" and "precision").
Action Limit	A type of control limit which is specified by a value on a control chart. If the value is exceeded, then corrective action must be taken. Action limits are usually placed at plus or minus 3 standard deviations from the expected or mean value.
Analyte	Denotes 'that which is to be analyzed for' in chemical, but not physical or biological determinations.
Analytical Error	The error, E, of an analytical result, R, is defined as; E=R-T, where T is the true value
Analytical Method	Denotes a set of written instructions specifying an analytical procedure to be followed by an analyst to obtain a numerical estimate of the concentration of a determinand (analyte) in each of one or more samples.
Analytical Response	A numerical observation which is obtained when a portion of a sample is presented to a measurement sub-system (e.g., spectrophotometric measurement of the absorbance of a solution). The magnitude is related to the amount or concentration of the determinand (analyte) in that portion.
Analytical Result	Denotes a numerical estimate of the concentration of a determinand (analyte) in a sample, and is obtained by carrying out once the procedure specified in an analytical method. Note that a method may specify analysis of more than one portion of a sample to produce one analytical result. The result can also be thought of as the final value reported to the user.
Analytical System	Denotes a combination of analyst, analytical method, equipment, reagents, standards, laboratory facilities, and any other components involved in carrying out an analytical procedure.
Batch	Batch usually refers to a set of consecutive determinations (analyses) made without interruption. The results are usually calculated from the same calibration curve or factor. Also called a "run".

Bias	The inaccuracy of an analytical result caused by a systematic error.
Blank	A determination which is intended to estimate the analytical response attributable to all factors other than the determinand (analyte) in the sample. Blanks are analyzed identically to samples, but do not contain the determinand (analyte) (e.g., in water analyses, pure water would be analyzed to determine the blank). Some writers use the term method blank with the same meaning given here for blank.
Calibration Standard(s)	Solution(s) of known concentration which are used in the calibration (standardization) procedure to determine the relation between response and concentration.
Certified Reference Material (CRM)	A reference material, one or more of whose property values are certified by a technically valid procedure accompanied by or traceable to a certificate or other documentation which is issued by a certifying body>
Check Standard	A solution of known concentration which is used to check the precision of analyses (and bias due to calibration). When used in conjunction with a control chart, it becomes a control standard.
Control Limit	A value specified on a control chart which is used to make decisions as to whether the results or control tests are acceptable. Two kinds of control limits are usually specified: warning limits and action limits.
Criterion of Detection	The smallest concentration which can be distinguished from a blank with no more than a 5% chance of reporting a false positive.
Data Quality Objectives (DQOs)	Qualitative and Quantitative statements of the quality of data needed to support specific decisions or regulatory actions.
Degrees of Freedom	A whole number expressing the amount of information available for a particular estimate; it is usually the number of independent results less the number of constraints (constraints being what else has to be estimated from the same set of results).
Determinand	Denotes 'that which is to be determined'. Covers chemical, physical, biological, or other analytical determinations.
External Standard Calibration	The use of independently prepared standards to determine the relationship between response and concentration. They are run separately from the sample(s). Also called external standardization.
Interference	A systematic error (bias) in the analytical result caused by the presence of a substance in the sample (or added to the sample during analysis).
Internal	A calibration procedure in which the responses of analytes are determined

Standardization	relative to internal standard(s) which is (are) added to every sample. Two solutions – calibration and spiking – are required. Used to reduce bias due to calibration.
Isotope Dilution	A type of internal Standardization procedure in which the internal standards are isotopically labeled analogs of the analytes. Isotope dilution techniques are characterized by very low bias due to calibration.
Limit of detection (or detection limit)	The smallest concentration for which there is at least a 95% chance it will be detected as a positive (i.e., there is only a 5% chance of obtaining a false negative).
Percent recovery	That percent of a known amount of material "spiked" or added to a sample being analyzed which is recovered at the end of analysis. See "spike" and "recovery". $$\% \text{ Recovery} = 100\,(R_2 - R_1)/A$$ Where R_2 and R_1 are the results for the sample (without the spike) and the spiked sample, respectively, and A is the equivalent concentration added in the spiked sample.
Population	The collection of all possible analytical results.
Precision	A qualitative term used to denote the scatter of results. Precision is said to improve as the scatter among results becomes smaller. Random error can also be referred to as imprecision. Usually measured as standard deviation.
Quality assurance	The total integrated program for assuring the reliability of monitoring and measurement data.
(Analytical) Quality Control	The routing application of statistically-based procedures to evaluate and control the accuracy of results from analytical measurements.
Random error	Indicated when repeated analyses of identical portions of a homogeneous sample does not give a series of identical results. The results differ among themselves and are more of less scattered about some value. They are termed random because the sign and magnitude of the error of any particular result vary at random, and cannot be predicted exactly.
(Analytical) Recovery	An estimate, usually expressed in percent, of an analytical result in comparison with a true or reference value for the analyte, (e.g., an analytical recovery of 95% for compound X means that the result was 95 percent of the true or reference value for X in the sample). It is a direct indication of analytical performance.
(Physical) Recovery	An estimate, usually expressed in percent of the amount of standard or analyte present at the final stage of analysis (e.g., final extract) compared with the amount present in the original sample. It can be an indirect indication of analytical performance.

Reference material (RM)	A material or substance, one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.
Relative standard Deviation (RSD)	The standard deviation relative to the mean. Also called "coefficient of variation". It is calculated as either s/x or 100s/x. The latter is sometimes referred to a percent relative standard deviation of %RSD.
Spike	The addition of known amount of analyte to a sample for the purpose of judging, from the analytical percent recovery, whether there is bias due to interference present in the sample. Also referred to as fortification of the sample. See "percent recovery".
Standard	A solution of known concentration. There are two types of standards: (1) check (or control), and; (2) calibration.
Standard Operating Procedure (SOP)	A detailed, written description of a procedure designed to systematize the the performance of the procedure.
Standard Deviation	A constant which describes the width of the normal distribution of results. An actual standard deviation is denoted by "σ " Whereas an estimate of the standard deviation is denoted by "s". For a sample of "n" replicate results taken from a population for analyses of a sample of known concentration, the estimate of the standard deviation is: $s=\sqrt{[\Sigma(x_i-\xi)^2/(n-1)]} = \sqrt{[(\Sigma x_i^2- (\Sigma x_i)^{2/n})/(n-1)]}$ Where "x" is a result and " ξ" is the mean of "n" results. For duplicate analyzed of "m" pairs of unknown samples, the estimate of the standard deviation of the difference (d) for the two samples in each pair is: $s=\sqrt{[(\Sigma d^2 - (\Sigma d)^2/m)(m-1)]}$ For spike recoveries on "n" samples, the estimate of the standard deviation of the percent recovery (p) is: $s=\sqrt{[(\Sigma p^2 - (\Sigma p)^2/n)(n-1)]}$
Standard Reference Material (STM)	A sample of known concentration, also called a certified reference material, issued by the National Institute for Standards and Technology (NIST).
Statistical Sample	The results of one or more determinations (taken from the population of all possible results.

Statistics	Certain single values computed from the results, which characterize certain properties of the results. Each statistic has its own frequency distribution which is defined by a particular mathematical function. Examples of statistics are the mean (x), estimate of the standard deviation (s), and the frequently- used t- and f- statistics
Surrogate Standard	A type of check standard, which is added to every sample in a known amount at the start of processing. The surrogate is not one of the target compounds for the analysis, but should have analytical properties similar to those compounds. The surrogate compounds are not expected to be present in environmental samples.
Systematic errors	Errors which are indicated by a tendency of results to be greater or smaller than the true value. Usually bias can be considered to be equivalent to systematic error.
Target compound	A compound, which is expected to be in an environmental sample, or for which the analysis is being conducted.
Warning Limits	A type of control limit which is specified by a value on a control chart, usually ±2s distant from the expected or mean value. Action is required when results fall outside the warning limits too frequently. A single value outside a warning limit does not require action, but should alert one to a possible problem.

APPENDIX B

BENCH SHEET FOR REPLICATES OF STANDARD SOLUTIONS

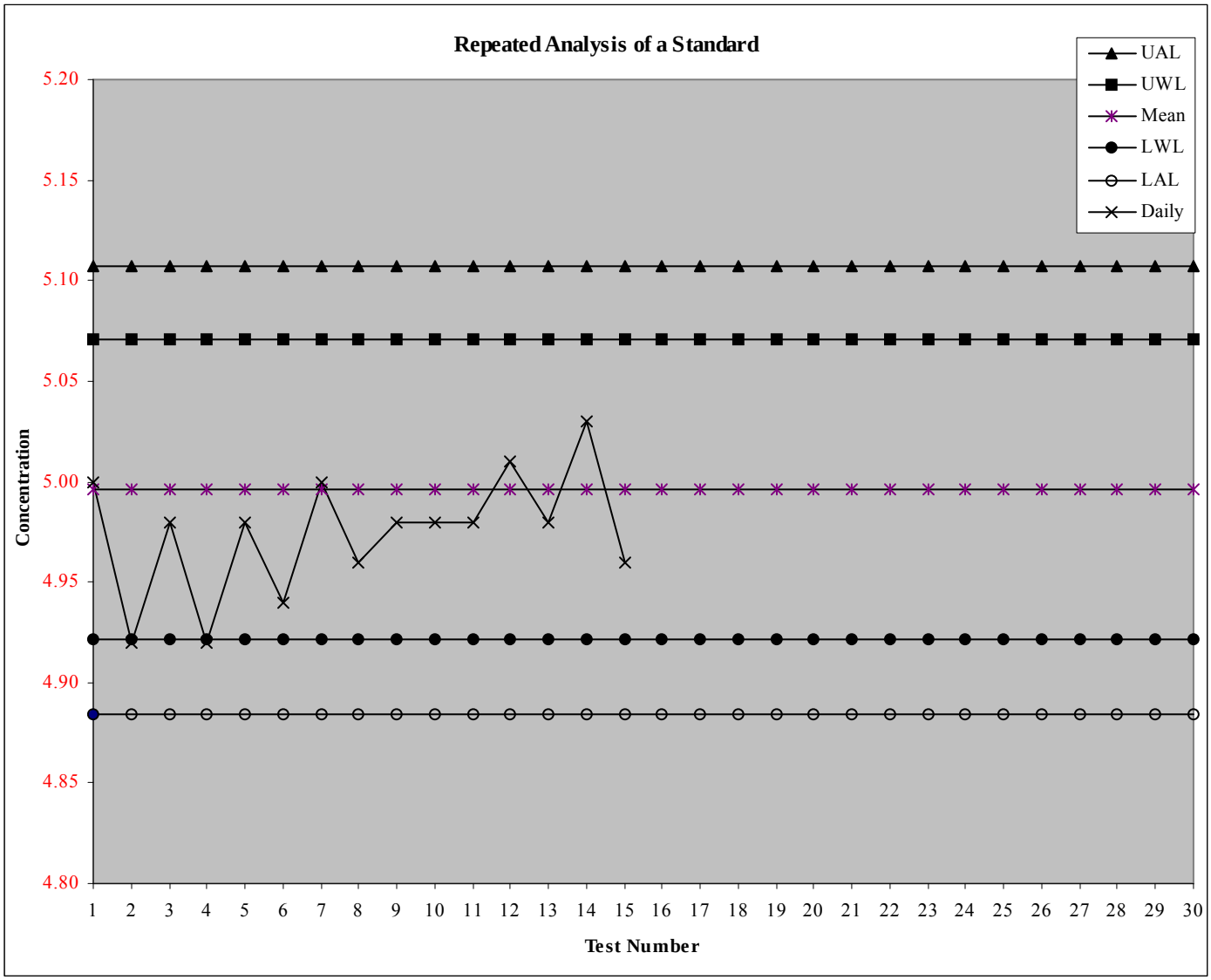
Parameters_____
Methods_____

TEST	DATE	RESULT(X)	X - 8	$(X - 8)^2$
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
		$\sum X =$		$\sum (X-8)^2 = A$
		$8 = \sum X / n =$	Where "n is the number of tests.	

Standard Deviation = s $= \sqrt{[A/(n-1)]} =$ [ ]

Completed Computerized pH Worksheet

pH 5.00 buffer			
Preliminary Tests		Daily Tests	
Test #	Result	Test #	Result
1	4.95	1	5.00
2	4.95	2	4.92
3	4.95	3	4.98
4	5.02	4	4.92
5	5.03	5	4.98
6	4.97	6	4.94
7	4.99	7	5.00
8	5.00	8	4.96
9	4.94	9	4.98
10	4.99	10	4.98
11	5.01	11	4.98
12	5.02	12	5.01
13	5.02	13	4.98
14	5.07	14	5.03
15	5.00	15	4.96
16	5.02	16	
17	5.02	17	
18	5.05	18	
19	4.98	19	
20	4.94	20	
STATISTICS for Preliminary tests		21	
		22	
		23	
		24	
Std Deviation	0.04	25	
Mean	5.00	26	
UAL	5.11	27	
UWL	5.07	28	
LWL	4.92	29	
LAL	4.88	30	



pH control chart

APPENDIX C

WWTP LABORATORY QUALITY ASSURANCE CHECKLIST

		Yes	No	Comments
	General			
1.	Is Quality Assurance Manual up-to-date, available to all lab personnel?	_____	_____	_____
	Laboratory Procedures			
1.	Are EPA-approved methods (I.e., Standard Methods) used and readily available to all lab personnel?	_____	_____	_____
2.	Is calibration and maintenance of instruments/ equipment satisfactory?	_____	_____	_____
3.	Are QC procedures in the QA manual used consistently?	_____	_____	_____
4.	Are QC procedures in the QA manual adequate to evaluate whether or not the lab is in control?	_____	_____	_____
	Laboratory Facilities and Equipment			
1.	Is distilled or deionized water available (as required by the method)?	_____	_____	_____
2.	Is dry, uncontaminated, compressed air available?	_____	_____	_____
3.	Is the fume hood sufficiently ventilated?	_____	_____	_____
4.	Is the laboratory sufficiently lighted?	_____	_____	_____
5.	Are adequate electrical sources available in the lab	_____	_____	_____
6.	Are instruments/equipment in good condition?	_____	_____	_____
7.	Are trouble shooting procedures and written requirements for daily operation of instruments available to each instrument operator?	_____	_____	_____
8.	Are standards and blanks available to perform required QC checks?	_____	_____	_____
9.	Does a written schedule for required equipment maintenance exist?	_____	_____	_____
10.	Is proper volumetric glassware used?	_____	_____	_____
11.	Is glassware properly cleaned?	_____	_____	_____
12.	Are solvents and standard reagents properly stored?	_____	_____	_____
13.	Are calibration and check standards frequently checked?	_____	_____	_____
14.	Are standards discarded after recommended shelf-life has expired?	_____	_____	_____

15. Are blanks run each day for appropriate analysis (e.g., BOD, TSS, Chlorine residual)? _____ _____ _____

16. Are sufficient SOPs on hand for lab operations (E.G., cleanup, hazard response)? _____ _____ _____

17. Are gas cylinders replaced at 100-200 psi? _____ _____ _____

Laboratory's Precision, Accuracy, and Control Procedures

1. Are duplicates analyzed for all analyses and results recorded? _____ _____ _____

2. Are control samples required by the QA Manual introduced into the run of actual samples to ensure valid data are being generated? _____ _____ _____

3. Are control charts maintained and used routinely? _____ _____ _____

4. Is the lab within control (i.e., is precision good)? _____ _____ _____

Data Handling and Reporting

1. Are round-off rules documented and uniformly applied? _____ _____ _____

2. Are significant figures established for each analytical procedure? _____ _____ _____

3. Are results checked by at least one person other than the analyst? _____ _____ _____

4. Are correct formulas used to calculate final results? _____ _____ _____

5. Do report forms exist to provide complete data documentation and permanent records and to facilitate data processing? _____ _____ _____

6. Are data reported in proper form and units? _____ _____ _____

7. Are lab records maintained for a three-year period? _____ _____ _____

8. Is all data recorded in ink with corrections initialed? _____ _____ _____

9. Is a list of initials identifying to whom they belong filed in the lab? _____ _____ _____

10. Are Lab notebooks and pre-printed data forms bound permanently to provide good and defensible documentation? _____ _____ _____

11. Does an efficient filing system exist? _____ _____ _____

Laboratory Personnel

1. Are enough analysts present to perform necessary analyses? _____ _____ _____

2. Do analysts have on-hand necessary references for procedures being used? _____ _____ _____

3. Are analysts trained in procedures performed? _____ _____ _____

APPENDIX D

SUMMARY OF ANALYTICAL METHODS

1. pH (sm. #4500-H B)

pH is the numerical value of the acidity or basically of the tested solution. A value of 7.0 indicates the solution is neutral. Values less than 7.0 are acidic; greater than 7.0 are basic. Sudden changes in pH values may be the result of illegal discharges of acid or base into the wastewater system. Extreme shifts in pH may cause damage to the treatment facility and/or the biological treatment process.

This lab uses the electrometric method for pH measurements. The manufacturer's instructions are followed closely on use of the pH meter and on storage and preparation for use of the electrodes. Electrodes are kept wet by returning them to the storage solution whenever the pH meter is not being used (electrodes are never stored in distilled water). To prepare the electrodes for use, remove them from the storage solution, rinse with distilled water, and gently blot them dry with soft tissue. Bring both the sample and buffer solutions to room temperature, record the temperature, and adjust the temperature on pH meter to this temperature.

Calibrate the pH meter by immersing the electrodes in a buffer solution which is within 2 pH units of the expected sample pH, and setting the meter to read the pH of the buffer solution. Remove the electrodes, rinse and blot dry, and immerse in a second buffer which is approximately 3 pH units from the first buffer and which brackets the expected sample pH. If the meter reads more than 0.1 pH units from the value expected for the second buffer, look for trouble with the electrodes or potentiometer. Repeat this calibration procedure for every batch of pH analyses. Because samples normally analyzed in the WWTP lab are slightly basic, buffers normally maintained in stock are 7.00, 4.00 and 10.0.

When reading pH of the sample, establish equilibrium between the electrodes and the sample by swirling the sample to ensure homogeneity. Values are reported to the nearest 0.1pH units. Typical intra-laboratory tests result in standard deviations of ± 0.1 to ±0.2 pH units over the pH range.

2. Biological Oxygen Demand (SM # 5210 b)

This test measures the amount of organic material in a sample by measuring the oxygen consumed by microorganisms in biodegrading organic constituents in the sample. BOD is an important measure of the quality of discharged water as high BOD can result in undesirable effects on receiving waters. The test is part biological, and part chemical. The biological part cannot be calibrated, but chemical part which consists of measuring dissolved oxygen (DO) before and after an incubation period, can. DO is measured using a DO meter.

Samples analyzed in this lab generally are in the 6.5 to 7.5 pH range, normally have not been oxidized by ozone, are not extremely hot or cold, do not contain toxics, and do not contain supersaturated dissolved oxygen. Therefore, BWWTP samples normally do not require any special

pretreatment as described in standard methods. When residual chlorine is present, as it sometimes is, the dechlorination procedure in Standard Methods 5210 B paragraph 4e is followed prior to BOD analysis. Past experience has shown that samples often contain materials that are not degraded at normal rates by the microorganisms in settled sewage at the BWWTP. It is therefore desirable to seed BOD samples taken from the final settling tank effluent (as in paragraph 4d, Method 5210, Std Meth, ED. 20). Currently, the source of the seed is settled plant influent. Seed blanks are analyzed with each run to determine the seed factor.
If oxygen demand is expected to be greater than 8 mg/L (and it generally is in the BWWTP served by this lab), the sample must be diluted. Amount of sample added to a 300-ml bottle is determined from charts in the written method. Care must be taken in adding dilution water to avoid air bubbles that would contribute to the D.O. determination.

Tests are done on two or more dilutions, and each test includes a D.O. determination on bottles containing only dilution water. D.O. is measured at the beginning of the test and after a 5-day (± 4 hours) incubation period. Incubation is in the dark at 20±1 ºC (as measured with a certified thermometer). During periods when BOD may vary widely, it is advisable to prepare additional sets of dilution bottles, the second set containing half the amount of sample, and the third set containing double the amount of sample as determined from the dilution chart (Table D-1, below). This is to be sure there will be measurable DO at the end of the incubation period.

TABLE D-1
BOD DILUTIONS

Sample added to 300-ml BOD bottle	Expected BOD range Minimum	Maximum
3	210	560
6	105	280
9	70	187
12	53	140
15	42	112
18	35	94
21	30	80
24	26	70
27	24	62
30	21	56
45	14	37
60	11	28
75	8	22
150	4	12

3. Total Suspended Solids (SM # 2540 D.)

The measurement of suspended solids, or suspended matter, in wastewater at various stages in the treatment gives a good indication of the efficiency of treatment. Total suspended solids may be determined by filtering a sample through a glass fiber filter and drying the residual to a constant weight at 103-105 º C.

This lab uses a filter funnel attached to a vacuum pump. Other apparatus used in the determination include a drying oven, disposable aluminum dishes, an analytical balance, and a desiccator. The glass fiber filter is first dried at 103-105 º C for one hour, cooled in a desiccator to room temperature, and weighed. The sample (10 to 500 milliliters, depending on expected suspended matter content, which should be at least one, but preferably between 2.5 and 100 milligrams) is filtered through the glass fiber filter and dried again, desiccated, and weighed. The cycle of drying, cooling desiccating, and weighing is repeated until a constant weight is obtained or until the weight loss is less than 4% of the previous weight or 0.5 mg, whichever is less. Difference in the two weights is the amount of suspended solids in the sample.

From a standpoint of quality control, the analytical balance is the most important instrument although care must be taken to assure the drying oven is actually operating at 103-105 º C, and that the desiccator is free from moisture.

4. Chlorine Residual (SM # 4500-Cl D.)

Either a Hach DR 3900, or a Hach DR2000 spectrophotometer are used to determine free chlorine residual, total chlorine residual, and or combined chlorine residual.

5. Fecal Coliforms (SM #9222 D)

Fecal coliforms are found only in the gut and feces of warm blooded animals. Determination of fecals in wastewater is a principal indicator of sanitary quality of the water and the effectiveness of the treatment process. The LPWRF Quality Assurance Lab takes special care in following standard methods pertaining to condition of laboratory apparatus, washing and sterilization, approved reagents, materials, dilution water, and sampling.

Samples for fecal colifrom tests must be taken in sterile bottles. The bottles will be wide-mouthed, made of glass or plastic, of any suitable size and shape. Glass bottles are preferred but, if they are not available, polypropylene bottles of sufficient thickness that can be sterilized repeatedly may be used. Bottles and all other glassware are cleansed thoroughly in our laboratory steamscrubber dishwasher, and sterilized as per the latest edition of Standard Methods. Sterilization indicator tape is used on all autoclaved labware.

Diack brand wax sterilization ampules are used monthly to double check that complete sterilization has taken place. Sodium thiosulfate is added to each bottle before sterilization to neutralize any residual chlorine. Samples are analyzed immediately after collection, but, if necessary, may be stored at below 4º C and analyzed within six hours of receipt in the lab.

This lab uses a standard filtering apparatus with Gelman 47 mm membrane filters for the fecal test, disposable plastic culture dishes (w/pad), commercially prepared M-FC Broth (Gelman), and incubation conditions of 24 ± 2 hours at 44.5ºC ± 0.2ºC. A water bath providing a uniform 44.5ºC is used. When incubation is complete, fecal coliform cultures are removed from the incubator and counted immediately, but not later than 20 minutes. All partially or totally blue colonies are counted. Grey-to-cream colonies indicate nonfecal coliforms and are not counted.

Monthly, the lab splits a sample with commercial laboratory as an interlab check on the accuracy of the fecal coliform tests.

TOTAL COLIFORM:

Total coliform refers to a group of gram negative, non spore forming rod shaped bacteria that will ferment lactose broth at 35 degrees C in 24 to 48 hours.
These are widely distributed in nature. Total coliform generally refers to the genera Escherichia, Endobacter, Citrobacter and Klebsiella. All of these, except Eschericha, can exist as free living saprophytes in addition to being intestinal organisms.

Samples for Total colifrom tests must be taken in sterile bottles. The bottles will be wide-mouthed, made of glass or plastic, of any suitable size and shape. Glass bottles are preferred but, if they are not available, polypropylene bottles of sufficient thickness that can be sterilized repeatedly may be used. Bottles and all other glassware are cleansed thoroughly in our laboratory steamscrubber dishwasher, and sterilized as per the latest edition of Standard Methods. Sterilization indicator tape is used on all autoclaved labware. Diack brand wax sterilization ampules are used monthly to double check that complete sterilization has taken place. Sodium thiosulfate is added to each bottle before sterilization to neutralize any residual chlorine. Samples are analyzed immediately after collection, but, if necessary, may be stored at below 4º C and analyzed within six hours of receipt in the lab.

This lab uses a standard filtering apparatus with Gelman 47 mm membrane filters for the fecal test, disposable plastic culture dishes (w/pad), commercially prepared M-ENDO Broth (Gelman), and incubation conditions of 24 ± 2 hours at 35.0ºC ± 0.2ºC. A water bath providing a uniform 35.0ºC is used. When incubation is complete, total coliform cultures are removed from the incubator and counted immediately, but not later than 20 minutes. All metallic green colonies are counted.

APPENDIX E

CHAIN OF CUSTODY PROCEDURES

AS FOUND IN *STANDARD METHODS (#1060 B.2)*

Properly designed and executed chain of custody forms will ensure sample integrity from collection to data reporting. This includes the ability to trace possession and handling of the sample from the time of collection through analysis and final disposition. This process is referred to as " chain of custody " and is required to demonstrate sample control when the data are to be used for regulation or litigation. Where litigation is not involved, chain of custody procedures are useful for routine control of samples.

A sample is considered to be under a person's custody if it is in the individual's physical possession, in the individual's sight, secured and tamper-proofed by that individual, or secured in an area restricted to authorized personnel. The following procedures summarize the major aspects of chain of custody.

a. **Sample labels (including bar-coded labels): Use labels to prevent sample misidentification. Gummed paper labels or tags generally are adequate. Include at least the following information: a unique sample number, sample type, name of collector, date and time of collection, place of collection, and sample preservative. Also include date and time of preservation for comparison to date and time of collection. Affix tags or self-adhesive labels to the sample containers before, or at the time of, sample collection.**
b. **Sample seals: Use sample seals to detect unauthorized tampering with samples up to the time of analysis. Use self-adhesive paper seals that include at least the following information: sample number (identical with number on sample label), collector's name, and date and time of sampling. Plastic shrink seals also may be used.**
   Attach seal in such a way that it is necessary to break it to open the sample container or the sample shipping container (e.g., a cooler). Affix seal to container before sample leaves custody of sampling personnel.
   Include address of field contact; producer of material being sampled and address, if different from location; type of sample; and method, date, and time of preservation. If the sample is wastewater, identify process producing waste stream. Also provide suspected sample composition, including concentration; number and volume of sample(s) taken; description of sampling point and sampling method; date and time of collection; collector's sample identification number(s); sample distribution and how transported; references such as maps or photographs of the sampling site; field observations and measurements; and signatures of personnel responsible for observations. Because sampling situations vary widely, it is essential to record sufficient information so that one could reconstruct the

sampling event without reliance on the collector's memory. Protect logbook and keep it in a safe place.

c. **Chain of custody record: Fill out a chain of custody record to accompany each sample or group of samples. The record includes the following information: sample number; signature of collector; date, time and address or location of sample collection; sample type; sample preservation requirements; signatures of persons involved in the chain of possession; and inclusive dates and times of possession.**

d. **Sample analysis request sheet: The sample analysis request sheet accompanies samples to the laboratory. The collector completes the field portion of such a form that includes most of the pertinent information noted in the logbook. The laboratory portion of such a form is to be completed by laboratory personnel and include: name of person receiving the sample, laboratory sample number, date of sample receipt, condition of each sample (i.e., if it is cold or warm, whether the container is full or not, color, if more than one phase is present, etc.), and determinations to be performed.**

e. **Sample delivery to the laboratory: Deliver sample(s) to laboratory as soon as practicable after collection, typically within 2 days. Where shorter sample holding times are required, make special arrangements to insure timely delivery to the lavatory. Where samples are shipped by a commercial carrier, include the waybill number in the sample custody documentation. Insure that samples are accompanied by a complete chain of custody record and a sample analysis request sheet. Deliver sample to sample custodian.**

f. **Receipt and logging of sample: In the laboratory, the sample custodian inspects the condition and seal of the sample and reconciles label information and seal against the chain of custody record before the sample is accepted for analysis. After acceptance, the custodian assigns a laboratory number, logs sample in the laboratory log book and/or computerized laboratory information management system, and stores it in a secured storage room or cabinet or refrigerator at the specified temperature until it is assigned to an analyst.**

g. **Assignment of sample for analysis: The laboratory supervisor usually assigns the sample for analysis. Once the sample is in the laboratory, the supervisor or analyst is responsible for its care and custody.**

BLAINE WASTEWATER TREATMENT PLANT
Quality Control Laboratory

Laboratory Procedure # 9
Fecal Coliform

(Reference-Standard Methods 9222D)

Revised 1/8/10 by Christina Ness

PURPOSE

Sampling is a critical part of sanitary water testing. It is important that the sample accurately represents the body of the water studied. To assess the sanitary quality of water, three major groups of microorganisms are used as indicators of bacterial pollution. These are coliforms, fecal coliforms, and fecal streptococci. For the assessment of potable water acceptability, coliform analysis is the mandatory procedure accepted throughout North America and most countries in the world. The fecal coliform and fecal streptococci procedures are used for wastewater and other non-potable waters. The latter two are also used to indicate whether a source of bacterial contamination is from warm-blooded or cold-blooded animals.

DISCUSSION

The term "coliform" refers to a group of gram-negative, aerobic to facultative anaerobic, non-spore forming, rod-shaped bacteria that will ferment lactose at 35ºC in 24 to 48 hours.
They are widely distributed in nature and many are native to the gut of warm-blooded animals and man.

"Total Coliform" generally refers to the genera *Escherichia, Endobacter, Citrobacter, and Klebsiella.* All of these, except *Eschericha*, can exist as free-living saprophytes in addition to being intestinal organisms.

"Fecal Coliform" are primarily *Escherichia, and Klebsiella* spp. They are distinguished from total coliforms by having the ability to ferment lactose at elevated temperatures, as well as at 35ºC, the optimum for coliforms. The elevated temperature of 44.5ºC +/-0.2ºC has been shown to be the best temperature to select coliforms specifically of fecal origin. Any total coliform count may include fecal coliform organisms.

Fecal coliform analysis is a more definitive test for recent fecal pollution than the total coliform test, and fecal coliform is the standard test organism(s) used in many laboratories testing treated sewage, untreated public water supplies, and such primary contact waters as swimming areas.

It is a common assumption that all chlorinated effluents contain stressed or damaged fecal coliforms that cannot be recovered by the conventional membrane filter test using M-FC medium and incubation at 44.5ºC. These can vary from 10 to 90 % of the total coliform population.. The sodium thiosulfate added to the sample bottle cannot reverse this. This test gives results equivalent to the multiple-tube test with untreated effluents and most fresh surface waters, but the combination of high temperature and inhibitory ingredients in the medium is sufficient to prevent multiplication of organisms that have been damaged but not killed. Similar effects have been found in saline waters and in effluents containing heavy metals and toxic organic compounds, and they can be accentuated by excessive holding time and failure to cool samples that cannot be analyzed because it uses a more moderated incubation temperature.

EPA Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the clean Water Act (correction to CFR Part 136 dated January 4, 1985) states "Since the membrane filter technique usually yields low and variable recovery from chlorinated wastewater, the MPN method will be required to resolve any controversies."

The ideal solution, and the one of choice, is the use of liquid media in a multiple-tube system that starts out at close to normal body temperature. This test is the reference standard, and if there is any disagreement, multiple-tube will prevail over the membrane filter test. <u>This procedure should be used for chlorinated effluents if there is the possibility that data may be challenged in legal or enforcement actions.</u> With this method there is sample dilution in an enriched medium and damaged organisms are able to recover at a more suitable incubation temperature before being exposed to 44.5ºC. Unfortunately, the multiple-tube method is not practical for smaller laboratories such as ours.

The method that we recommend is a periodic comparison of membrane filter results with MPN. The testing may be done by either the EC or the A-1 method. A laboratory accredited by the Department of Ecology or certified by the Department of Health for this test is acceptable. In either case, it is very important that there be no more than 6 hours of sample transit time.

There are modified methods that are permitted for fecal coliforms in certain instances. These are listed in Standard Methods (17th ed.) 9212B, page 9-44. Briefly, they are:

1. M-FC agar overlaid with non-selective medium (lactose broth + 1.5 percent agar) immediately before use. Incubate 2 hours at 35ºC, then at 44.5ºC.
2. Incubate membrane 4 hours at 35ºC on pad with lactose or phenol red lactose broth, transfer membrane to M-FC agar, and incubate 22 hours at 44.5ºC.
3. Standard M-FC medium; incubate 5 hours at 35ºC, then 18 hours at 44.5ºC.
4. Use m-T7 medium, 8 hours at 37ºC. then 12 hours at 44.5º.

Standard Methods requires that with modifications such as these, not less than 10 % of the positive-appearing colonies be picked and verified. However, methods such as these

are more likely to produce false positives and the picking and verifying will indicate only the number of false positives. These are more sensitive tests and are acceptable without verification as long as the numbers if fecal coliforms are below the permitted levels. If numbers are above, verification may be necessary to determine whether there is a permit violation. Deletion of rosolic acid is also included in Standard Methods as such a modification, but its likelihood of producing false positives is much less than the four methods listed above.

There are several things that a lab can do to improve recovery of these "stressed" fecal coliforms from chlorinated effluents and still use the M-FC test. The simplest one is to use a type of membrane filter developed especially for this purpose. The Millipore type HC filters used in our lab are designed to retain organisms within the filter rather than upon the surface, helping to prevent evaporation and heat damage to chlorine-injured fecal coliforms during the critical first few hours at high temperature of the test.

Another simple technique is the elimination of rosolic acid from M-FC medium. Rosolic acid is added to suppress growth of background non-coliform organisms, but it also can suppress growth of injured fecal coliforms. This modification usually works well with most chlorinated effluents, but occasionally it can cause high numbers and even complete overgrowth of membranes by other organisms. Laboratories preparing the medium from the dehydrated form can simply omit the rosolic acid. For laboratories using M-FC in ampoules, it is a matter of choosing a supplier; Millipore's and Hach's contain rosolic acid, Gelman's does not. If samples other than chlorinated effluents are ran, rosolic acid will probably be needed.

Damage to organisms can result from substances other than chlorine. Samples containing heavy metals (copper, nickel, zinc, etc.) can be toxic to organisms, especially if there is a delay between sampling and analysis. Industrial wastes entering the system or combined sewers allowing entry of urban runoff are prime sources All sludges, also, would be expected to contain inhibitory levels of heavy metals. Some types of membranes themselves may absorb heavy metals from the samples and further inhibit recovery of fecal coliforms. With such samples it is necessary that EDTA be routinely added to sample bottles before sterilization.

In the presence of toxic metals, it is also recommended that 0.1 % peptone water (Standard Methods 17th ed., pages 9-31 or 16th ed., page 855) be used in place of standard buffered water for sample dilution and filter funnel rinsing. This can be difficult to work with because of its foaming tendency, but it does not reduce the effect of the toxicity.

Upsets in the biological, physical, or chemical treatment processes of a plant can cause large shifts in the quality of the effluent. Crowding and insufficient media to support full development of colonies can result if the bacterial concentrations on the filter exceeds the upper limit of the ideal range (20-60 colonies/100 ml). Consequently, there could be large swings in the final level of fecal coliform bacteria. This is frequently reflected in the membrane filter (MF) test when the standard dilution/filtration series used results in plates that have colonies that are "too numerous to count" (TNTC). If you obtain TNTC

counts on all plates for a a given sample run, use the listed procedure to determine the reporting value:

SAMPLING:

Three types of sample containers may be used. A wide-mouthed, 4 oz. (120 ml) borosilicate glass bottle with glass stopper of screw cap closure, or an autoclavable non-toxic polypropylene bottle of the same capacity is acceptable. A 4 oz. capacity, plastic "Whirl Pak" disposable bag is a third choice. Glass or polypropylene containers must be sterilized prior to use. If the sample to be tested contains residual chlorine, the appropriate amount of sodium thiosulfate dechlorination agent must be added to the bottle (or bag) prior to sample collection. This is usually .01 ml of a 10% solution of sodium thiosulfate for a 4-oz bottle, and is generally added prior to sterilization of the container. The closed glass or plastic containers should be capped with a sterile marker during storage and transit until opened for sampling.

When collecting samples, leave ample space (1" or 2.5 cm) in the bottle to facilitate mixing of the sample by shaking. Avoid contaminating the mouth of the bottle with hands of other non-sterile objects. Do not overfill or rinse sample bottles.

STORAGE AND PRESERVATION

Ideally, the sample should be processed within one hour after collection. When this is not possible, it should be iced or refrigerated below 10ºC (preferably between 1ºC and to 4ºC), then processed within six hours. If the latter is not possible, serious consideration should be given to field examination at the site of collection.

PREPARATION

1. Prepare m-FC broth as directed on the bottle of dehydrated media, or, preferably, obtain ampules containing 2.0 ml of ready to use broth. The ampules must be stored in the refrigerator until shelf life expires, at which point they are discarded. If the ampules contents color changes for any reason (lightens darkens or becomes cloudy), discard them.

2. Set water bath incubator temperature at 44.5ºC-± .2ºC. Make sure it will maintain this temperature, as it is critical to the test! The thermometer must be in water, not above it!
3. Choose sample filtration volumes which will produce 20-60 blue colonies upon incubation. If there is no previous coliform data, begin filtering 1 ml, 3 ml, and 10 ml portions of sample, respectively. When past coliform data is available, determine which sample plus a smaller or a larger volume to filter.

MAKING DILUTIONS

Where sample volumes require a range between 1 ml and 100 ml, it is frequently more convenient, time- saving, and less expensive to introduce the sample directly into the filtration funnel. First, filter about 20 to 30 ml of sterile phosphate buffer through the filtration unit funnel. Then, add the required sample volume into the filter funnel. Rinse the filter and funnel with about 30 ml of sterile buffer. Complete the necessary procedures called for in the analysis.

Accurate measurement of sample volumes less than 1.0 ml requires dilutions in sterile buffered water. The most convenient way is to sterilize 9.0 or 99.0 ml of buffer in dilution bottles. To make a 1:10 dilution, aseptically pipette 1.0 ml of sample into a 9.0-ml sterile buffer and mix thoroughly. Using 1.0 ml from this for filtration equals 0.1 ml of the original sample. Use 0.5 ml from this for filtration equals 0.5 ml of the original sample. To make a 1:100 ml dilution, either pipette 1.0 ml of sample from a 1:10 dilution into another 9.0 ml sterile buffer, or pipette 1.0 ml of undiluted sample into 99 ml sterile buffer and mix thoroughly. Remove 1.0 ml for filtration. Using 1.0 ml from this for filtration equals 0.01 ml of the original sample. By using this basic technique, any dilution is possible.

Diluted samples should result in fewer organisms on the membrane filter. For instance, if a 10-ml sample produces 200 fecal coliform colonies, a 1.0-ml sample should produce about 20 fecal coliform colonies

.

MEMBRANE FILTRATION PROCEDURE

1. Add 2 ml of broth to each Petri dish needed for analysis (typically a blank and three dilutions). Allow the broth to soak in, then pour out the excess, leaving only one extra drop when the dish is tilted.

2. Aseptically assemble sterile filter holder.

4. Dip forceps in alcohol to sterilize, then ignite forceps in a flame, burning off the alcohol (flame sterilize).

5. With sterile forceps, aseptically remove a sterile membrane filter from its package. Center the membrane, grid side up on the base of the filter funnel holder. Replace funnel top.

6. Shake sample vigorously (25 up and down motions in seven seconds).

7. Measure the selected sample volume and pour into filter funnel.

8. Apply vacuum to the filter. Try to limit vacuum to 15 inches of mercury.

9. When all of the sample has passed through the membrane filter, rinse the sides of the filter funnel with three successive 20-30 ml volumes of sterile phosphate buffer by adding each rinse of buffer while applying vacuum.

10. Using flame sterilized forceps, carefully remove the filter from the holder base. Transfer the filter to the pre- prepared petri dish. Use a rolling motion when laying the membrane filter on the absorbent pad so air is not trapped between them.

11. A buffer blank is run with each sample set. The filter in the blank determination is rinsed with buffer solution to assure that the buffer is not contaminated. In house duplicates are run weekly.

12. Occasionally run the blank after sample filtering. Note on Lab Sheet.

INCUBATION

1. Place petri dishes in a "Whirl-Pak" bag (or other suitable waterproof container), press out extra air, and fasten closed.

2. Submerge the "Whirl-Pak" (or other container) containing the petri dishes upside down in a water bath (temperature controlled to 44.5ºC, ±-0.2ºC) and weight it down to keep the contents completely submerged beneath the surface of the water (place the bag under the wire rack with a weight on it). Keep the bag opening above water to prevent flooding the Petri dishes.
3. Check the temperature of the waterbath with a certified thermometer immersed to its proper depth. Assure that water level is at immersion mark on thermometer. Record the temperature on the lab bench sheet along with sample volumes and time of filtration

4. Incubate under the above conditions for 24 hours (±2 hours).

CLEAN-UP

1. Remove equipment to sink or cleaning area.
2. Disinfect counter
3. Clean glassware and equipment before sterilization or reuse.

4. Wrap used petri dishes in paper towels and tape with sterile indicating tape for sterilization.

STERILIZATION

Autoclave wrapped used petri dishes and equipment needed for future testing at the same time.

1. Always use Sterility indicator tape on each sterilization run, and every other run, use a "Diack" sterility indicator.
   a. If you don't find a melted "Diack" from the previous autoclave run, go ahead and use a "Diack".
   b. If you find a "Diack" that has melted indicating proper sterilization, place a check mark in "Diack" column on the day it was found melted on lab worksheet as proof of sterilization. This indicates the used petri dishes from the last fecal analysis were sterile before discarding. Then discard used sterile Petri dishes.
   c. If either sterility indicator does not confirm sterility, do not perform fecal analysis or discard used petri dishes. Set up autoclave and resterilize.

2. Wrap disposable Petri dishes containing expended membrane filters in paper towels and tape with sterile indicating tape. Autoclave wrapped expended petri dishes. It is dangerous to delay autoclaving the expended petri dishes because they have the potential of spreading disease-causing organisms.
3. After insuring sterilization of the membrane filters (the indicating tape will have "sterile" showing), discard them.

COUNTING COLONIES

1. Following incubation, count all the blue colonies (all shades of blue) by sweeping back and forth along each row of the grid. Do not use higher magnification since these colonies are large enough to be seen with the naked eye. Higher magnification may cause you to count tiny particles that have been stained blue. Do not count gray colonies.
4. Record counts on the lab data sheet and retain these sheets for the files.

CALCULATING and REPORTING COUNTS

Calculation of Results: Count the number of colonies in each dish (up to a maximum of 200, then select the dish(es) with the number of colonies in the acceptable range and calculate count per 100 ml according to the general formula:

The acceptable range of colonies for Fecal coliform analysis is 20-60 colonies/100 ml.

The acceptable range of colonies for Total coliform analysis is 20-80 colonies/100 ml.

Colony counts or the limits cited above must be identified as outside of this range, either as estimated (E), or more than (>).

If there are no results because of confluency, lab accidents, etc.
Report as: No Result and specify reason in the plant logbook for reference and later entry in the monthly cause and remedy narrative submitted with the monthly reports..

7. Reporting Results: Report bacterial densities per 100 ml of sample

$$\text{Counts per 100 ml} = \frac{\text{number of colonies counted} \times 100}{\text{Volume of sample filtered, in ml}}$$

1. Counts with one dish within the acceptable limits

.

As an example, you found that filtration of volumes of 25, 50, and 75 ml produce fecal coliform colony counts of 19, 40, and 80 colonies respectively.
After selecting the membrane filter with an acceptable count (20-60 colonies for fecal coliforms), in this case the dish with the 40 colony count, the analyst calculate the number of colonies per 100 ml as follows:

$$\frac{40 \text{ colonies} \times 100}{50 \text{ ml sample}} = 80 \text{ colonies/ 100 ml}$$

Report as: 13 colonies per 100 ml.

2. All counts below lower limit.

If all MF counts are below the lower limit (less than 20), add the colony counts together. Then add the sample volumes together. Then divide the total number of colonies by the total sample volume.

For example, if the sample volumes of 25, 50, and 75 ml produced colony counts of over 3, 6 and 10, Calculate the number of colonies per 100 ml as follows:

$$\frac{3+6+10 \text{ colonies } 20{,}000}{25+50+75 \text{ ml of sample}} = \frac{19 \text{ colonies} \times 100}{150 \text{ ml}} = 12.66 \text{ colonies/ 100 ml}$$

Report as: 13 colonies per 100 ml. (Always round up to the next whole colony.)

3. More Than One Acceptable Count

If two or more plates have acceptable counts, independently carry counts to final reporting units as in example 1, then average the results for the final reported value.

For example, if the sample volumes of 25, 50, and 75 ml produced colony counts of over 20, 45 and 70, Calculate the number of colonies per 100 ml as follows:

$$\frac{20 \text{ colonies} \times 100}{25 \text{ ml of sample}} = 80 \text{colonies/ 100 ml}$$

$$\frac{45 \text{ colonies} \times 100}{50 \text{ ml of sample}} = 90 \text{colonies/ 100 ml}$$

$$\frac{80 \text{ colonies} + 90 \text{ colonies}}{2 \text{ acceptable counts}} = 85 \text{ colonies/ 100 ml}$$

Report as: 85 colonies per 100 ml.

The 70 ml sample would not be used as it is out of the ideal range and there are other counts in the desired range

4. Reporting colony counts between 60 and 200 colonies

If all counts over 60, but less than 200, take the plate with the smallest volume and report that value as being estimated.

For example, if the sample volumes of 25, 50, and 75 ml produced colony counts of over 69, 96 and 180, Calculate the number of colonies per 100 ml as follows:

$$\frac{69 \text{ colonies} \times 100}{25 \text{ ml of sample}} = 276 \text{ estimated colonies/ 100 ml}$$

Report as: E 276 colonies per 100 ml.

5. Reporting colony counts when no counts are in the 20-60 range with one or more TNTC

If no counts are in the 20-60 range and there is at least one count over 200 (TNTC), add the colony counts together. Then add the sample volumes together. Then divide the total number of colonies by the total sample volume.

For example, if the sample volumes of 25, 50, and 75 ml produced colony counts of over 18, 110 and 200, Calculate the number of colonies per 100 ml as follows:

$$\frac{18+110 \text{ colonies}}{25+50 \text{ ml of sample}} = \frac{128 \text{ colonies} \times 100}{75 \text{ ml}} = 170.66 \text{ colonies/ 100 ml}$$

Report as: 171 colonies per 100 ml. (Always round up to the next whole colony.)

6. Reporting too numerous to count (TNTC) results

Count the number of colonies on each plate to the best of your ability. If the number of fecal coliform colonies counted is greater than 200 on each of the plates, then assume the final count to be at least 200 organisms per plate. Calculate the approximate number of fecal coliform colonies using the count of 200 and the smallest filtration volume. Use the greater than (>) symbol on the approximated number reported for the test when recording the test results.

For example, if the sample volumes of 25, 50, and 100 ml produced colony counts of over 200 in all dilutions, Calculate the number of colonies per 100 ml that would have been reported if there had been 200 colonies on the filter representing the **smallest** filtration volume, thus:

$$\frac{200 \text{ colonies} \times 100}{25 \text{ ml sample}} = \frac{20{,}000}{25} = 800$$

Reported as: (More than) 800 colonies per 100 ml = >800.

Whatcom County
Fecal Coliform Monitoring Group

STANDARD OPERATING PROCEDURE

DIRECT GRAB SAMPLE COLLECTION WITH SAMPLE BOTTLE FOR FECAL COLIFORM ANALYSIS

* Adapted from City of Portland (Oregon), Environmental Protection Agency, and Washington State Department of Ecology SOPs

1.0 Purpose and Scope

This document is the Whatcom County Fecal Coliform Monitoring Group Standard Operating Procedure (SOP) for collecting grab samples directly into sample bottles or containers for the purposes of surface water and/or stormwater sampling and analysis.

2.0 Scope and Applicability

The procedures described in this document pertain to the proper collection of grab samples for laboratory analysis of fecal coliform and/or nutrients. Samples are intended to be collected from surface water and stormwater monitoring sites with direct access to the flow stream or sample medium. It is intended that this document pertain only to surface water and stormwater sampling sites that allow access to the entire flow stream and/or when sampling is done just beneath the water's surface (i.e. up to 12 inches beneath the water's surface). If the flow stream is well-mixed and the chemistry is relatively uniform, the methods described in this SOP are sufficient to represent the water body. The procedures in this SOP may also be used to collect bacteria samples such as E. coli, Enterococci, etc. Standard Methods contains analytical procedures for laboratory analysis.

3.0 Definitions

Fecal coliform- A group of bacteria that inhabit the intestinal tract of warm-blooded animals and remain viable in freshwater for a variable period of time. The presence of fecal coliform bacteria in surface water indicates fecal contamination by a warm-blooded animal; harmful bacteria and viruses associated with fecal contamination may also be present.

4.0 Equipment and Materials

The following is a list of equipment for collecting surface water and stormwater grab samples.

- Sterile, plastic sample bottles (provided by lab)
- Extension sampler pole and/or bridge sampler and sampling ropes
- Cooler containing ice
- Field data sheet or field log book
- Chain-of-Custody form
- Site files detailing sampling locations, sample site information, site identification codes, and past site visits
- Orange safety vest for sites accessed from roadways
- Anti-bacterial hand sanitizer
- Latex gloves (recommended)

4.1 Sample Containers

The typical bacteria sample containers are 125mL or 250mL pre-autoclaved polypropylene bottles. Masking tape with black lines sealing the bottle lid indicates the bottle was autoclaved. Pre-autoclaved

bottles should not be used if the tape is missing or black lines are not present. The laboratory may provide one-time use sterile plastic bottles. These bottles will have a label sealing the lid and should not be used if the seal has been broken.

When chlorine is suspected to be present in the sample water, bottles with sodium thiosulfate (thiosulfate) added should be requested from the laboratory. Thiosulfate will not affect samples if chlorine is not present.

5.0 Procedures

The following procedures relate to the direct collection of surface water and stormwater samples with the sample container itself.

5.1 Field Preparation

1. Gather necessary field/sampling documentation and site-specific information.
2. Reference the project checklist and/or field file to determine appropriate number of sample bottles and assemble sampling/safety equipment.
3. Obtain sample bottles from the certified laboratory that will analyze the samples. Care should be used at all times to avoid contamination of the inside of the sample bottle or bottle cap. The sample needs to be placed in ice in a cooler as soon as possible after collection. *Note: Non-drinking water bacteria samples have a maximum holding time of 24 hours (APHA 2000). The holding time goal is sample delivery to lab within 6 hours and sample processed within 2 hours for a total holding time of 8 hours.*
4. Identify the sites for field duplicates and/or lab duplicates on the field data sheet.

5.2 General Sampling Techniques

Bacteria sampling requires careful attention to sampling methods to avoid contamination of the bottle and water sample and ensure a representative sample is collected. The following guidance should be consistently considered:

1. Avoid contamination of the inside of the bottle cap and mouth. These should not be touched by hands or any other surface that may have bacteria exposure.
2. The bottle should not be rinsed or have water poured from another container that is not sterilized.
3. Avoid disturbing the sediment from the stream bed, particularly in slow moving waters.
4. Avoid sample collection from the surface layer (top inch of water column), near the streambank, and from eddies and side channels. In shallow depths, make notes on field form if a surface sample is unavoidable.
5. Avoid sample collection from stagnant waters (generally less than 0.1 ft/s) and eddies.
6. Collect samples from the active part of the stream where there is sufficient mixing to ensure the sample is representative.
7. If sample is collected from a boat, collect upstream of the boat's engine to avoid hydrocarbon contamination.

5.3 Sample Collection

<u>Hand Dip Method:</u> This method is typically used to collect samples within reach of the water surface (when standing in or near the stream or from a small boat).

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Move to a well mixed location, such as the deepest part of the active channel or another location where a representative sample may be collected. Do not contaminate the sample location by wading upstream of it. *Note: Use the Extension Pole Method if sampling from a lake or wide stream.*

3. Hold the base of the sample bottle with one hand and remove the bottle cap. Invert the bottle, reach upstream, and submerge the bottle into the water about 6 inches, and then tip the bottle mouth upstream and toward the water surface. Allow the bottle to fill to approximately the shoulder and take it out of the water. If the bottle is overfilled, immediately dump some water from the bottle. *Note: In shallow surface water, ensure that the sample bottle does not touch or disturb the stream bed, potentially contaminating the sample. Submerge the sample bottle to approximately the midpoint of the water column and tip upwards toward the direction of the flow. Samples should be collected far enough below the surface to avoid contamination from surface film and detritus. If a surface sample is unavoidable, note this on the field data sheet.*
4. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

<u>Extension Pole Method:</u> This method is typically used to reach a more representative or undisturbed sample location from the stream bank, or when sampling a lake or slow moving stream.

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Secure the sample bottle in the extension pole clamp.
3. Move to a location where a representative sample can be reached with the pole.
4. Remove the bottle cap avoiding contamination of the cap or inside of the bottle.
5. Position the bottle over the desired sampling location.
5. Invert the bottle and in one quick motion submerge the mouth of the bottle into the water column to a depth of approximately six inches. Slowly move the bottle upstream with the bottle mouth tipped toward the surface until the bottle fills to the bottle shoulder. For lake sampling, slowly move the tipped bottle away from the bottle entry point until it fills. If the bottle is overfilled, immediately dump some water from the bottle. *Note: In shallow surface water, ensure that the sample bottle does not touch or disturb the stream bed, potentially contaminating the sample. Submerge the sample bottle to approximately the midpoint of the water column and tip upwards toward the direction of the flow. Samples should be collected far enough below the surface to avoid contamination from surface film and detritus. If a surface sample is unavoidable, note this on the field data sheet.*
6. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

<u>Hand Collection from Pipe Method:</u> This method is typically used to collect samples within reach of the end of a stormwater pipe. A sample of stormwater discharge should be taken as a single uninterrupted event (i.e., grabbed at one time) from a single stormwater outfall.

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Move to the end of a stormwater pipe where there is moderate flow with turbulence, if possible, so the stormwater discharge will be well-mixed and representative. When sampling a stormwater system, samples should be sampled from the system discharge point first to ensure samples are not contaminated by upstream sampling.
3. Hold the base of the sample bottle with one hand and remove the bottle cap. Hold the bottle under the stormwater discharge with its opening positioned into the flow of water so that water enters directly into the bottle without flowing over the bottle or hands during sampling to prevent contaminating the sample.
4. Allow the bottle to fill to approximately the shoulder and take it out of the water. If the bottle is overfilled, immediately dump some water from the bottle. *Note: Ensure that the sample bottle does not touch the outfall pipe, potentially contaminating the sample.*
5. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

Bridge Sampling Method: This method is typically used to collect samples when standing on a bridge or boat.

1. Label the bottle with sample site ID, date, and time of sample collection prior to collecting the sample.
2. Secure the sample bottle in the bridge sampler and attach the sampling rope.
3. Move to a location where a representative sample can be reached with the sampler.
4. Remove the bottle cap avoiding contamination of the cap or inside of the bottle. Hold the cap with your free hand or set the cap upside down on a surface to avoid contamination of the inside of the cap (e.g. road, bridge, or clipboard).
5. Position the bottle and sampler over the desired sampling location. Lower the sampler to the water surface and allow the bottom of the sampler to touch the water surface to remove any debris from the bottom of the bottle and sampler. Raise the sampler off the water surface to allow debris to wash downstream. *Note: Ensure debris is not dislodged from the bridge while lowering the sampler. This step is intended to prevent sample contamination from any debris attached to the sampler.*
6. Without submerging the mouth of the bottle, lower the sampler into the water and allow the current to position the sampler so the bottle is on the upstream side. Rapidly lower the sampler so the mouth of the bottle to a depth of approximately 6 inches. The rapid motion is intended to minimize collection of the surface film. If the bottle is overfilled, immediately dump some water from the bottle. *Note: In shallow surface water, ensure that the sampler does not touch or disturb the stream bed, potentially contaminating the sample. If a surface sample is unavoidable, note this on the field data sheet*.
7. Replace the cap securely, avoiding contamination to the inside of the bottle or cap.

5.4 Field Processing

1. Ensure the bottle label matches the desired sample site and includes the correct date and time.
2. Place the sample bottle in cooler.
3. Record the sample site ID, data, time of sample collection on the field data sheet and *Chain of Custody* form. Other notes on conditions of the sampling sites, adjacent land activities, or sample collection methods should be recorded on the field data sheet.
4. If the range of bacteria concentration can be estimated before sampling, let the lab microbiologist know so that a set of dilutions that bracket the expected concentration range can be prepared.

6.0 Records Management

Field sheets, chain of custody forms, and field/laboratory results will be stored in project files at Whatcom County Public Works- Natural Resources. After approximately six years, the documents will be boxed and archived. Laboratory results will be reviewed and verified. Data will be entered into an Excel spreadsheet for the project and saved on the Whatcom County network.

7.0 Quality Control and Quality Assurance

QA/QC procedures will be addressed thoroughly on a project-by-project basis in the Quality Assurance Project Plan (QAPP) or monitoring plan. General QA/QC procedures will include field notes, sample identification, representative sampling, field duplicates, comparability, and completeness. Personnel collecting water samples will have received training on the sampling protocol.

Data quality control consists of two elements: 1) Sample collection, handling, and storage will be conducted in a manner consistent with the methods outlined in the SOP and 2) field quality control samples will be collected with each sampling run. The field duplicate is a replicated sample collected immediately after the routine sample at the same location. One field duplicate (field quality control sample) will be collected in a side-by-side manner, per every “set” of samples to assess field

YSI **Environmental**

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YSI 556 MPS

Multi Probe System

Operations Manual

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1. Safety

1.1 General Safety Information

Read all safety information in this manual carefully before using the YSI 556 Multi-Probe System (MPS). Reagents that are used to calibrate and check this instrument may be hazardous to your health. Take a moment to review *Appendix D Health and Safety*.

⚠ **WARNING**

Warnings are used in this manual when misuse of the instrument could result in death or serious injury to a person.

⚠ **CAUTION**

Cautions are used in this manual when misuse of the instrument could result in mild or serious injury to a person and/or damage to equipment.

⚠ **IMPORTANT SAFETY INSTRUCTIONS!**

⚠ **SAVE THESE INSTRUCTIONS!**

⚠ In essence, the most important safety rule for use of the YSI 556 MPS is to utilize the instrument ONLY for purposes documented in this manual. This is particularly true of the YSI 6117 rechargeable battery pack that contains nickel metal hydride (NiMH) batteries. The user should be certain to read all of the safety precautions outlined below before using the instrument.

⚠ YSI 6117 Rechargeable Battery Pack Safety Information

⚠ **Restrictions on Usage**

1. Never dispose of the battery pack in a fire.
2. Do not attempt to disassemble the YSI 6117 battery pack.
3. Do not tamper with any of the electronic components or the batteries within the battery pack. Tampering with either the electronic circuitry or the batteries will result in the voiding of the warranty and the compromising of the system performance, but, more importantly, can cause safety

hazards which result from overcharging such as overheating, venting of gas, and loss of corrosive electrolyte.

4. Do not charge the battery pack outside the 0–40°C temperature range.
5. Do not use or store the battery at high temperature, such as in strong direct sunlight, in cars during hot weather, or directly in front of heaters.
6. Do not expose the battery pack to water or allow the terminals to become damp.
7. Avoid striking or dropping the battery pack. If the pack appears to have sustained damage from these actions or malfunctions after an impact or drop, the user should not attempt to repair the unit. Instead, contact YSI Customer Service. Refer to *Appendix E Customer Service*.
8. If the battery pack is removed from the YSI 556 MPS, do not store it in pockets or packaging where metallic objects such as keys can short between the positive and negative terminals.

⚠ Precautions for Users with Small Children

Keep the battery pack out of reach of babies and small children.

⚠ Danger Notifications – Misuse creates a STRONG possibility of death or serious injury.

FAILURE TO CAREFULLY OBSERVE THE FOLLOWING PROCEDURES AND PRECAUTIONS CAN RESULT IN LEAKAGE OF BATTERY FLUID, HEAT GENERATION, BURSTING, AND SERIOUS PERSONAL INJURY.

1. Never dispose of the battery pack in a fire or heat it.
2. Never allow the positive and negative terminals of the battery pack to become shorted or connected with electrically conductive materials. When the battery pack has been removed from the YSI 556 MPS, store it in a heavy plastic bag to prevent accidental shorting of the terminals.

3. Never disassemble the battery pack and do not tamper with any of the electronic components or the batteries within the battery pack. The battery pack is equipped with a variety of safety features. Accidental deactivation of any of these safety features can cause a serious hazard to the user.
4. The NiMH batteries in the battery pack contain a strong alkaline solution (electrolyte). The alkaline solution is extremely corrosive and will cause damage to skin or other tissues. If any fluid from the battery pack comes in contact with a user's eyes, immediately flush with clean water and consult a physician immediately. The alkaline solution can damage eyes and lead to permanent loss of eyesight.

⚠ Warning Notifications – Misuse creates a possibility of death or serious injury

1. Do not allow the battery pack to contact freshwater, seawater, or other oxidizing reagents that might cause rust and result in heat generation. If a battery becomes rusted, the gas release vent may no longer operate and this failure can result in bursting.
2. If electrolyte from the battery pack contacts the skin or clothing, thoroughly wash the area immediately with clean water. The battery fluid can irritate the skin.

⚠ Caution Notifications – Misuse creates a possibility of mild or serious injury or damage to the equipment.

1. Do not strike or drop the battery pack. If any impact damage to the battery pack is suspected, contact YSI Customer Service. Refer to *Appendix E Customer Service*.
2. Store the battery pack out of reach of babies and small children.
3. Store the battery pack between the temperatures of -20 and 30°C.
4. Before using the battery pack, be sure to read the operation manual and all precautions carefully. Then store this information carefully to use as a reference when the need arises.

⚠ YSI 616 Cigarette Lighter Charger Safety Information

1. This section contains important safety and operating instructions for the YSI 556 MPS cigarette lighter battery charger (YSI 616; RadioShack Number 270-1533E). BE SURE TO SAVE THESE INSTRUCTIONS.
2. Before using the YSI 616 cigarette lighter charger, read all instructions and cautionary markings on battery charger, battery pack, and YSI 556 MPS.
3. Charge the YSI 6117 battery pack with the YSI 616 cigarette lighter charger ONLY when the YSI 6117 is installed in the YSI 556 MPS.
4. Do not expose charger to rain, moisture, or snow.
5. Use of an attachment not recommended or sold by the battery charger manufacturer may result in a risk of fire, electric shock, or injury to persons.
6. To reduce risk of damage to cigarette lighter and cord, pull by cigarette lighter rather than cord when disconnecting charger.
7. Make sure that the cord is located so that it will not be stepped on, tripped over, or otherwise subjected to damage or stress.
8. Do not operate charger with damaged cord or cigarette lighter connector – replace it immediately.
9. Do not operate charger if it has received a sharp blow, been dropped, or otherwise damaged in any way; contact YSI Customer Service. Refer to *Appendix E Customer Service*.
10. Do not disassemble charger other than to change the fuse as instructed. Replace the part or send it to YSI Product Service if repair is required (refer to *Appendix E Customer Service*). Incorrect reassembly may result in a risk of electric shock or fire.
11. To reduce risk of electric shock, unplug charger before attempting any maintenance or cleaning. Turning off controls will not reduce this risk.

⚠ YSI 556 MPS Water Leakage Safety Information

The YSI 556 MPS has been tested and shown to comply with IP67 criterion, i.e. submersion in 1 meter of water for 30 minutes with no leakage into either the battery compartment or the main case. However, if the instrument is submersed for periods of time in excess of 30 minutes, leakage may occur with subsequent damage to the batteries, the rechargeable battery pack circuitry, and/or the electronics in the main case.

If leakage into the battery compartment is observed when using alkaline C cells, remove batteries, dispose of batteries properly, and dry the battery compartment completely, ideally using compressed air. If corrosion is present on the battery terminals, contact YSI Customer Service for instructions. Refer to *Appendix E Customer Service*.

If leakage into the battery compartment is observed when using the YSI 6117 rechargeable battery pack, remove the battery assembly and set aside to dry. Return the battery pack to YSI Product Service for evaluation of possible damage. Finally dry the battery compartment completely, ideally using compressed air. If corrosion is present on the battery terminals, contact YSI Customer Service for instructions. Refer to *Appendix E Customer Service*.

⚠ **CAUTION:** If water has contacted the rechargeable battery pack, do not attempt to reuse it until it has been evaluated by YSI Product Service (refer to *Appendix E Customer Service*). Failure to follow this precaution can result in serious injury to the user.

If it is suspected that leakage into the main cavity of the case has occurred, remove the batteries immediately and return the instrument to YSI Product Service for damage assessment. Refer to *Appendix E Customer Service*.

⚠ **CAUTION:** Under no circumstances should the user attempt to open the main case.

2. General Information

2.1 Description

The rugged and reliable YSI 556 MPS (Multi-Probe System) combines the versatility of an easy-to-use, easy-to-read handheld unit with all the functionality of a multi-parameter system. Featuring a waterproof, impact-resistant case, the YSI 556 MPS simultaneously measures dissolved oxygen, conductivity, temperature, and optional pH and ORP. A simple cellular phone style keypad and large display make the instrument easy to use. The YSI 556 MPS is compatible with YSI EcoWatch™ for Windows™ software.

The YSI 556 MPS assists the user in conforming to Good Laboratory Practice (GLP) standards which help ensure that quality control/quality assurance methods are followed. Battery life is displayed with a fuel gauge, and the user can choose standard alkaline batteries or an optional rechargeable battery pack.

The 1.5 MB memory can store more than 49,000 data sets. Other options include a flow cell and barometer. The internal barometer can be user-calibrated and displayed along with other data, used in dissolved oxygen calibrations, and logged to memory for tracking changes in barometric pressure.

Features

- Waterproof - meets IP67 specifications
- Field-replaceable DO electrode module; pH and pH/ORP sensors
- Compatible with Ecowatch™ for Windows™ data analysis software
- Assists with Good Laboratory Practice Standards (GLP)
- Choice of DO membrane material for different applications
- Easy-to-use, screw-on cap DO membranes
- User-upgradable software from YSI website
- Three-year warranty on the instrument; one-year on the probe modules
- Available with 4,10, and 20 m cable lengths
- Stores over 49,000 data sets, time and date stamped

- Auto temperature compensating display contrast
- Optional barometer
- Optional rechargeable battery pack or standard alkaline batteries

2.2 Unpacking the Instrument

1. Remove the instrument from the shipping box. Note that the probe module and sensors are shipped in a separate box and will be unpacked later in Section *3.2 Unpacking the Probe Module*.

   NOTE: Do not discard any parts or supplies.

2. Use the packing list to ensure all items are present.

3. Visually inspect all components for damage.

   NOTE: If any parts are missing or damaged, contact your YSI Service Center immediately. Refer to *Appendix E Customer Service* or www.ysi.com.

2.3 Features of the YSI 556 Multi-Probe System

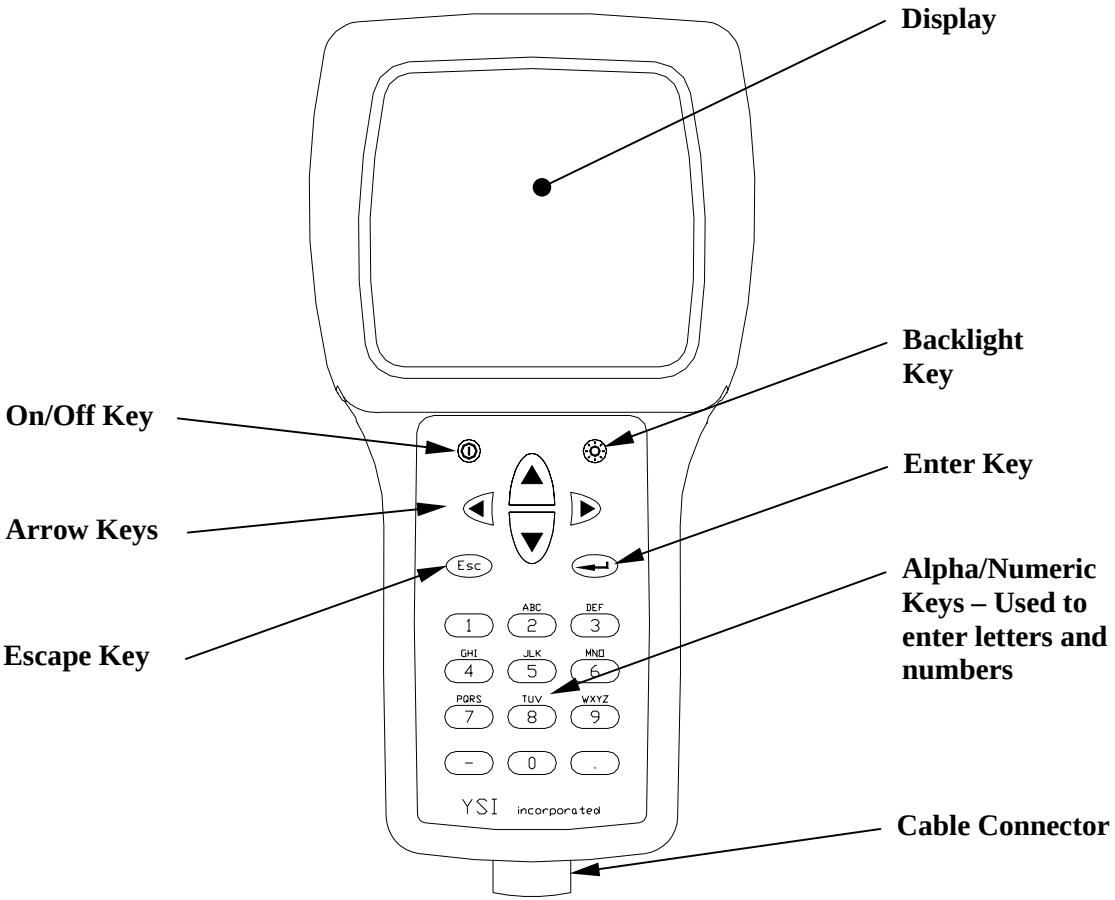


Figure 2.1 Front View of YSI 556 MPS

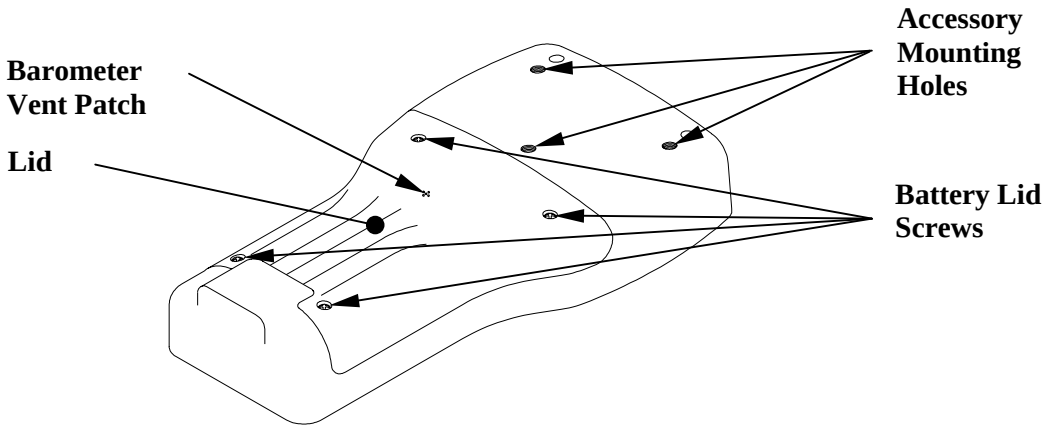


Figure 2.2 Back View of YSI 556 MPS

2.4 Batteries

2.4.1 Battery Life

Standard Alkaline Batteries

With the standard battery configuration of 4 alkaline C cells, the YSI 556 MPS will operate continuously for approximately 180 hours. Assuming a standard usage pattern when sampling of 3 hours of “on time” in a typical day, the alkaline cells will last approximately 60 days.

Optional Rechargeable Battery Pack

When fully charged, the optional rechargeable battery pack will provide approximately 50 hours of battery life.

2.4.2 Inserting 4 C Batteries

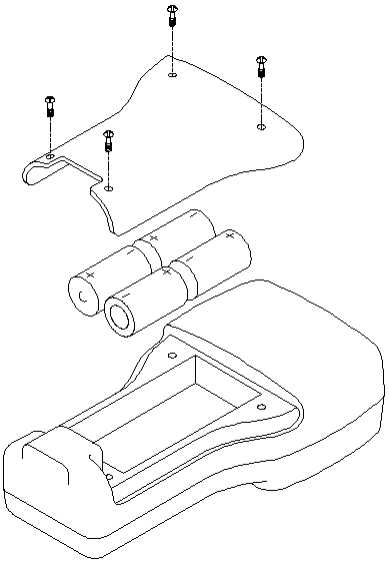


Figure 2.3 Inserting C Cells

⚠ **CAUTION: Install batteries properly to avoid damage to the instrument.**

1. Loosen the four screws in the battery lid on the back of the instrument using any screwdriver.

2. Remove the battery lid.

3. Insert four C batteries between the clips following the polarity (+ and -) labels on the bottom of the battery compartment.

4. Check gasket for proper placement on the battery lid.

5. Replace the battery lid and tighten the 4 screws securely and evenly.

   NOTE: Do not over-tighten the screws.

2.4.3 Inserting Optional Rechargeable Battery Pack

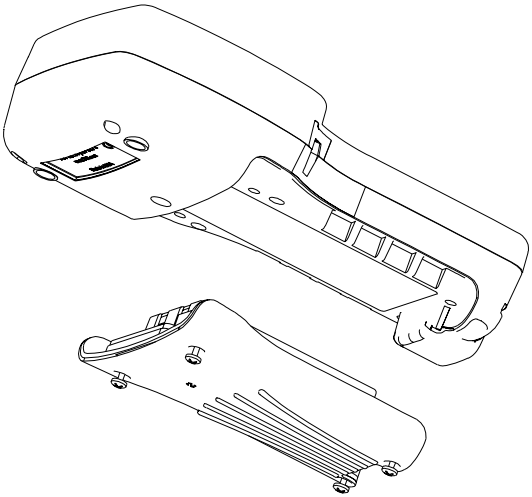


Figure 2.4 Inserting Battery Pack

⚠ **CAUTION: Read all cautions and warnings that come with the battery pack *before* using the battery pack.**

1. Loosen the four screws in the battery lid on the back of the instrument using any screwdriver.

2. Remove the C battery lid and store for future use. Remove C batteries, if installed.

3. Check for proper placement of gasket on the rechargeable battery pack and lid.

4. Install the rechargeable battery pack and lid and tighten the 4 screws securely and evenly.

   NOTE: Do not over tighten the screws.

2.4.4 Charging the Optional Rechargeable Battery Pack

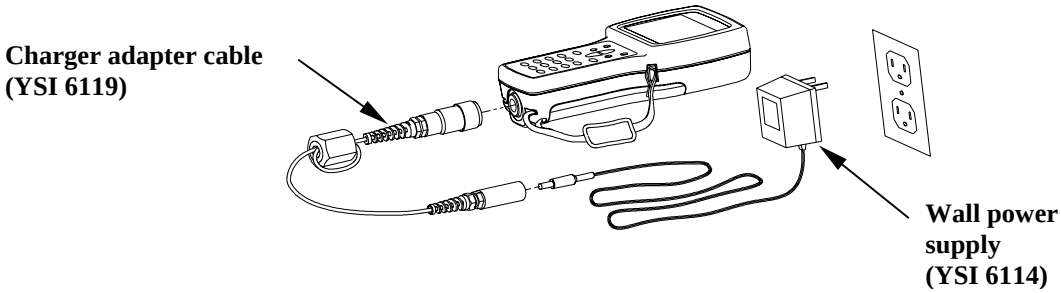


Figure 2.5 Charging the Battery Pack

⚠ **CAUTION: Do not use or store the battery pack at extreme temperatures such as in strong direct sunlight, in cars during hot weather or close to heaters.**

1. Install the rechargeable battery pack into the instrument as described in Section *2.4.3 Inserting Optional Rechargeable Battery Pack*.

2. Attach the charger adapter cable (YSI 6119) to the instrument.

   NOTE: Wall power supplies for use in countries outside the US and Canada can be found in *Appendix B Instrument Accessories*.

3. Insert the barrel connector of the wall power supply into the barrel of the adapter cable.

⚠ **CAUTION: Do not charge the battery pack continuously for more than 48 hours.**

⚠ **CAUTION: Do not drop or expose to water.**

⚠ **CAUTION: Do not charge the battery pack at temperatures below 0°C or above 40°C.**

4. Plug the wall power supply into an AC power outlet for approximately 2 hours to obtain an 80% to 90% charge and for 6 hours to get a full charge.

   NOTE: The battery pack can be recharged whether the instrument is on or off.

2.4.5 Storing the Battery Pack

Remove the battery pack from the instrument when the instrument will not be used for extended periods of time to prevent over discharge of the battery pack.

Store the battery pack in a heavy plastic bag to prevent accidental shorting of the terminals. Store between –20 and 30°C.

2.4.6 Optional Cigarette Lighter Charger

⚠ **CAUTION: Read all warnings and cautions that come with the charger before using the charger.**

⚠ **CAUTION: Only use cigarette lighter charger when *rechargeable* battery pack is inserted into instrument.**

⚠ **CAUTION: Do not mishandle cigarette lighter charger. Do not expose to moisture.**

1. Plug the barrel connector of the cigarette lighter charger into the mating end of the YSI 6119 Charger Adapter Cable.

2. Attach the MS-19 end of the YSI 6119 Charger Adapter Cable to the instrument.

3. Make one of the following modifications to the other end of the charger:

   Slide the adapter ring off the plug to use the device with an American or Japanese vehicle.

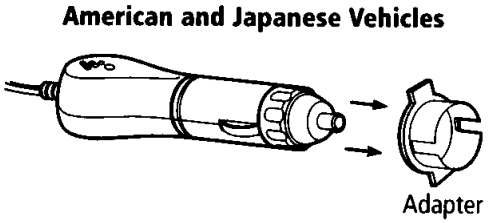


Figure 2.6 Charger Plug Adapter Use

Leave the adapter ring on the plug and position it so that the slots on the adapter ring line up with the plug's spring clips to use the device on a European vehicle.

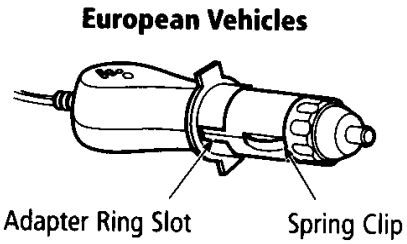


Figure 2.7 European Charger Plug Adapter Use

NOTE: If the charger stops working properly, refer to Section *13 Troubleshooting*.

2.5 Power On

Press and release the on/off button in the upper left corner of the instrument keypad to turn the instrument on or off. See Figure 2.1 Front View of YSI 556 MPS.

2.6 Setting Display Contrast

The display contrast automatically compensates for temperature changes. However, under extreme temperature conditions you may wish to optimize the display by manual adjustment as follows:

1. Press and *hold down* the backlight key in the upper right corner of the keypad and press the “up” arrow to increase (darken) the contrast.

2. Press and *hold down* the backlight key in the upper right corner of the keypad and press the “down” arrow to decrease (lighten) the contrast.

2.7 Backlight

Press and *release* the backlight key in the upper right corner of the keypad to turn the backlight on or off. See Figure 2.1 Front View of YSI 556 MPS.

NOTE: The backlight turns off automatically after two minutes of non-use.

2.8 General Screen Features

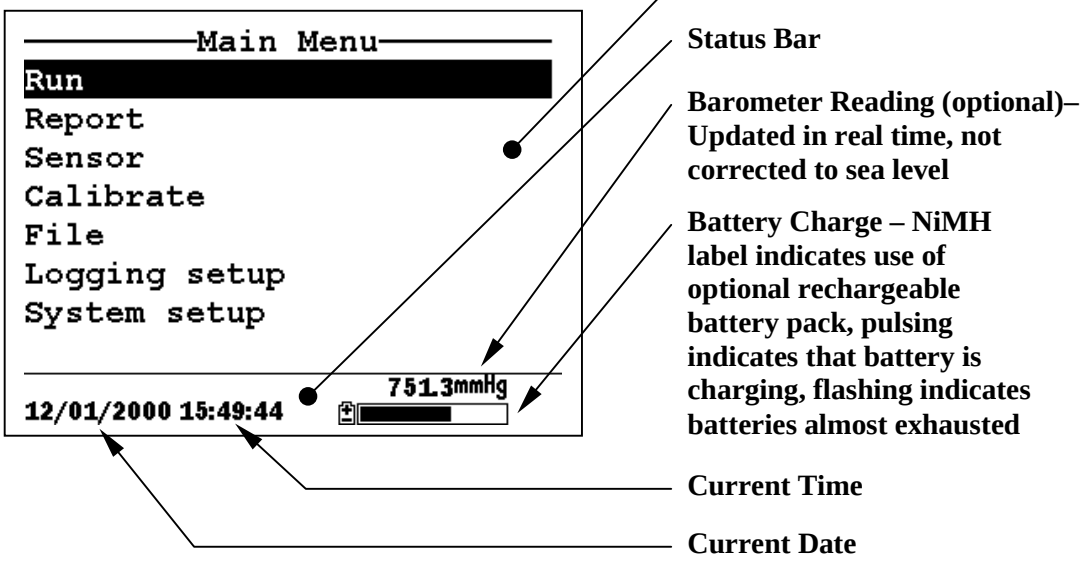


Figure 2.8 Main Menu Screen

2.9 Keypad Use

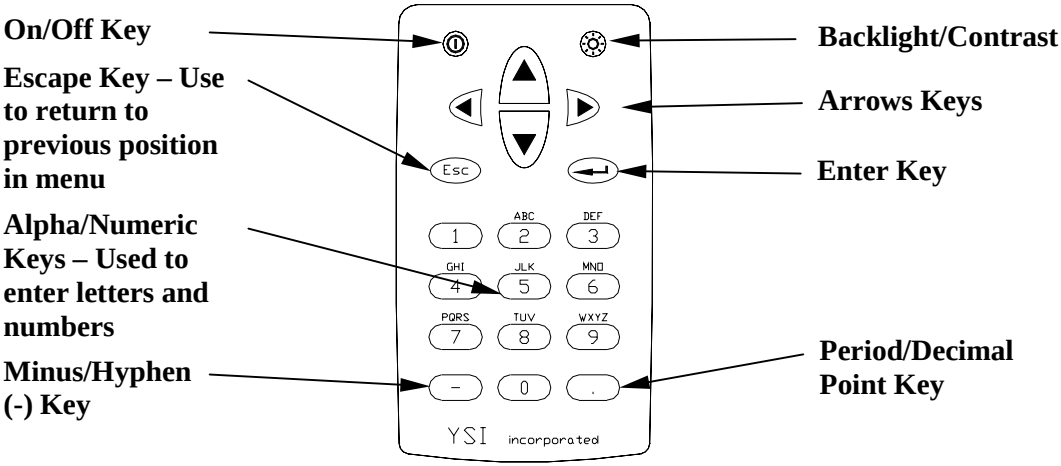


Figure 2.9 Keypad Features

KEY	LETTER/ NUMBER
1	1
2	ABC2abc3
3	DEF3def3
4	GHI4ghi4
5	JKL5jkl5
6	MNO6mno6
7	PQRS7pqrs7
8	TUV8tuv8
9	WXYZ9wxyz9
0	0

Figure 2.10 Keypad Letters & Numbers

1. See Figure 2.10 Keypad Letters & Numbers and press the appropriate key repeatedly until letter or number desired appears in display.

NOTE: Press the key repeatedly in rapid succession to get to the desired letter or number. If you pause for more than a

second, the cursor automatically scrolls to the right to prepare for the next input.

EXAMPLE 1: Press the **6** key *once* and *release* to display an uppercase “M.”

EXAMPLE 2: Press the **6** key *four times* and *release* to display the number “6.”

EXAMPLE 3: Press the **6** key *five times* and *stop* to display a lowercase “m.”

2. Press the left arrow key to go back and reenter a number or letter that needs to be changed.

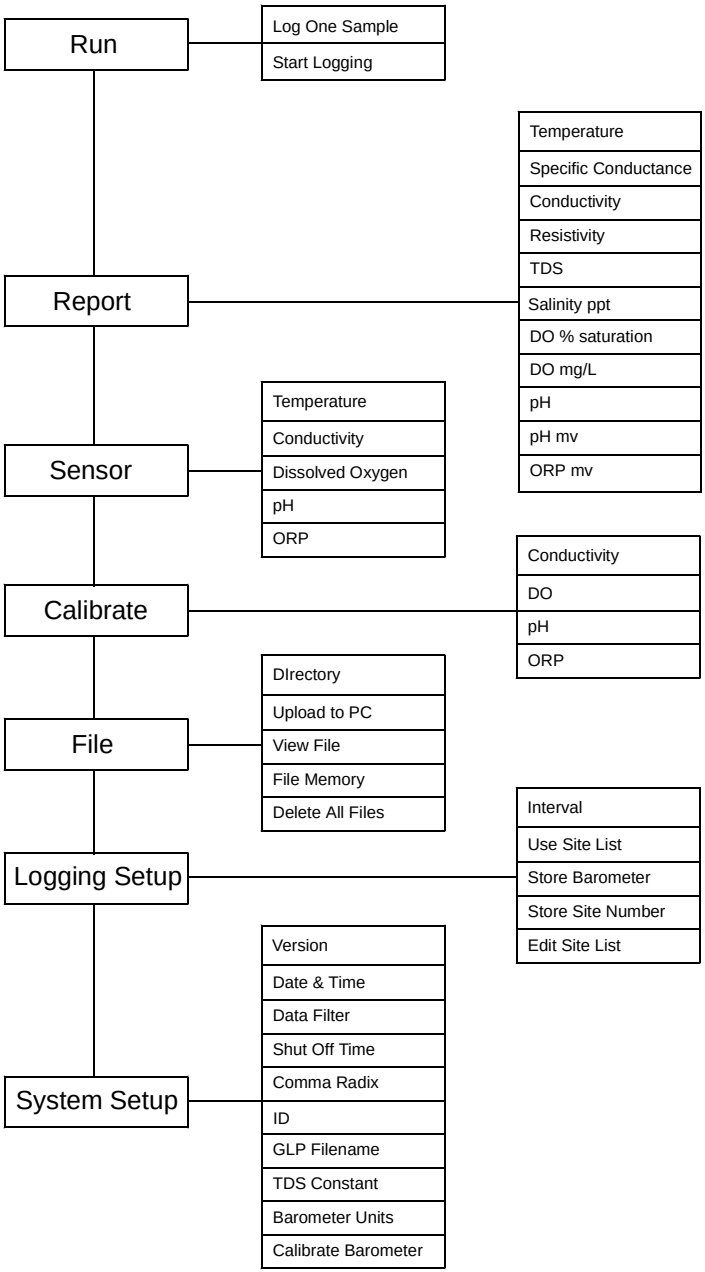
3. Press the **Enter** key when your entry is complete.

   NOTE: The instrument software permits only numeric entries in many instances, such as when setting the clock or entering calibration parameters.

2.10 Instrument Reset

The YSI 556 MPS is characterized by sophisticated software that should provided trouble-free operation. However as with all high-capability software packages, it is always possible that the user will encounter circumstances in which the instrument does not respond to keypad entry. If this occurs, the instrument function can easily be restored by removing and then reapplying battery power. Simply remove either your C-cells or rechargeable battery pack from the battery compartment, wait 30 seconds and then replace the batteries. See Section *2.4 Batteries* for battery removal/reinstallation instructions.

2.11 Menu Flowchart



3. Probe Module

3.1 Introduction

The YSI 5563 Probe module is used for measuring dissolved oxygen, temperature, conductivity, and optional pH and ORP. The probe module is rugged, with the sensors enclosed in a heavy duty probe sensor guard with attached sinking weight. A 4, 10 or 20 meter cable is directly connected to the probe module body making it waterproof. An MS-19 connector at the end of the cable makes the YSI 5563 fully compatible with the YSI 556 Multi-Probe System.

3.2 Unpacking the Probe Module

1. Remove the YSI 5563 Probe module from the shipping boxes.

   NOTE: Do not discard any parts or supplies.

2. Use the packing list to ensure all items are present.

3. Visually inspect all components for damage.

   NOTE: If any parts are missing or damaged, contact your YSI Service Center immediately. Refer to *Appendix E Customer Service* or www.ysi.com.

3.3 Features of the YSI 5563 Probe Module

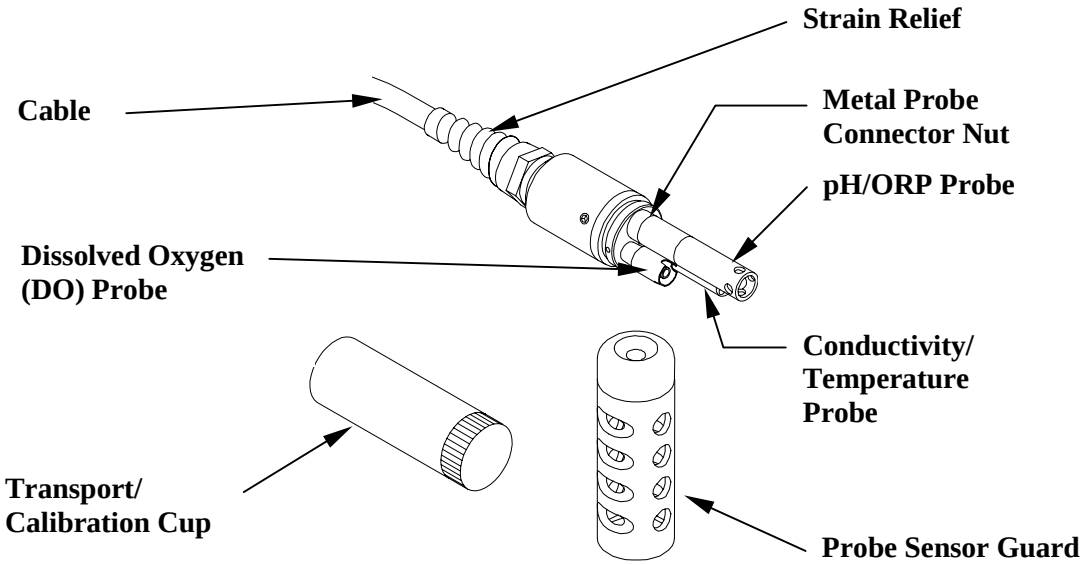


Figure 3.1 Probe Module

3.4 Preparing the Probe Module

To prepare the probe module for calibration and operation, you need to install the sensors into the connectors on the probe module bulkhead. In addition to sensor installation, you need to install a new DO membrane cap.

3.4.1 Sensor Installation

Whenever you install, remove or replace a sensor, it is extremely important that the entire probe module and all sensors be thoroughly dried prior to the removal of a sensor or a sensor port plug. This will prevent water from entering the port. Once you remove a sensor or plug, examine the connector inside the probe module sensor port. If any moisture is present, use compressed air to completely dry the connector. If the connector is corroded, return the probe module to your dealer or directly to YSI Customer Service. Refer to *Appendix E Customer Service*.

Conductivity/Temperature and pH, pH/ORP Sensor Installation

1. Unscrew and remove the probe sensor guard.

2. Using the sensor installation tool supplied in the YSI 5511 maintenance kit, unscrew and remove the sensor port plugs.

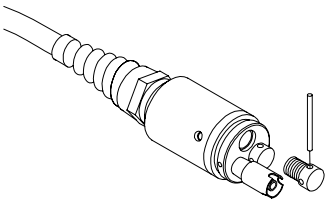


Figure 3.2 Port Plug Removal

3. Locate the port with the connector that corresponds to the sensor that is to be installed.

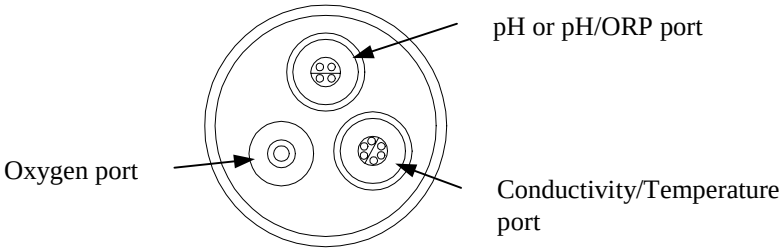


Figure 3.3 Sensor Port Identification

4. Apply a thin coat of o-ring lubricant (supplied in the YSI 5511 maintenance kit) to the o-rings on the connector side of the sensor (see Figure 3.4 O-Ring Lubrication).

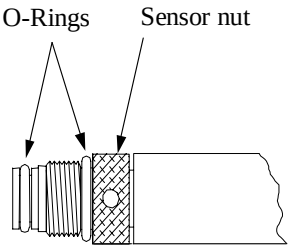


Figure 3.4 O-Ring Lubrication

⚠ **CAUTION:** Make sure that there are NO contaminants between the O-ring and the sensor. Contaminants that are present under the O-ring may cause the O-ring to leak.

5. Be sure the probe module sensor port is free of moisture and then insert the sensor into the correct port. Gently rotate the sensor until the two connectors align.

6. With connectors aligned, screw down the sensor nut using the sensor installation tool.

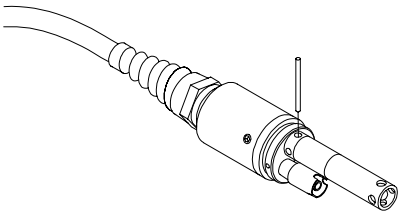


Figure 3.5 Sensor Installation

⚠ **CAUTION:** Do not cross thread the sensor nut. Tighten the nut until it is flush with the face of the probe module bulkhead. Do not over tighten.

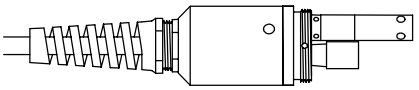


Figure 3.6 Bulkhead Seating

7. Repeat steps 3-6 for any other sensors.

8. Replace the probe sensor guard.

Dissolved Oxygen Sensor Installation

The YSI 5563 comes with the DO sensor already installed. Refer to Section *11.1.2 DO Sensor Replacement* for instructions on installing the YSI 558 Replaceable DO Module Kit.

3.4.2 Membrane Cap Selection

The YSI 5563 is shipped with a YSI 5909 kit that contains membrane caps made with 2 mil polyethylene (PE), a material which should be ideal for most field applications of the 556. However, YSI also offers membrane caps made with two other materials (1 mil polyethylene and 1 mil Teflon) which some users may also prefer. All membranes available for the 556/5563 system provide comparable accuracy if used properly. The difference between the two thicknesses of PE is found in the trade-off of flow dependence and response time as described below. Teflon is offered because some users may prefer to continue using the traditional membrane material used by YSI. To avoid confusion, the membrane caps are color coded as described below and can be ordered in kits as noted:

1 mil Teflon – Black Caps (Kit = YSI 5906)
1 mil Polyethylene (PE) – Yellow Caps (Kit = YSI 5908)
2 mil Polyethylene (PE) – Blue Caps (Kit = YSI 5909)

The 1 mil Teflon caps will offer traditional, reliable performance for most dissolved oxygen applications. The 1 mil PE caps will provide a significantly faster dissolved oxygen response (as long as your 556 Data Filter is set correctly as described below in Sections 10.2 and 10.3.1)) while also giving readings which are significantly less flow dependent than the 1 mil Teflon caps. Finally, 2 mil PE caps will show a large reduction in flow dependence over 1 mil Teflon while not significantly increasing the response time. Generally, one of the PE caps is likely to provide better performance for your application.

IMPORTANT: No matter which type of membrane cap you select, you will also have to confirm your selection in the 556

software from the Sensor menu as described in Section *4 Sensors*.

3.4.3 Membrane Cap Installation

NOTE: The YSI 5563 DO sensor (already installed in the probe module) was shipped dry. A shipping membrane was installed to protect the electrode. **A new membrane cap must be installed before the first use**.

1. Unscrew and remove the probe sensor guard.
2. Unscrew, remove, and discard the old membrane cap.
3. Thoroughly rinse the sensor tip with distilled water.
4. Prepare the electrolyte according to the directions on the electrolyte solution bottle.
5. Hold the new membrane cap and fill it at least 1/2 full with the electrolyte solution.
6. Screw the membrane cap onto the sensor moderately tight. A small amount of electrolyte should overflow.

⚠ **CAUTION:** Do not touch the membrane surface.

7. Screw the probe sensor guard on moderately tight.

3.5 Transport/Calibration Cup

The YSI 5563 Probe module has been supplied with a convenient transport/calibration cup. This cup is an ideal container for calibration of the different sensors, minimizing the amount of solution needed. Refer to Section *6 Calibrate*.

3.5.1 Transport/Calibration Cup Installation

1. Remove probe sensor guard, if already installed.

2. Ensure that an o-ring is installed in the o-ring groove on the threaded end of the probe module body.

3. Screw the transport/calibration cup on the threaded end of the probe module and securely tighten.

   NOTE: Do not overtighten as this could cause damage to the threaded portions.

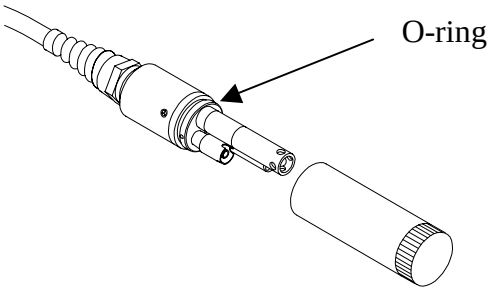


Figure 3.7 Transport/Calibration Cup Installation

3.6 Instrument/Cable Connection

Attach the cable to the instrument as follows:

1. Line up the pins and guides on the cable with the holes and indentations on the cable connector at the bottom of the YSI 556 instrument. See Figure 2.1 Front View of YSI 556 MPS.

2. Holding the cable firmly against the cable connector, turn the locking mechanism clockwise until it snaps into place.

Remove the cable from the instrument by turning the cable connector counterclockwise until the cable disengages from the instrument.

The Sensors Enabled screen allows the user to enable or disable each of the sensors and select which membrane material will be used for the dissolved oxygen sensor. Disabled sensors will not be displayed on the screen in real time or logged to files.

1. Press the **On/off** key to display the run screen.

2. Press the **Escape** key to display the main menu screen.

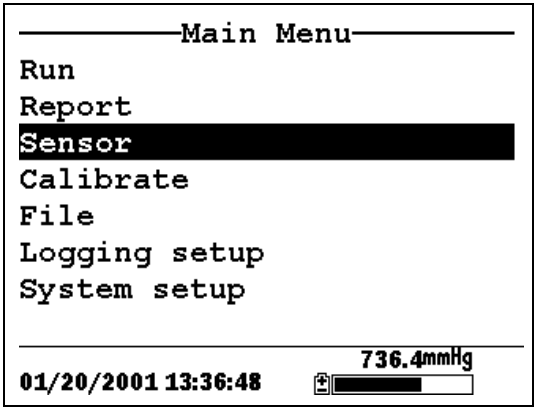


Figure 4.1 Main Menu Screen

3. Use the arrow keys to highlight the **Sensor** selection.

4. Press the **Enter** key to display the sensors enabled screen.

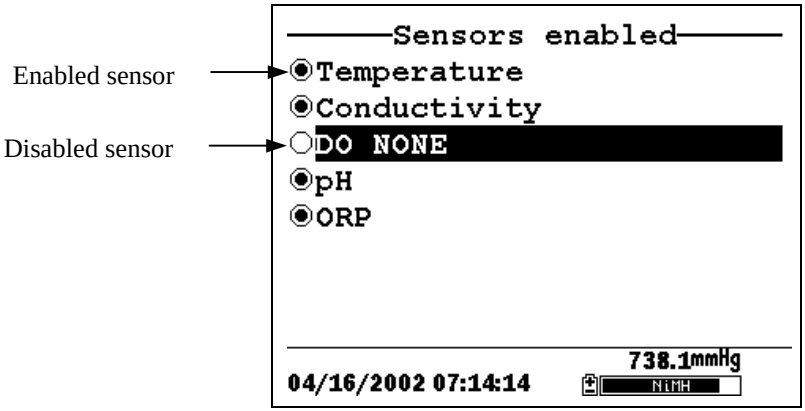


Figure 4.2 Sensors Enabled Screen Before DO Membrane Selection

A black dot to the left of a sensor indicates that sensor is enabled. Sensors with an empty circle are disabled.

Highlight the “DO None” entry as shown above and press **Enter** to display the membrane choice screen. Consult Section *3.4.2 Membrane Cap Selection* for information on the advantages of each type of membrane material. Blue membrane caps using 2 mil polyethylene (PE) were shipped with your YSI 5563 and are likely to be the best choice for most 556 field applications.

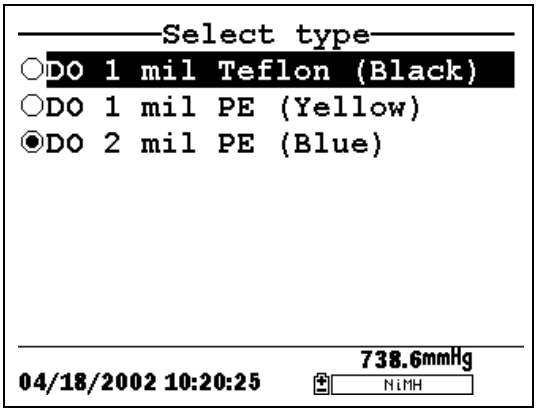


Figure 4.3 Membrane Selection Screen

Highlight the desired membrane choice – in this case, 2 mil PE -- and press Enter to activate your selection with a dot to the left of the screen. Then press **Escape** to return to the Sensor menu that now shows your DO membrane selection.

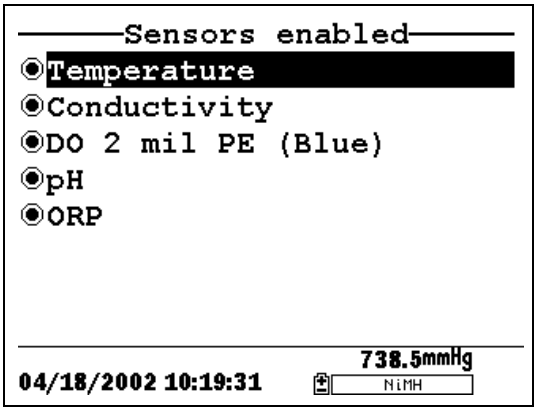


Figure 4.4 Sensors Enabled Screen After DO Membrane Selection

NOTE: The Temperature sensor cannot be disabled. Most other sensors require temperature compensation for accurate readings. In addition, the conductivity sensor must be activated in order to obtain accurate dissolved oxygen mg/L readings.

5. Use the arrow keys to highlight the sensor you want to change, then press the **Enter** key to enable or disable it.

6. Repeat step 5 for each sensor you want to change.

7. Press the **Escape** key to return to the main menu screen.

5. Report

The Report Setup screen allows the user to select which sample parameters and units the YSI 556 MPS will display on the screen. It does NOT determine which parameters are logged to memory. Refer to Section *4 Sensors*.

1. Press the **On/off** key to display the run screen.

2. Press the **Escape** key to display the main menu screen.

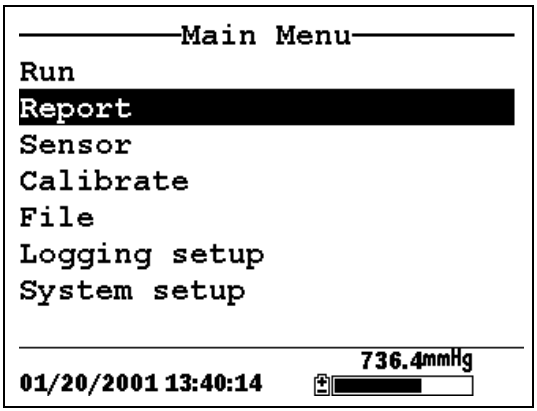


Figure 5.1 Main Menu

3. Use the arrow keys to highlight the **Report** selection.

4. Press the **Enter** key to display the report setup screen.

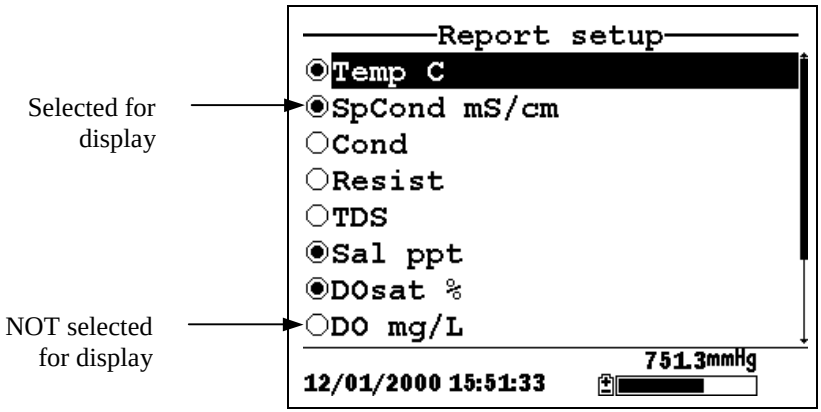


Figure 5.2 Report Setup Screen

NOTE: A black dot to the left of a parameter indicates that parameter is selected for display. Parameters with an empty circle will not be displayed.

NOTE: You may have to scroll down past the bottom of the screen to see all the parameters.

5. Use the arrow keys to highlight the parameter you want to change, then press the **Enter** key. If you can't find the parameter you want, even after scrolling down past the bottom of the screen, the sensor used for that parameter is disabled. Refer to Section *4 Sensors*.

6. If you selected Temperature, Specific Conductivity, Conductivity, Resistance or Total Dissolved Solids, the Units screen will appear.

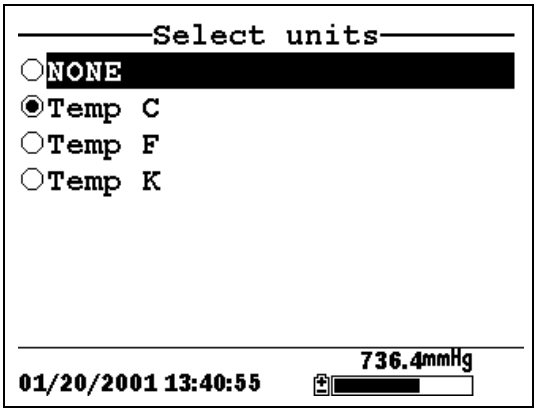


Figure 5.3 Units Screen

7. Use the arrow keys to select the units desired, then press the **Enter** key to return to the report setup screen.

   If you selected Salinity, Dissolved Oxygen %, Dissolved Oxygen mg/L, pH, pH mv or ORP mv, the selection dot will simply toggle on or off.

8. Repeat steps 5 and 6 for each parameter you want to change.

NOTE: All parameters may be enabled at the same time.

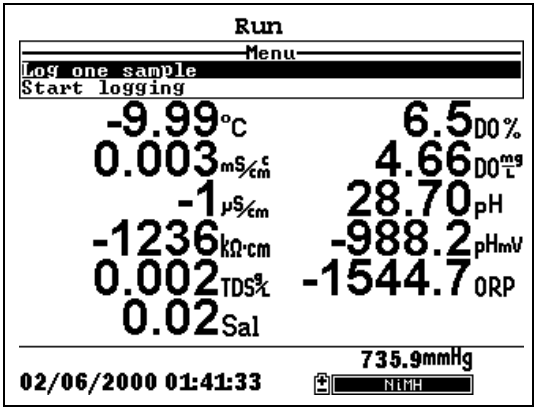


Figure 5.4 All Parameters Displayed

9. Press the **Escape** key to return to the Main menu screen.

6. Calibrate

All of the sensors, except temperature, require periodic calibration to assure high performance. You will find specific calibration procedures for all sensors that require calibration in the following sections. If a sensor listed is not installed in your probe module, skip that section and proceed to the next sensor until the calibration is complete.

⚠ **CAUTION:** Reagents that are used to calibrate and check this instrument may be hazardous to your health. Take a moment to review *Appendix D Health and Safety*. Some calibration standard solutions may require special handling.

6.1 Getting Ready to Calibrate

6.1.1 Containers Needed to Calibrate the Probe Module

The transport/calibration cup that comes with your probe module serves as a calibration chamber for all calibrations and minimizes the volume of calibration reagents required.

Instead of the transport/calibration cup, you may use laboratory glassware to perform calibrations. If you do not use the transport/calibration cup that is designed for the probe module, you are cautioned to do the following:

- ✓ Perform all calibrations with the Probe Sensor Guard installed. This protects the sensors from possible physical damage.
- ✓ Use a ring stand and clamp to secure the probe module body to prevent the module from falling over. Most laboratory glassware has convex bottoms.
- ✓ Ensure that all sensors are immersed in calibration solutions. Many of the calibrations factor in readings from other sensors (e.g., temperature sensor). The top vent hole of the conductivity sensor must also be immersed during some calibrations.

6.1.2 Calibration Tips

1. If you use the Transport/Calibration Cup for dissolved oxygen (DO) calibration, make certain to loosen the seal to allow pressure equilibration before calibration. The DO calibration is a water-saturated air calibration.
2. The key to successful calibration is to ensure that the sensors are completely submersed when calibration values are entered. Use recommended volumes when performing calibrations.
3. For maximum accuracy, use a small amount of previously used calibration solution to pre-rinse the probe module. You may wish to save old calibration standards for this purpose.
4. Fill a bucket with ambient temperature water to rinse the probe module between calibration solutions.
5. Have several clean, absorbent paper towels or cotton cloths available to dry the probe module between rinses and calibration solutions. Shake the excess rinse water off of the probe module, especially when the probe sensor guard is installed. Dry off the outside of the probe module and probe sensor guard. Making sure that the probe module is dry reduces carry-over contamination of calibrator solutions and increases the accuracy of the calibration.
6. If you are using laboratory glassware for calibration, you do not need to remove the probe sensor guard to rinse and dry the sensors between calibration solutions. The inaccuracy resulting from simply rinsing the sensor compartment and drying the outside of the guard is minimal.
7. If you are using laboratory glassware, remove the stainless steel weight from the bottom of the probe sensor guard by turning the weight counterclockwise. When the weight is removed, the calibration solutions have access to the sensors without displacing a lot of fluid. This also reduces the amount of liquid that is carried between calibrations.
8. Make certain that port plugs are installed in all ports where sensors are not installed. It is extremely important to keep these electrical connectors dry.

6.1.3 Recommended Volumes

Follow these instructions to use the transport/calibration cup for calibration procedures.

- ✓ Ensure that an o-ring is installed in the o-ring groove of the transport/calibration cup bottom cap, and that the bottom cap is securely tightened.

  NOTE: Do not over-tighten as this could cause damage to the threaded portions.
- ✓ Remove the probe sensor guard, if it is installed.
- ✓ Remove the o-ring, if installed, from the probe module and inspect the installed o-ring on the probe module for obvious defects and, if necessary, replace it with the extra o-ring supplied.
- ✓ Some calibrations can be accomplished with the probe module upright or upside down. A separate clamp and stand, such as a ring stand, is required to support the probe module in the inverted position.
- ✓ To calibrate, follow the procedures in the next section, Calibration Procedures. The approximate volumes of the reagents are specified below for both the upright and upside down orientations.
- ✓ When using the Transport/Calibration Cup for dissolved oxygen % saturation calibration, make certain that the vessel is vented to the atmosphere by loosening the bottom cap or cup assembly and that approximately 1/8” of water is present in the cup.

Sensor to Calibrate	Upright	Upside Down
Conductivity	55ml	55ml
pH/ORP	30ml	60ml

Table 6.1 Calibration Volumes

6.2 Calibration Procedures

6.2.1 Accessing the Calibrate Screen

1. Press the **On/off** key to display the run screen.
2. Press the **Escape** key to display the main menu screen.
3. Use the arrow keys to highlight the **Calibrate** selection.

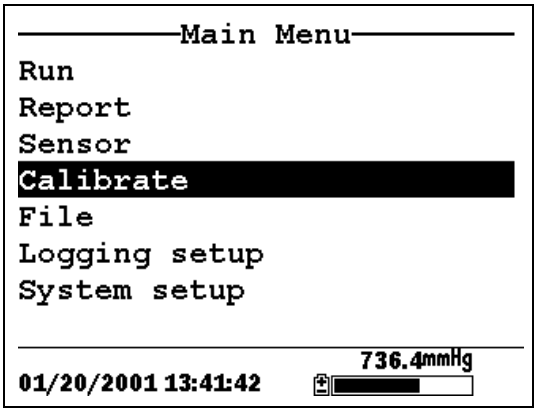


Figure 6.1 Main Menu

4. Press the **Enter** key. The Calibrate screen is displayed.

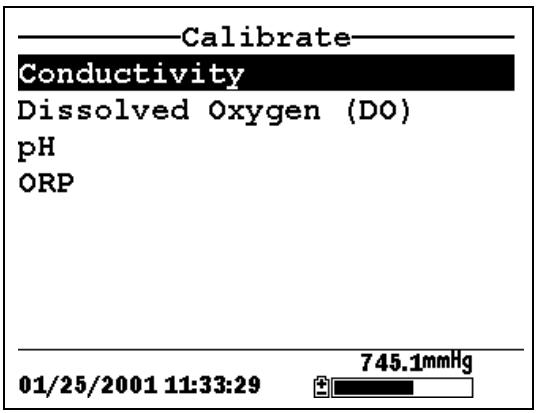


Figure 6.2 Calibrate Screen

6.2.2 Conductivity Calibration

This procedure calibrates specific conductance (recommended), conductivity and salinity. Calibrating any one option automatically calibrates the other two.

1. Go to the calibrate screen as described in Section 6.2.1 Accessing the Calibrate Screen.

2. Use the arrow keys to highlight the **Conductivity** selection. See Figure 6.2 Calibrate Screen.

3. Press **Enter.** The Conductivity Calibration Selection Screen is displayed.

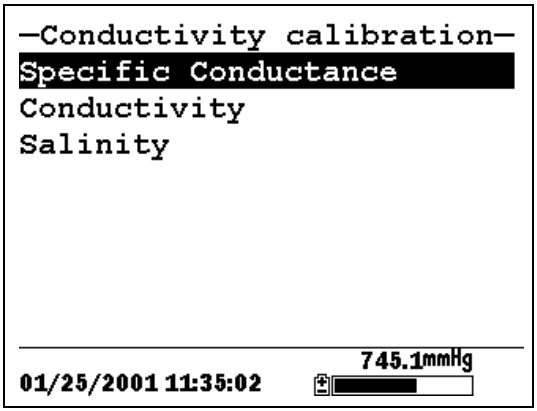


Figure 6.3 Conductivity Calibration Selection Screen

4. Use the arrow keys to highlight the Specific Conductance selection.

5. Press **Enter.** The Conductivity Calibration Entry Screen is displayed.

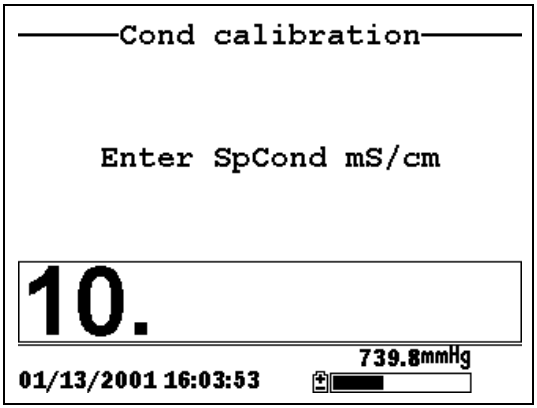


Figure 6.4 Conductivity Calibration Entry Screen

6. Place the correct amount of conductivity standard (see Table 6.1 Calibration Volumes) into a clean, dry or pre-rinsed transport/calibration cup.

⚠ **WARNING**: Calibration reagents may be hazardous to your health. See *Appendix D Health and Safety* for more information.

NOTE: For maximum accuracy, the conductivity standard you choose should be within the same conductivity range as the samples you are preparing to measure. However, we do not recommend using standards less than 1 mS/cm. For example:

- ✓ For fresh water use a 1 mS/cm conductivity standard.
- ✓ For brackish water use a 10 mS/cm conductivity standard.
- ✓ For seawater use a 50 mS/cm conductivity standard.

NOTE: Before proceeding, ensure that the sensor is as dry as possible. Ideally, rinse the conductivity sensor with a small amount of standard that can be discarded. Be certain that you avoid cross-contamination of solutions. Make certain that there are no salt deposits around the oxygen and pH/ORP sensors, particularly if you are employing standards of low conductivity.

7. Carefully immerse the sensor end of the probe module into the solution.

8. Gently rotate and/or move the probe module up and down to remove any bubbles from the conductivity cell.

   NOTE: The sensor must be completely immersed past its vent hole. Using the recommended volumes from Table 6.1 Calibration Volumes, should ensure that the vent hole is covered.

9. Screw the transport/calibration cup on the threaded end of the probe module and securely tighten.

   NOTE: Do not overtighten as this could cause damage to the threaded portions.

10. Use the keypad to enter the calibration value of the standard you are using.

    NOTE: Be sure to enter the value in **mS/cm at 25°C**.

11. Press **Enter**. The Conductivity Calibration Screen is displayed.

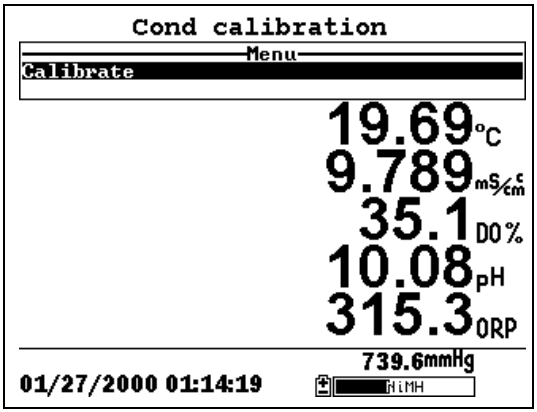


Figure 6.5 Conductivity Calibration Screen

12. Allow at least one minute for temperature equilibration before proceeding. The current values of all enabled sensors

will appear on the screen and will change with time as they stabilize.

13. Observe the reading under Specific Conductance. When the reading shows no significant change for approximately 30 seconds, press **Enter**. The screen will indicate that the calibration has been accepted and prompt you to press **Enter** again to Continue.

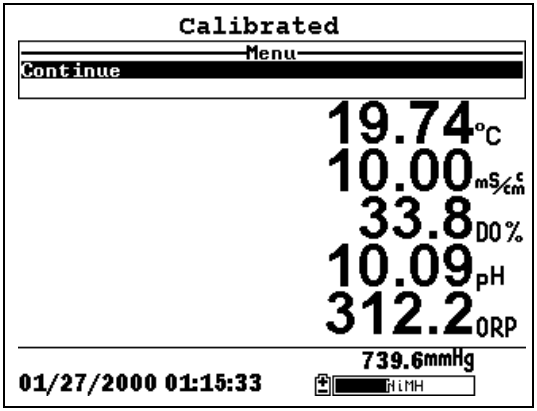


Figure 6.6 Calibrated

14. Press **Enter**. This returns you to the Conductivity Calibrate Selection Screen, See Figure 6.3 Conductivity Calibration Selection Screen.

15. Press **Escape** to return to the calibrate menu. See Figure 6.2 Calibrate Screen.

16. Rinse the probe module and sensors in tap or purified water and dry.

6.2.3 Dissolved Oxygen Calibration

This procedure calibrates dissolved oxygen. Calibrating any one option (% or mg/L) automatically calibrates the other.

1. Go to the calibrate screen as described in Section *6.2.1 Accessing the Calibrate Screen*.

NOTE: The instrument must be on for at least 20 minutes to polarize the DO sensor before calibrating.

2. Use the arrow keys to highlight the **Dissolved Oxygen** selection. See Figure 6.2 Calibrate Screen.
3. Press **Enter**. The dissolved oxygen calibration screen is displayed.

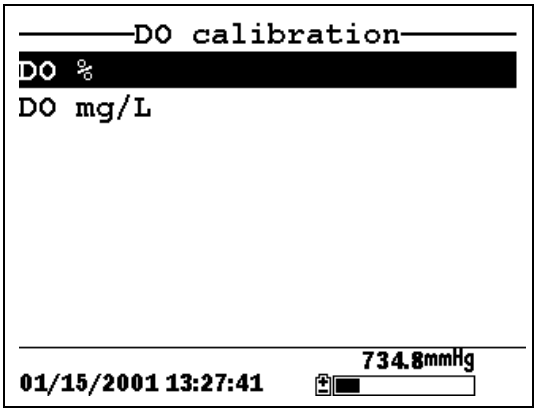


Figure 6.7 DO Calibration Screen

DO Calibration in % Saturation

1. Use the arrow keys to highlight the DO% selection.
2. Press **Enter**. The DO Barometric Pressure Entry Screen is displayed.

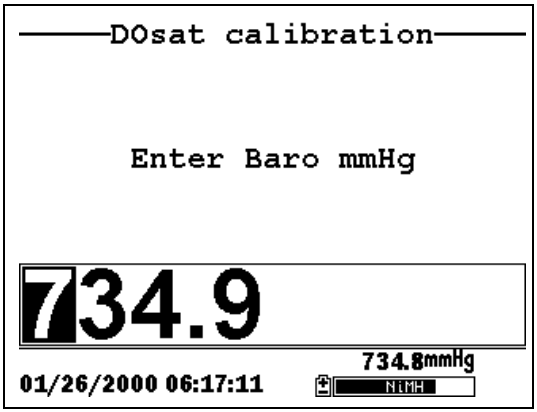


Figure 6.8 DO Barometric Pressure Entry Screen

3. Place approximately 3 mm (1/8 inch) of water in the bottom of the transport/calibration cup.

4. Place the probe module into the transport/calibration cup.

   NOTE: Make sure that the DO and temperature sensors are **not** immersed in the water.

5. Engage only 1 or 2 threads of the transport/calibration cup to ensure the DO sensor is vented to the atmosphere.

6. Use the keypad to enter the current local barometric pressure.

   NOTE: If the unit has the optional barometer, no entry is required.

   NOTE: Barometer readings that appear in meteorological reports are generally corrected to sea level and must be uncorrected before use (refer to Section *10.10 Calibrate Barometer, Step 2*).

7. Press **Enter**. The DO% saturation calibration screen is displayed.

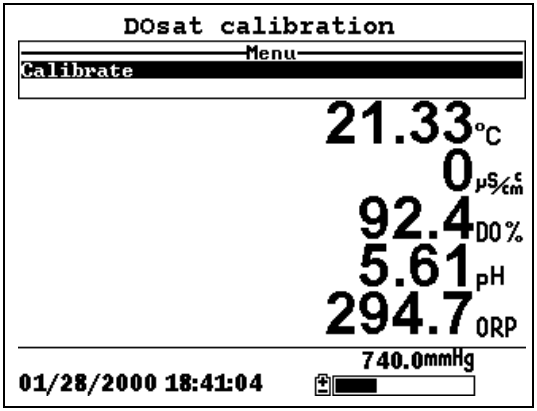


Figure 6.9 DO Sat Calibration Screen

8. Allow approximately ten minutes for the air in the transport/calibration cup to become water saturated and for

the temperature to equilibrate before proceeding. The current values of all enabled sensors will appear on the screen and will change with time as they stabilize.

9. Observe the reading under DO %. When the reading shows no significant change for approximately 30 seconds, press **Enter**. The screen will indicate that the calibration has been accepted and prompt you to press **Enter** again to Continue. See Figure 6.6 Calibrated.

10. Press **Enter**. This returns you to the DO calibration screen, See Figure 6.7 DO Calibration Screen.

11. Press **Escape** to return to the calibrate menu. See Figure 6.2 Calibrate Screen.

12. Rinse the probe module and sensors in tap or purified water and dry.

DO Calibration in mg/L

DO calibration in mg/L is carried out in a water sample which has a known concentration of dissolved oxygen (usually determined by a Winkler titration).

1. Go to the DO calibrate screen as described in Section *6.2.3 Dissolved Oxygen Calibration*, steps 1 through 3.

2. Use the arrow keys to highlight the **DO mg/L** selection.

3. Press **Enter**. The DO mg/L Entry Screen is displayed.

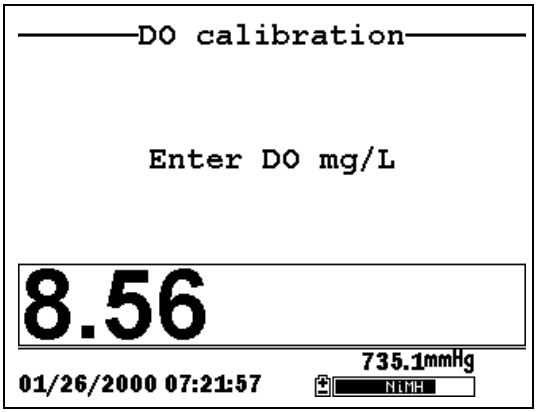


Figure 6.10 DO mg/L Entry Screen

4. Place the probe module in water with a known DO concentration.

   NOTE: Be sure to completely immerse all the sensors.

5. Use the keypad to enter the known DO concentration of the water.

6. Press **Enter**. The Dissolved Oxygen mg/L Calibration Screen is displayed.

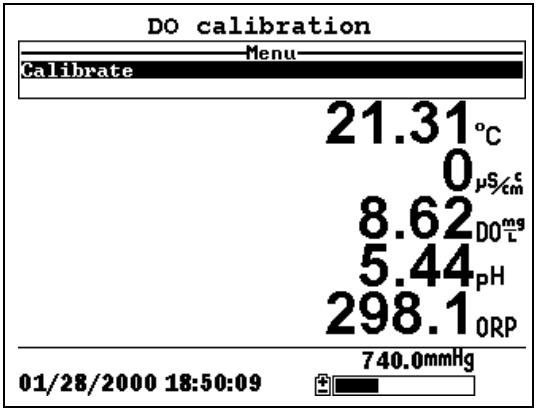


Figure 6.11 DO mg/L Calibration Screen

7. Stir the water with a stir bar, or by rapidly moving the probe module, to provide fresh sample to the DO sensor.

8. Allow at least one minute for temperature equilibration before proceeding. The current values of all enabled sensors will appear on the screen and will change with time as they stabilize.

9. Observe the DO mg/L reading, when the reading is stable (shows no significant change for approximately 30 seconds), press **Enter**. The screen will indicate that the calibration has been accepted and prompt you to press **Enter** again to Continue.

10. Press **Enter**. This returns you to the DO calibration screen. See Figure 6.7 DO Calibration Screen.

11. Press **Escape** to return to the calibrate menu. See Figure 6.2 Calibrate Screen.

12. Rinse the probe module and sensors in tap or purified water and dry.

6.2.4 pH Calibration

1. Go to the calibrate screen as described in *Section 6.2.1 Accessing the Calibrate Screen*.

2. Use the arrow keys to highlight the **pH** selection. See Figure 6.2 Calibrate Screen.

3. Press **Enter**. The pH calibration screen is displayed.

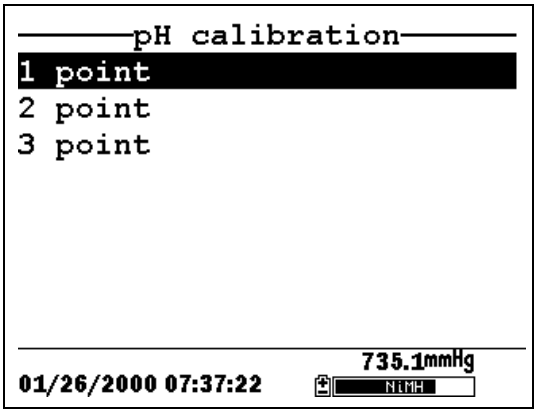


Figure 6.12 pH Calibration Screen

- Select the **1-point** option only if you are adjusting a previous calibration. If a 2-point or 3-point calibration has been performed previously, you can adjust the calibration by carrying out a one point calibration. The procedure for this calibration is the same as for a 2-point calibration, but the software will prompt you to select only one pH buffer.
- Select the **2-point** option to calibrate the pH sensor using only two calibration standards. Use this option if the media being monitored is known to be either basic or acidic. For example, if the pH of a pond is known to vary between 5.5 and 7, a two-point calibration with pH 7 and pH 4 buffers is sufficient. A three point calibration with an additional pH 10 buffer will not increase the accuracy of this measurement since the pH is not within this higher range.
- Select the **3-point** option to calibrate the pH sensor using three calibration solutions. In this procedure, the pH sensor is calibrated with a pH 7 buffer and two additional buffers. The 3-point calibration method assures maximum accuracy when the pH of the media to be monitored cannot be anticipated. The procedure for this calibration is the same as for a 2-point calibration, but the software will prompt you to select a third pH buffer.

4. Use the arrow keys to highlight the **2-point** selection.

5. Press **Enter**. The pH Entry Screen is displayed.

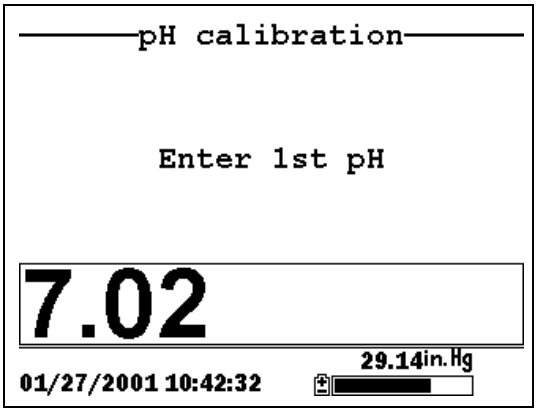


Figure 6.13 pH Entry Screen

6. Place the correct amount (see Table 6.1 Calibration Volumes) of pH buffer into a clean, dry or pre-rinsed transport/calibration cup.

⚠ **WARNING**: Calibration reagents may be hazardous to your health. See *Appendix D Health and Safety* for more information.

NOTE: For maximum accuracy, the pH buffers you choose should be within the same pH range as the water you are preparing to sample.

NOTE: Before proceeding, ensure that the sensor is as dry as possible. Ideally, rinse the pH sensor with a small amount of buffer that can be discarded. Be certain that you avoid cross-contamination of buffers with other solutions.

7. Carefully immerse the sensor end of the probe module into the solution.

8. Gently rotate and/or move the probe module up and down to remove any bubbles from the pH sensor.

NOTE: The sensor must be completely immersed. Using the recommended volumes from Table 6.1 Calibration Volumes, should ensure that the sensor is covered.

9. Screw the transport/calibration cup on the threaded end of the probe module and securely tighten.

NOTE: Do not overtighten as this could cause damage to the threaded portions.

10. Use the keypad to enter the calibration value of the buffer you are using **at the current temperature**.

NOTE: pH vs. temperature values are printed on the labels of all YSI pH buffers.

11. Press **Enter**. The pH calibration screen is displayed.

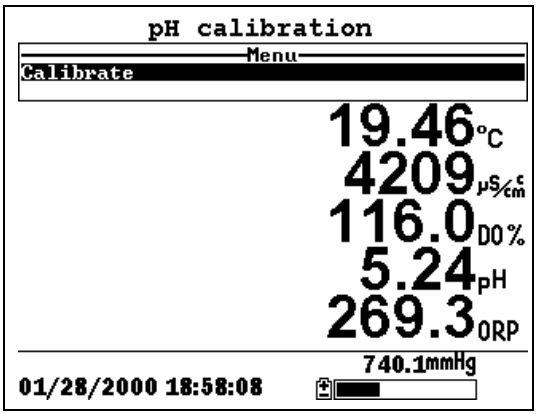


Figure 6.14 pH Calibration Screen

12. Allow at least one minute for temperature equilibration before proceeding. The current values of all enabled sensors will appear on the screen and will change with time as they stabilize.

13. Observe the reading under pH, when the reading shows no significant change for approximately 30 seconds, press **Enter**. The screen will indicate that the calibration has been accepted and prompt you to press **Enter** again to Continue.

14. Press **Enter**. This returns you to the Specified pH Calibration Screen, See Figure 6.13 pH Entry Screen.

15. Rinse the probe module, transport/calibration cup and sensors in tap or purified water and dry.

16. Repeat steps 6 through 13 above using a second pH buffer.

17. Press **Enter**. This returns you to the pH Calibration Screen, See Figure 6.12 pH Calibration Screen.

18. Press **Escape** to return to the calibrate menu. See Figure 6.2 Calibrate Screen.

19. Rinse the probe module and sensors in tap or purified water and dry.

6.2.5 ORP Calibration

1. Go to the calibrate screen as described in Section *6.2.1 Accessing the Calibrate Screen*.

2. Use the arrow keys to highlight the **ORP** selection. See Figure 6.2 Calibrate Screen.

3. Press **Enter**. The ORP calibration screen is displayed.

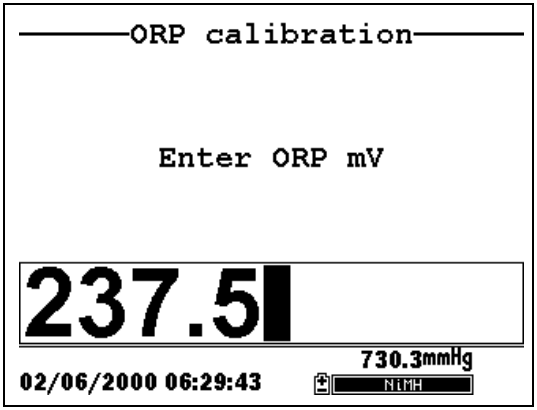


Figure 6.15 Specified ORP Calibration Screen

4. Place the correct amount (see Table 6.1 Calibration Volumes) of a known ORP solution (we recommend Zobell solution) into a clean, dry or pre-rinsed transport/calibration cup.

⚠ **WARNING**: Calibration reagents may be hazardous to your health. See *Appendix D Health and Safety* for more information.

NOTE: Before proceeding, ensure that the sensor is as dry as possible. Ideally, rinse the ORP sensor with a small amount of solution that can be discarded. Be certain that you avoid cross-contamination with other solutions.

5. Carefully immerse the sensor end of the probe module into the solution.

6. Gently rotate and/or move the probe module up and down to remove any bubbles from the ORP sensor.

NOTE: The sensor must be completely immersed. Using the recommended volumes from Table 6.1 Calibration Volumes should ensure that the sensor is covered.

7. Screw the transport/calibration cup on the threaded end of the probe module and securely tighten.

NOTE: Do not overtighten as this could cause damage to the threaded portions.

8. Use the keypad to enter the correct value of the calibration solution you are using at the current temperature. Refer to Table 6.2 Zobel Solution Values.

Temperature °C	Zobell Solution Value, mV
-5	270.0
0	263.5
5	257.0
10	250.5
15	244.0
20	237.5
25	231.0
30	224.5
35	218.0
40	211.5
45	205.0
50	198.5

Table 6.2 Zobel Solution Values

9. Press **Enter**. The ORP calibration screen is displayed.

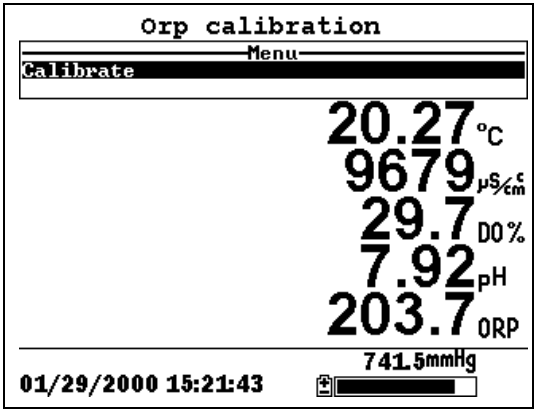


Figure 6.16 ORP Calibration Screen

10. Allow at least one minute for temperature equilibration before proceeding. The current values of all enabled sensors will appear on the screen and will change with time as they stabilize.

NOTE: Verify that the temperature reading matches the value you used in Table 6.2 Zobel Solution Values.

11. Observe the reading under ORP, when the reading shows no significant change for approximately 30 seconds, press **Enter**. The screen will indicate that the calibration has been accepted and prompt you to press **Enter** again to Continue.

12. Press **Enter**. This returns you to the Calibrate Screen. See Figure 6.2 Calibrate Screen.

13. Rinse the probe module and sensors in tap or purified water and dry.

6.3 Return to Factory Settings

1. Go to the calibrate screen as described in Section *6.2.1 Accessing the Calibrate Screen*.

2. Use the arrow keys to highlight the **Conductivity** selection. See Figure 6.2 Calibrate Screen.

   NOTE: We will use the Conductivity sensor as an example; however, this process will work for any sensor.

3. Press **Enter.** The Conductivity Calibration Selection Screen is displayed. See Figure 6.3 Conductivity Calibration Selection Screen.

4. Use the arrow keys to highlight the **Specific Conductance** selection.

5. Press **Enter.** The Conductivity Calibration Entry Screen is displayed. See Figure 6.4 Conductivity Calibration Entry Screen.

6. Press and hold the **Enter** key down and press the **Escape** key.

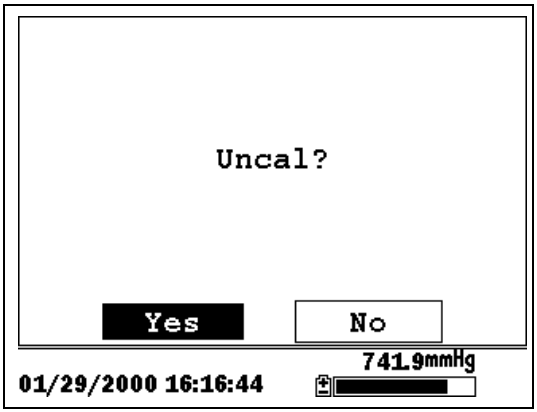


Figure 6.17 ORP Calibration Screen

7. Use the arrow keys to highlight the **YES** selection.

CAUTION: This returns a sensor to the factory settings. For example, in selecting to return specific conductance to the factory setting, salinity and conductivity will automatically return to their factory settings.

8. Press **Enter**. This returns you to the Conductivity Calibrate Selection Screen, See Figure 6.3 Conductivity Calibration Selection Screen.

9. Press **Escape** to return to the calibrate menu. See Figure 6.2 Calibrate Screen.

7. Run

The Run screen displays data from the sensors in real-time and allows the user to log sample data to memory for later analysis. Refer to Section *9 Logging* for details on logging sample data.

7.1 Real-Time Data

NOTE: Before measuring samples you must prepare the probe module (refer to Section *3.4 Preparing the Probe Module*), attach the probe module to the instrument (refer to Section *3.6 Instrument/Cable Connection*) and calibrate the sensors (refer to Section *6 Calibrate*).

1. Press the **On/off** key.

OR select Run from the main menu to display the run screen.

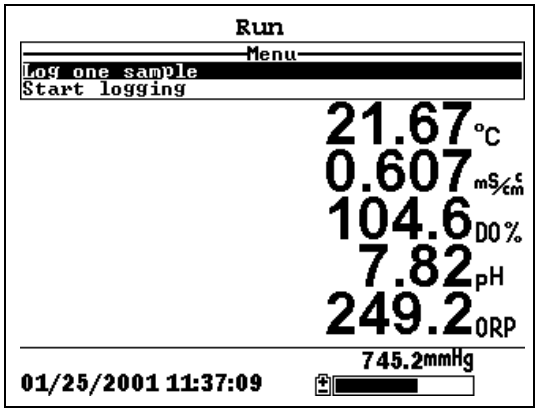


Figure 7.1 Run Screen

2. Make sure the probe sensor guard is installed.

3. Place the probe module in the sample. Be sure to completely immerse all the sensors.

4. Rapidly move the probe module through the sample to provide fresh sample to the DO sensor.

5. Watch the readings on the display until they are stable.

6. Refer to Section *9 Logging* for instructions on logging sample data.

8. File

The File menu allows the user to view, upload or delete sample data and calibration record files stored in the YSI 556 MPS.

8.1 Accessing the File Screen

1. Press the **On/off** key to display the run screen.

2. Press the **Escape** key to display the main menu screen.

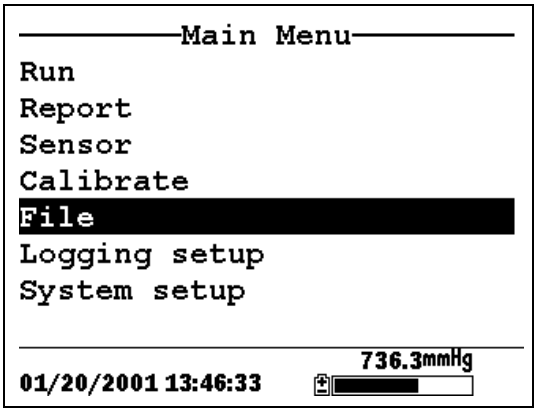


Figure 8.1 Main Menu Screen

3. Use the arrow keys to highlight the **File** selection.

4. Press the **Enter** key. The file screen is displayed.

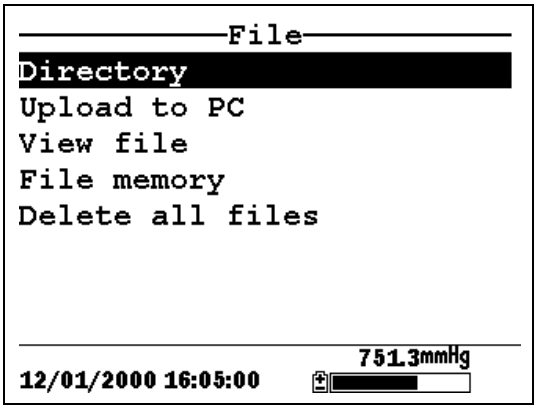


Figure 8.2 File Screen

8.2 Directory

1. Go to the file screen as described in Section *8.1 Accessing the File Screen*.

2. Use the arrow keys to highlight the **Directory** selection. See Figure 8.2 File Screen.

3. Press the **Enter** key. The file list screen is displayed.

   NOTE: Files are listed in the order in which they are logged to memory. Sample Data files have the file extension **.dat**, while Calibration Record files have the file extension **.glp**.

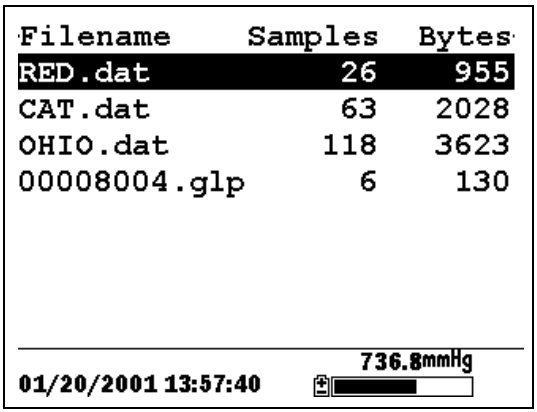


Figure 8.3 File List Screen

4. Use the arrow keys to highlight a file.

5. Press the **Enter** key. The file details screen is displayed.

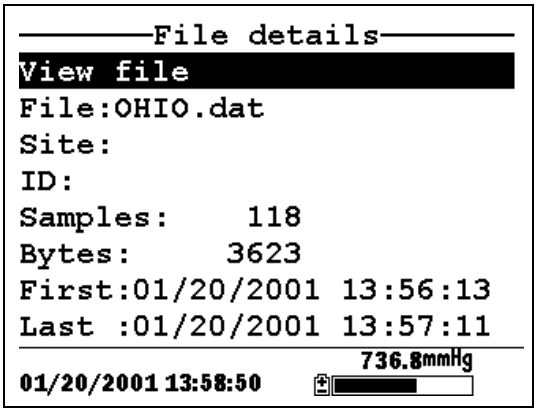


Figure 8.4 File Details Screen

6. Press the **Enter** key to view the file data. Refer to Section *8.3 View File* for details.

7. Press the **Escape** key repeatedly to return to the main menu screen.

8.3 View File

1. Go to the file screen as described in Section *8.1 Accessing the File Screen*. See Figure 8.2 File Screen.

2. Use the arrow keys to highlight the **View file** selection.

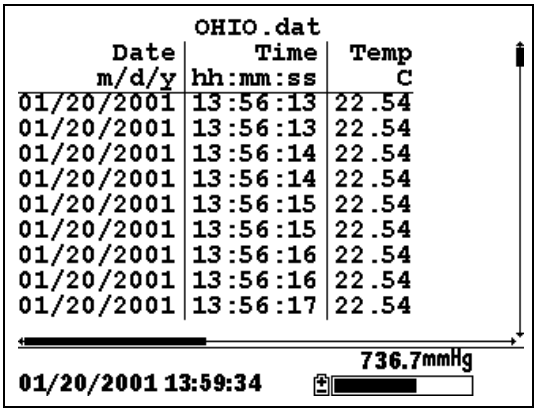
3. Press the **Enter** key. A list of files is displayed. See Figure 8.3 File List Screen.

4. Use the arrow keys to highlight an individual file.

   NOTE: You may have to scroll down to see all the files.

5. Press the **Enter** key. The file data is displayed with the file name at the top of the display.

   NOTE: If no file name was specified, the data is stored under the default name NONAME1.dat.



OHIO.dat

Date m/d/y	Time hh:mm:ss	Temp C
01/20/2001	13:56:13	22.54
01/20/2001	13:56:13	22.54
01/20/2001	13:56:14	22.54
01/20/2001	13:56:14	22.54
01/20/2001	13:56:15	22.54
01/20/2001	13:56:15	22.54
01/20/2001	13:56:16	22.54
01/20/2001	13:56:16	22.54
01/20/2001	13:56:17	22.54

736.7mmHg

01/20/2001 13:59:34

Figure 8.5 File Data Screen

6. Use the arrow keys to scroll horizontally and/or vertically to view all the data.

7. Press the **Escape** key repeatedly to return to the main menu screen.

8.4 Upload to PC

EcoWatch™ for Windows™ must be used as the PC software interface to the YSI 556 MPS. Refer to *Appendix G EcoWatch* for more information. EcoWatch for Windows is available at no cost via a download from the YSI Web Site (www.ysi.com) or by contacting YSI Customer Support. Refer to *Appendix E Customer Service.*

8.4.1 Upload Setup

1. Disconnect the YSI 5563 Probe Module from the YSI 556 MPS instrument.

2. Connect the YSI 556 MPS to a serial (Comm) port of your computer via the 655173 PC Interface cable as shown in the following diagram:

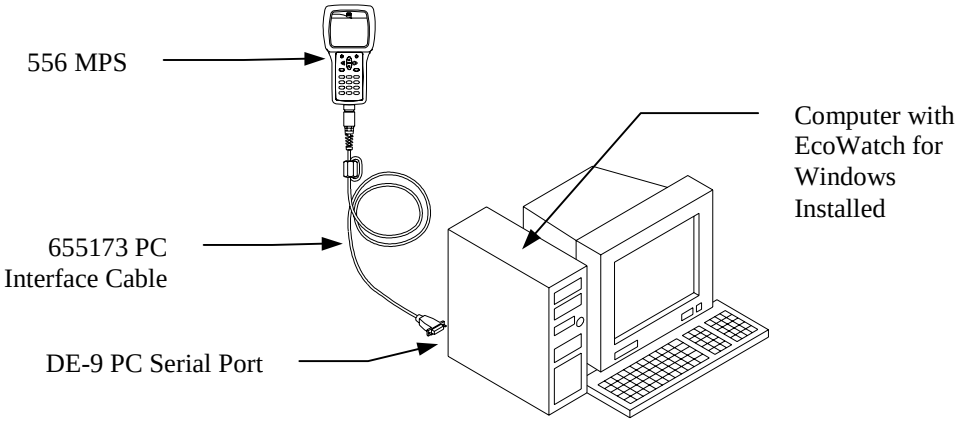


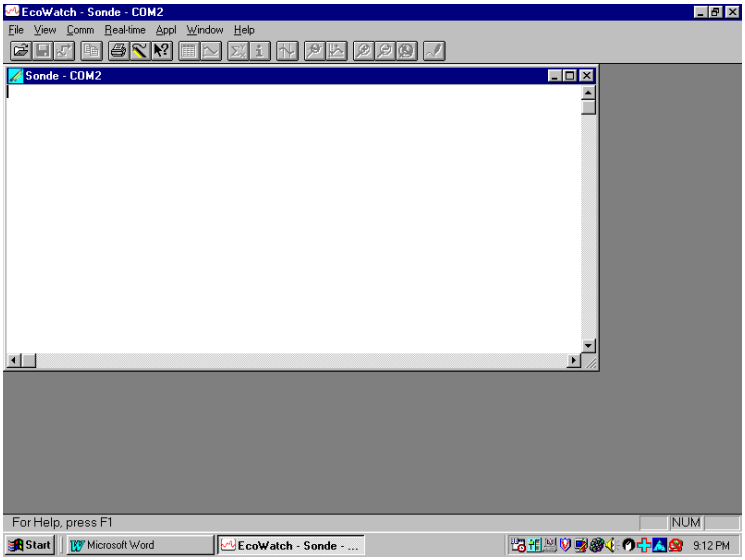
Figure 8.6 Computer/Instrument Interface

3. Open EcoWatch for Windows on your computer.

NOTE: See *Appendix G EcoWatch* for installation instructions.

4. Click on the sonde/probe icon in the upper toolbar.

5. Set the Comm port number to match the port the YSI 556 MPS is connected to. After this setup procedure, the following screen will be present on your PC monitor:



8.4.2 Uploading a .DAT File

1. Setup the instrument as described in Section *8.4.1 Upload Setup*.

2. Go to the YSI 556 MPS file screen as described in Section *8.1 Accessing the File Screen.*

3. Use the arrow keys to highlight the **Upload to PC** selection. See Figure 8.2 File Screen.

4. Press the **Enter** key. The file list screen is displayed. See Figure 8.3 File List Screen.

5. Use the arrow keys to highlight the DAT file that you wish to transfer and press **Enter**, both the YSI 556 MPS and PC displays show the progress of the file transfer.

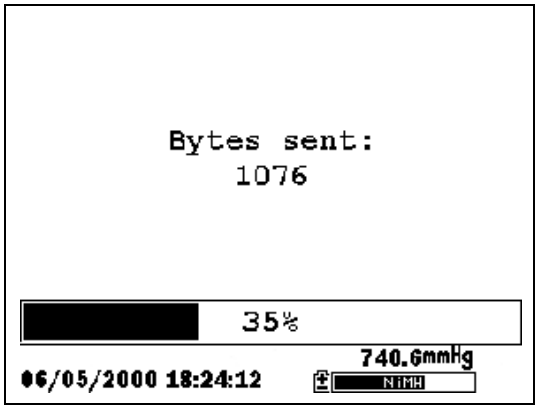
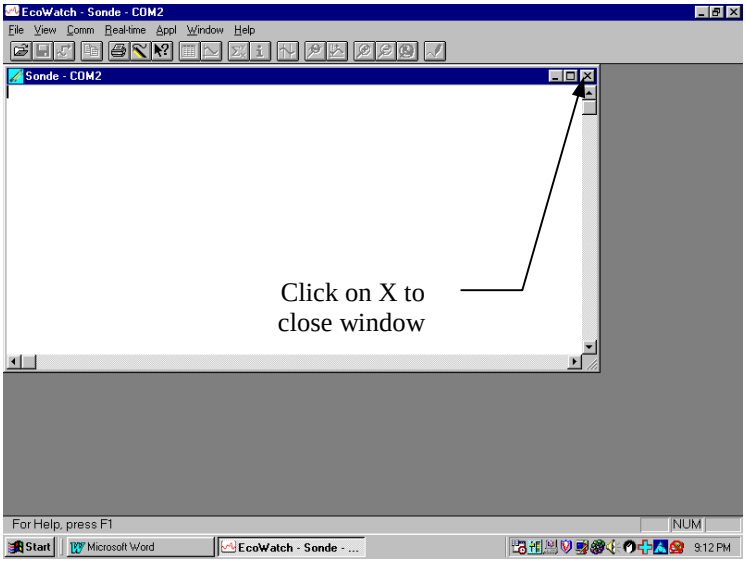


Figure 8.7 File Transfer Progress Screen

NOTE: After transfer, the file will be located in the C:\ECOWWIN\DATA folder of your PC, designated with a .DAT extension.

6. After the file transfer is complete, close the terminal window (small window on the PC) by clicking on the "X" at its upper right corner.



7. Press the **Escape** key on the YSI 556 MPS repeatedly to return to the main menu screen.

8.4.3 Uploading a Calibration Record (.glp) File

For more information on the calibration record, Refer to *Appendix H Calibration Record Information*.

1. Setup up the instrument as described in Section *8.4.1 Upload Setup*.

2. Go to the YSI 556 MPS file screen as described in Section *8.1 Accessing the File Screen.*

3. Use the arrow keys to highlight the **Upload to PC** selection. See Figure 8.2 File Screen.

4. Press the **Enter** key. The file list screen is displayed. See Figure 8.3 File List Screen.

5. Use the arrow keys to highlight the calibration record file that you wish to transfer and press **Enter.**

6. You will then be given a choice of uploading the file in three formats; **Binary, Comma & “” Delimited, and ASCII Text**.

   NOTE: The binary format is reserved for future YSI software packages.

7. Choose an option and press **Enter,** both the YSI 556 and PC displays show the progress of the file transfer.

   NOTE: After transfer, the file will be located in the C:\ECOWWIN\DATA folder of your PC, designated with the appropriate file extension.

   NOTE: To view the Calibration Record data after upload, simply open the .txt file in a general text editor such as Wordpad or Notepad.

8. After the file transfer is complete, close the terminal window (small window on the PC) by clicking on the “X” at its upper right corner.

9. Press the **Escape** key repeatedly to return to the main menu screen.

8.5 File Memory

1. Go to the file screen as described in Section *8.1 Accessing the File Screen*.

2. Use the arrow keys to highlight the **File memory** selection. See Figure 8.2 File Screen.

3. Press the **Enter** key. The file bytes used screen is displayed.

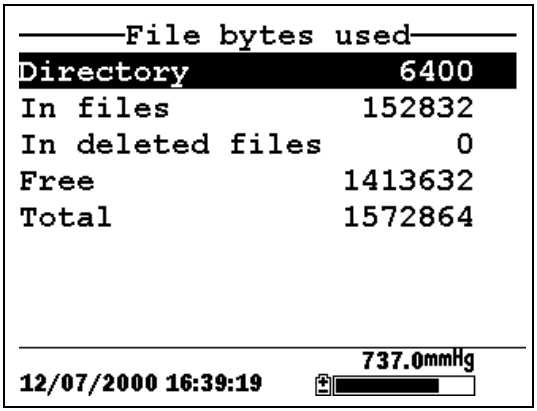


Figure 8.8 File Bytes Used Screen

4. The amount of free memory is listed in line 4 of the file bytes used screen.

   NOTE: If the amount of free memory is low, it may be time to delete all files (after first uploading all data to a PC). Refer to Section *8.6 Delete All Files*.

5. Press the **Escape** key repeatedly to return to the main menu screen.

8.6 Delete All Files

NOTE: It is not possible to delete individual files in order to free up memory. The only way to free up memory is to delete ALL files present. Take care to transfer all files to your computer (refer to Section *8.4 Upload to PC*) *before* deleting them.

1. Go to the file screen as described in Section *8.1 Accessing the File Screen*.

2. Use the arrow keys to highlight the **Delete all files** selection. See Figure 8.2 File Screen.

3. Press the **Enter** key. The Delete all Files screen is displayed.

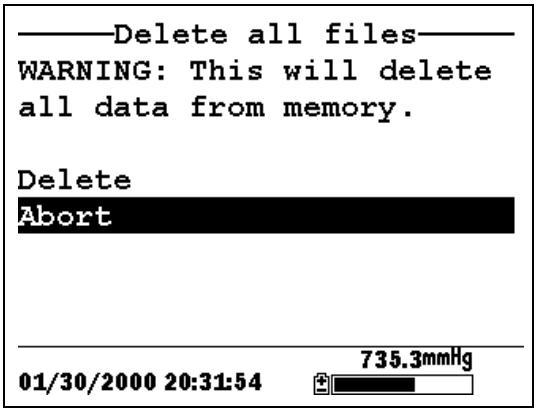


Figure 8.9 Delete All Files Screen

4. Use the arrow keys to highlight the **Delete** selection.

5. Press the **Enter** key.

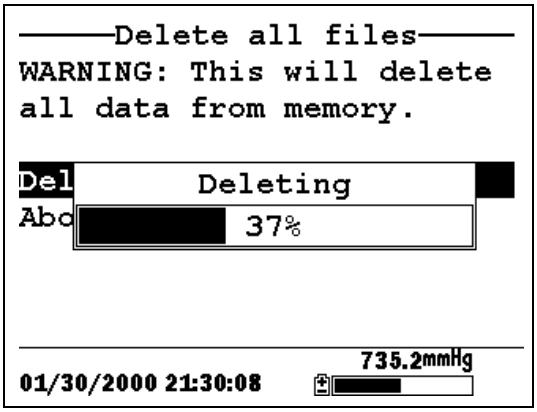


Figure 8.10 Deleting

The progress of file deletion is displayed in bar graph format.

NOTE: Deleting all files in the directory will not change any information in the site list.

6. Press the **Escape** key repeatedly to return to the main menu screen.

9. Logging

9.1 Accessing the Logging Setup Screen

1. Press the **On/off** key to display the run screen.

2. Press the **Escape** key to display the main menu screen.

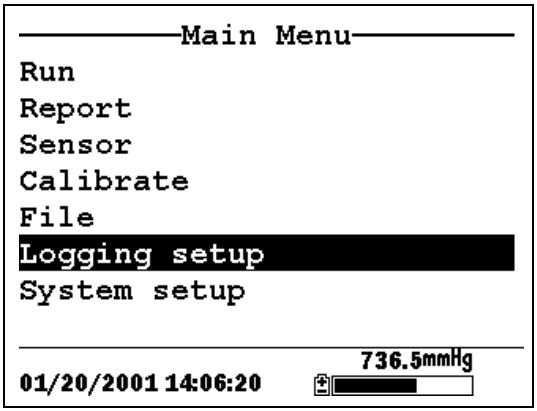


Figure 9.1 Main Menu

3. Use the arrow keys to highlight the **Logging setup** selection.

4. Press the **Enter** key. The logging setup screen is displayed.

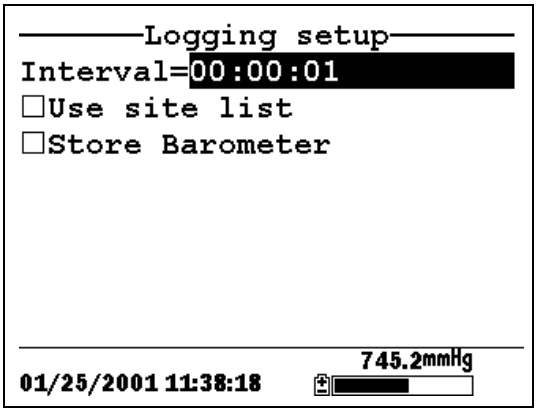


Figure 9.2 Logging Setup Screen

9.2 Setting Logging Interval

Follow steps below to set the interval for logging a data stream.

NOTE: If you do not specify an interval, the instrument will use a default interval setting of 1 second.

NOTE: It is not necessary to set a logging interval when logging a single sample.

1. Go to the logging setup screen as described in Section *9.1 Accessing the Logging Setup Screen*.

2. Use the keypad to enter an interval between 1 second and 15 minutes. Refer to Section *2.9 Keypad Use*.

   NOTE: The interval field has hour, minute and second entry fields. Any entry over 15 minutes will change automatically to a 15-minute setting.

3. Press the **Enter** key. The data stream interval is set.

4. Press the **Escape** key repeatedly to return to the main menu screen.

9.3 Storing Barometer Readings

NOTE: The **Store barometer** option is only available on instruments that are equipped with the optional barometer.

1. Go to the logging setup screen as described in Section *9.1 Accessing the Logging Setup Screen*.

2. Use the arrow keys to highlight the **Store barometer** selection. See Figure 9.2 Logging Setup Screen.

3. Press the **Enter** key until a check mark is entered in the box next to the store barometer selection if you want to log barometric readings.

   OR press the **Enter** key until the box next to the barometer selection is empty if you do not want to log barometric readings.

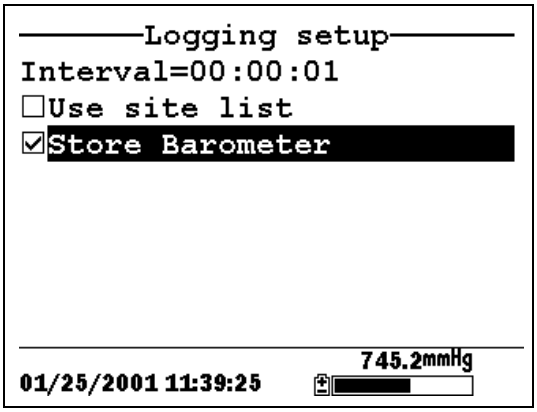


Figure 9.3 Store Barometer

4. Press the **Escape** key repeatedly to return to the main menu screen.

9.4 Creating a Site List

The site list option allows you to define file and site descriptions in the office or laboratory before moving to field logging studies. This is usually more convenient than entering the information at the site and is particularly valuable if you are visiting certain sites on a regular basis. The following section describes how to set up site lists which contain entries designated “Site Descriptions” that will be instantly available to the user in the field to facilitate the logging of data with pre-established naming of files and sites. There are two kinds of **Site Descriptions** available for use in Site lists:

- **Site Descriptions** associated with applications where data from a single site is always logged to a single file. This type is referred to as a “Single-Site Description” and is characterized by two parameters – a file name and a site name. Files logged to YSI 556 MPS memory under a **Single-Site Description** will be characterized primarily by the file name, but will also have the Site name attached, so that it is viewable in either the YSI 556 MPS **File directory** or in EcoWatch for Windows after upload to a PC

- **Site Descriptions** associated with applications where data from multiple sites are logged to a single file. This type is referred to as a "Multi-site Description" and is characterized by three parameters – a file name, a site name, and a site number. Files logged to YSI 556 MPS memory under a **Multi-site Description** are characterized by a file name, but not a site name, since multiple sites are involved. However, each data point has a Site Number attached to it so that the user can easily determine the sampling site when viewing the data from the YSI 556 MPS **File** menu or processing the data in EcoWatch for Windows after upload to a PC.

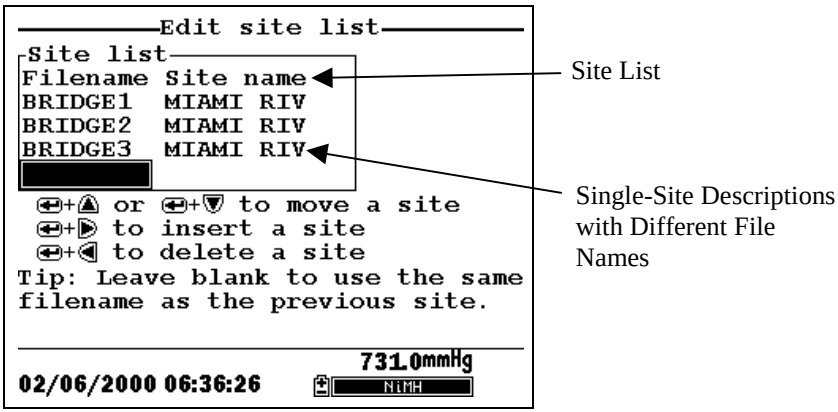


Figure 9.4 Single-Site Descriptions

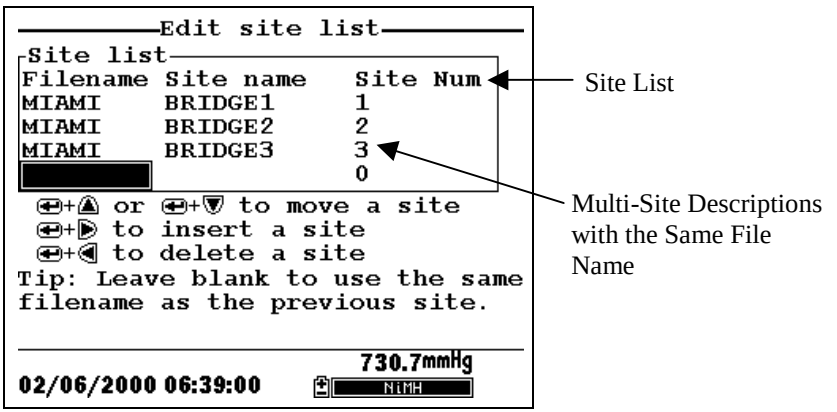


Figure 9.5 Multiple-Site Descriptions

NOTE: Site lists containing Single Site Descriptions are usually input with the designation **Store Site Number** INACTIVE in the YSI 556 MPS **Logging setup** menu. Thus, no site numbers appear in the first **Site list** example. Conversely, **Site lists** containing **Multi-Site Descriptions** MUST be input with the **Store Site Number** selection ACTIVE as shown in the second example.

To create a site list:

1. Go to the logging setup screen as described in Section *9.1 Accessing the Logging Setup Screen*.

2. Use the arrow keys to highlight the **Use site list** selection.

3. Press the **Enter** key. A check mark is entered in the box next to the use site list selection *and* two new entries appear on the logging setup screen. See Figure 9.6 Logging Setup Screen.

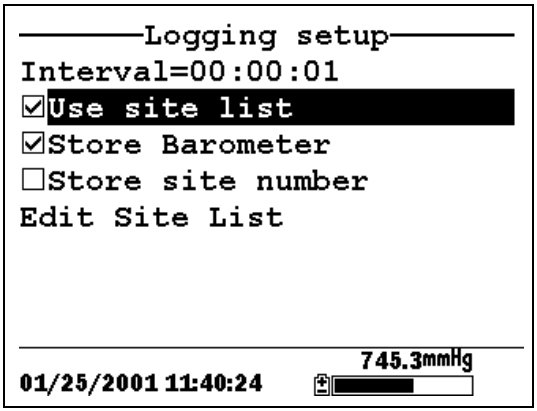


Figure 9.6 Logging Setup Screen

4. Use the arrow keys to highlight the **Store site number** selection.

5. If you are creating Multi-Site Descriptions (which require that the site **number** be stored in your data files), press the **Enter** key until a check mark appears in the box next to the store site number selection.

OR Press the **Enter** key until the box next to the store site number selection is empty, to create Single-Site Descriptions. The site **name** will be stored in the header of your data files.

6. Use the arrow keys to highlight the **Edit site list** selection.

7. Press the **Enter** key. The edit site list screen is displayed. See Figure 9.7 Edit Site List Screen. The **Filename** field is ready for input.

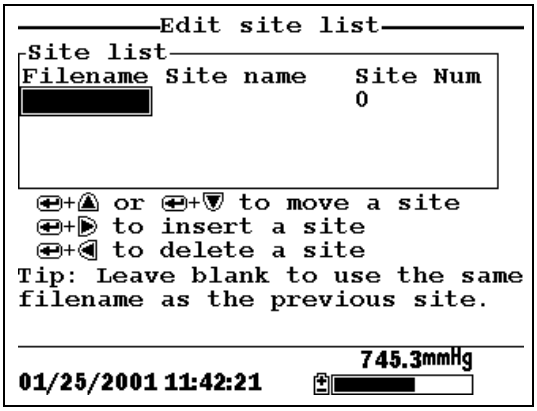


Figure 9.7 Edit Site List Screen

8. Use the keypad to enter a filename up to 8 characters in length. Refer to Section *2.9 Keypad Use*.

9. Press the **Enter** key. The cursor moves to the right for the entry of a **Site name**.

10. Use the keypad to enter a site name up to 11 characters in length. Refer to Section *2.9 Keypad Use*.

    NOTE: If the store site number selection is *not* checked, skip to Step 13.

11. Press the **Enter** key. The cursor moves to the site number entry position.

12. Use the keypad to enter a site number up to 7 characters in length. Refer to Section *2.9 Keypad Use*.

13. Press **Enter**. The cursor moves to the next filename entry position.

14. Repeat Steps 8 to 13 until all filenames and sites have been entered.

15. Press **Escape** repeatedly to return to the main menu screen.

9.5 Editing a Site List

1. Go to the logging setup screen as described in Section *9.1 Accessing the Logging Setup Screen*.

2. Use the arrow keys to highlight the **Edit Site List** selection. See Figure 9.6 Logging Setup Screen.

3. Press the **Enter** key. The edit site list screen is displayed.

4. Edit the site list using the keystrokes described below.

   NOTE: Editing the site list will not have any effect on files stored in the instrument memory.

<u>To MOVE a site:</u> **Use the arrow keys to highlight a site. Press the Up or Down arrow key while holding down the Enter key.**

<u>To INSERT a site above another site:</u> **Use the arrow keys to highlight the site. Press the** Right **arrow key while holding down the** Enter **key. Use keypad to input letters. Refer to Section *2.9 Keypad Use*.**

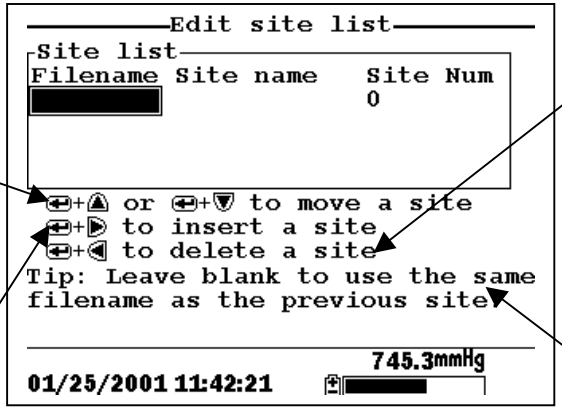


Figure 9.8 Keystrokes for Editing Site List

<u>To DELETE a site:</u> **Use the arrow keys to highlight a site. Press the Left arrow key while holding down the Enter key.**

<u>To use the same file name as the previous site:</u> **Leave the filename blank.**

9.6 Logging Data Without a Site List

1. Follow Steps 1 through 5 in Section 7.1 Real-Time Data.

2. Use the arrow keys to highlight the **Log one sample** selection on the run screen if only a single sample is being logged.

   OR Use the arrow keys to highlight the **Start logging** selection on the run screen if a data stream is being logged.

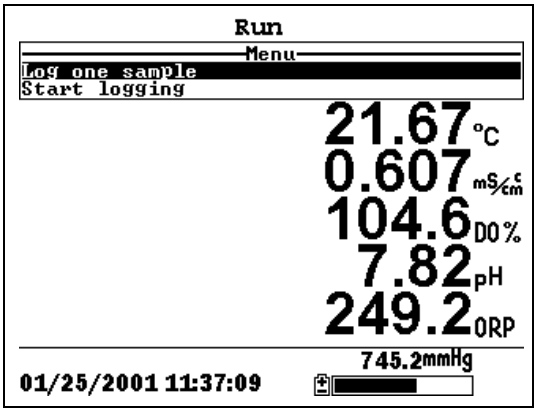


Figure 9.9 Run Screen

3. Press the **Enter** key. The Enter information screen is displayed.

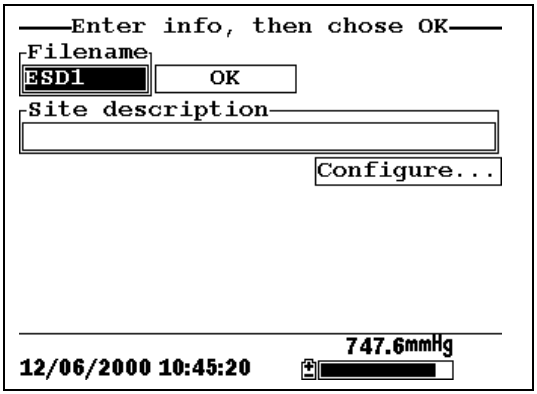


Figure 9.10 Enter Information Screen

NOTE: The last filename used will be displayed.

4. Use the keypad to enter a file name. Refer to Section *2.9 Keypad Use*.

   NOTE: The instrument will assign a default file name of NONAME if no file name is specified.

5. Press the **Enter** key to input the file name.

6. Use the arrow keys to highlight the **Site description** field in the enter information screen.

   NOTE: Entering a Site Description is optional. You may leave the Site Description blank and skip to Step 9.

7. Use the keypad to enter a site description name. Refer to Section *2.9 Keypad Use*.

8. Press the **Enter** key to input the site description.

   NOTE: If you want to change the logging setup, such as sampling interval or storing the barometer reading, use the arrow keys to highlight the **Configure** field, press the **Enter** key, then refer to Section *9.2 Setting Logging Interval* or *9.3 Storing Barometer Readings* for details.

9. Use the arrow keys to highlight the **OK** field in the center of the information screen.

10. Press the **Enter** key to start logging.

    NOTE: If the parameter mismatch screen is displayed, refer to Section *9.8 Adding Data to Existing Files*.

11. If a single point is being logged, the header on the run screen changes momentarily from **Menu** to **Sample logged** to confirm that the point was successfully logged. Skip to Step 13.

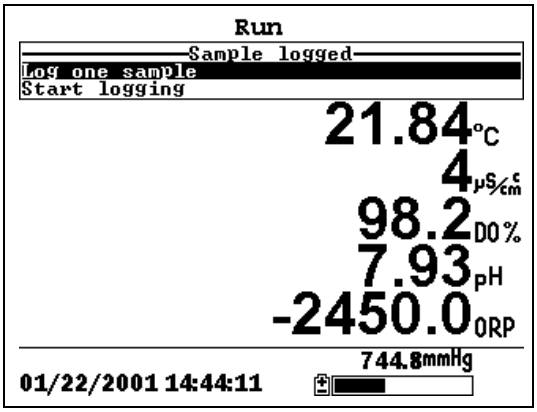


Figure 9.11 Sample Logged Screen

If a continuous stream of points is being logged, the start logging entry in the run screen changes from **Start logging** to **Stop logging**.

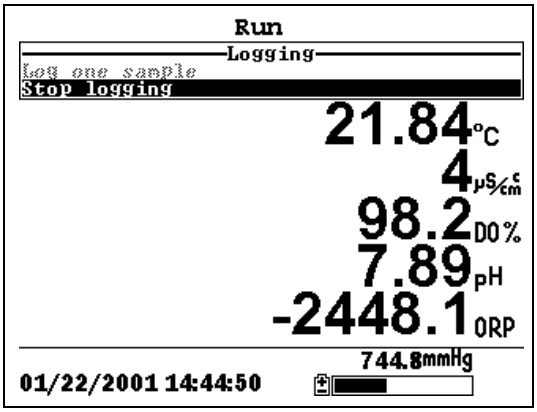


Figure 9.12 Logging Screen

12. At the end of the logging interval, press **Enter** to stop logging.

13. Refer to Section *8.3 View File* to view the data on the instrument display.

9.7 Logging Data With a Site List

1. If you have not already created a site list, refer to Section *9.4 Creating a Site List*.

2. Follow Steps 1 through 5 in Section 7.1 Real-Time Data.

3. Use the arrow keys to highlight the **Log one sample** selection on the run screen if only a single sample is being logged.

   OR Use the arrow keys to highlight the **Start logging** selection on the run screen if a data stream is being logged. See Figure 9.9 Run Screen.

4. Press the **Enter** key. The Pick a site screen is displayed.

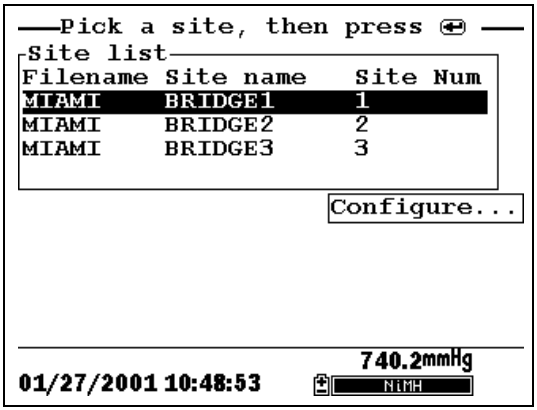


Figure 9.13 Pick a Site Screen

5. Use the arrow keys to highlight the **site** of your choice.

   NOTE: If the site of your choice is grayed out in the site list, refer to Section *9.8 Adding Data to Existing Files*.

   NOTE: Refer to Section *9.5 Editing a Site List* if you want to edit the site list.

6. Press the **Enter** key to start logging.

NOTE: If the parameter mismatch screen is displayed, refer to Section *9.8 Adding Data to Existing Files*.

7. If a single point is being logged, the header on the run screen changes momentarily from **Menu** to **Sample logged** to confirm that the point was successfully logged. See Figure 9.11 Sample Logged Screen. Skip to Step 9.

   If a continuous stream of points is being logged, the start logging entry in the run screen changes from **Start logging** to **Stop logging**. See Figure 9.12 Logging Screen.

8. At the end of the logging interval, press **Enter** to stop logging.

9. Refer to Section *8.3 View File* to view the data on the instrument display.

9.8 Adding Data to Existing Files

In order to add new data to an existing file, the current logging and sensor setup must be *exactly* the same as when the file was created. The following settings must be the same:

- **Sensors enabled** (refer to Section *4 Sensors*)
- **Store Barometer** (refer to Section *9.3 Storing Barometer Readings*)
- **Store Site Number** (refer to Section *9.4 Creating a Site List*)

If the current logging setup is not exactly the same as when the file was created, a parameter mismatch screen is displayed.

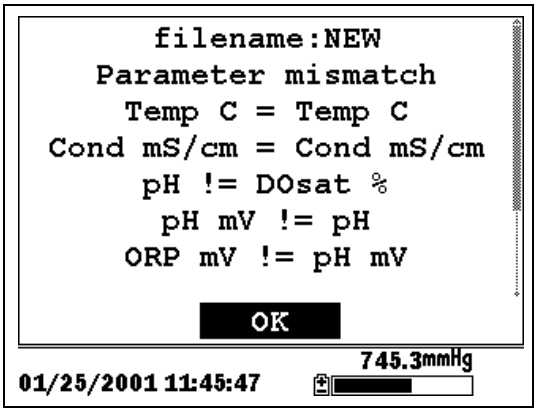


Figure 9.14 Parameter Mismatch Screen

NOTE: The right column shows parameters used when the file was created. The left column shows current parameters.

1. Press the **Down Arrow** key to scroll down and find the mismatch(es).

2. Use the following chart to resolve the mismatch(es).

Mismatch	Action	Reference
Sensor(s) missing from left column	Enable the missing sensor(s)	Section *4 Sensors*
Extra sensor(s) listed in left column	Disable the extra sensor(s)	Section *4 Sensors*
Barometer missing from left column, but present in right column	Enable the Store Barometer setting	Section *9.3 Storing Barometer Readings*
Barometer present in left column, but missing from right column	Disable the Store Barometer setting	Section *9.3 Storing Barometer Readings*
Store Site Number missing from left column, but present in right column	Enable the Store Site Number setting	Section *9.4 Creating a Site List*
Store Site Number present in left column, but missing from right column	Disable the Store Site Number setting	Section *9.4 Creating a Site List*

3. Return to Section *9.6 Logging Data Without a Site List* or *9.7 Logging Data With a Site List*.

10. System Setup

The YSI 556 MPS has a number of features that are user-selectable or can be configured to meet the user's preferences. Most of these choices are found in the **System setup** menu.

10.1 Accessing the System Setup Screen

1. Press the **On/off** key to display the run screen. See Figure 2.1 Front View of YSI 556 MPS.

2. Press the **Escape** key to display the main menu screen.

3. Use the arrow keys to highlight the **System setup** selection.

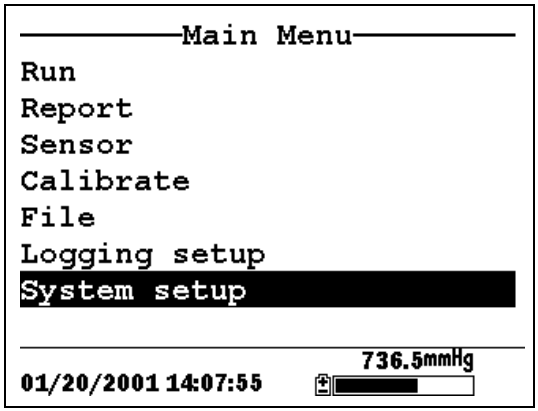


Figure 10.1 Main Menu

4. Press the **Enter** key. The system setup screen is displayed.

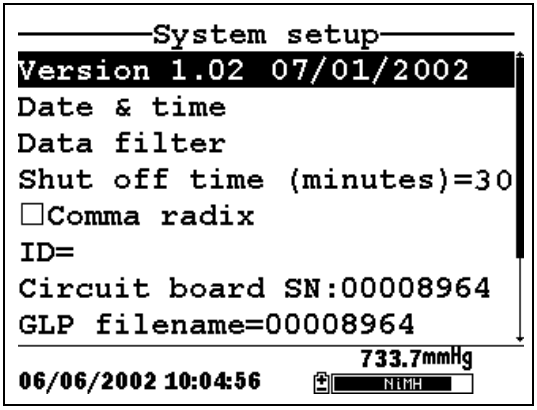


Figure 10.2 System Setup Screen

NOTE: The first line of the **System setup** menu shows the current software version of your YSI 556 MPS. As software enhancements are introduced, you will be able to upgrade your YSI 556 MPS from the YSI Web site. Refer to Section *11.2 Upgrading YSI 556 MPS* Software for details.

10.2 Date and Time Setup

1. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

2. Use the arrow keys to highlight the **Date & time** selection on the system setup screen. See Figure 10.2 System Setup Screen.

3. Press **Enter**. The date and time setup screen is displayed.

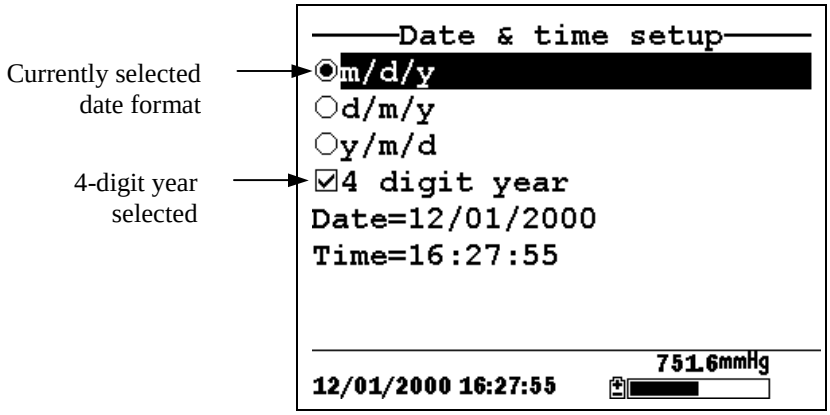


Figure 10.3 Date Setup Screen

NOTE: A black dot to the left of a date format indicates that format is selected.

4. Use the arrow keys to highlight your desired date format.

5. Press **Enter**.

6. Use the arrow keys to highlight the 4-digit year selection.

7. Press **Enter**. A check mark appears in the check box next to the 4-digit year selection.

   NOTE: If unchecked, a 2-digit year is used.

8. Use the arrow keys to highlight the **Date** selection.

9. Press **Enter**. A cursor appears over the first number in the date.

10. Enter the proper number from the keypad for the highlighted date digit. The cursor moves automatically to the next date digit. Refer to Section *2.9 Keypad Use* for more keypad information.

11. Repeat Step 10 until all date digits are correct.

12. Press **Enter** to input the specified date.

13. Use the arrow keys to highlight the **Time** selection.

14. Press **Enter**. A cursor appears over the first number in the time selection.

15. Enter the proper number from the keypad for the highlighted time digit. The cursor moves automatically to the next time digit.

    NOTE: Use military format when entering time. For example, 2:00 PM is entered as 14:00.

16. Repeat Step 15 until all time digits are correct.

17. Press **Enter** to input the correct time.

18. Press the **Escape** key repeatedly to return to the Main menu screen.

10.3 Data Filter

The Data Filter is a software filter that eliminates sensor noise and provides more stable readings.

NOTE: YSI recommends using the default values for the data filter for most field applications.

However, users who are primarily interested in a fast response from their dissolved oxygen sensor should consider a change of the default time constant setting of 8 seconds to one of 2 seconds. This change can be made according to the instructions in Section *10.3.1 Changing the Data Filter Settings* below. The disadvantage of lowering the time constant is that field pH readings may appear somewhat noisy if the cable is in motion.

10.3.1 Changing the Data Filter Settings

1. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

2. Use the arrow keys to highlight the **Data filter** selection. See Figure 10.1 Main Menu.

3. Press the **Enter** key. The Data filter setup screen is displayed.

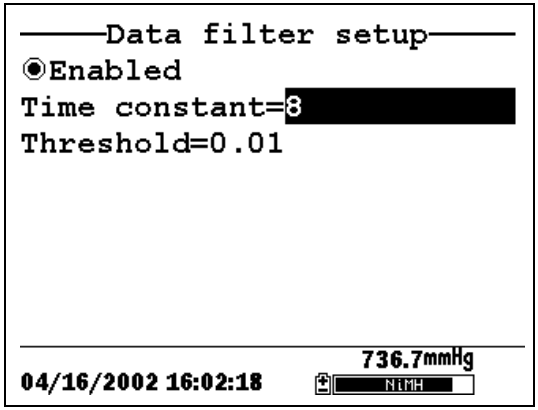


Figure 10.4 Data Filter Screen

4. With Enabled highlighted, press the **Enter** key to Enable or Disable the data filter. A black dot to the left of the selection indicates the data filter is enabled.

5. Use the arrow keys to highlight the **Time constant** field.

   NOTE: This value is the time constant in seconds for the software data filter. Increasing the time constant will result in greater filtering of the data, but will also slow down the apparent response of the sensors.

6. Use the keypad to enter a value. The default value is 8 and this value is ideal for most 556 field applications. As described in Section *10.3 Data Filter* above, users who wish to decrease the response time of the DO readings at the expense of some noise for the pH readings determined concurrently, should change the Time Constant to a value of 2.

7. Press the **Enter** key to enter the time constant.

8. Use the arrow keys to highlight the **Threshold** field.

   NOTE: This value determines when the software data filter will engage/disengage, speeding the response to large changes in a reading. When the difference between two consecutive readings is larger than the threshold, then the reading is displayed unfiltered. When the difference between two consecutive readings drops below the threshold, readings will be filtered again.

9. Use the keypad to enter a value. The default value is 0.01.

10. Press the **Enter** key to enter the threshold.

11. Press the **Escape** key repeatedly to return to the Main menu screen.

10.4 Shutoff Time

The YSI 556 MPS shuts off automatically after 30 minutes of inactivity. The shut off time may be changed as described below.

1. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

2. Use the arrow keys to highlight the **Shutoff time** selection on the system setup screen. See Figure 10.2 System Setup Screen.

3. Use the keypad to enter a value from 0 to 60 minutes. The default value is 30.

   NOTE: To disable the automatic shutoff feature, enter a zero (0).

4. Press the **Enter** key to enter the correct shutoff time.

5. Press the **Escape** key repeatedly to return to the main menu screen.

10.5 Comma Radix

The user can toggle between a period (default) and comma for the radix mark by selecting this item and pressing the **Enter** key as follows:

1. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

2. Use the arrow keys to highlight the **Comma radix** selection on the system setup screen. See Figure 10.2 System Setup Screen.

3. Press the **Enter** key. A check mark appears in the check box next to the comma radix selection indicating that the radix mark is a comma.

10.6 ID

This selection allows you to enter an identification name/number for your YSI 556 MPS. This ID name/number is logged in the header of each file.

1. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

2. Use the arrow keys to highlight the **ID** selection. See Figure 10.1 Main Menu.

3. Use the keypad to enter an alphanumeric ID up to 15 characters in length. Refer to Section *2.9 Keypad Use*.

4. Press the **Enter** key to enter the ID.

5. Press the **Escape** key repeatedly to return to the main menu screen.

10.7 GLP Filename

This selection allows you to enter a different filename for the YSI 556 MPS Calibration Record file.

NOTE: The default filename is the “556 PC board Serial Number.glp.”

6. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

7. Use the arrow keys to highlight the **GLP Filename** selection. See Figure 10.1 Main Menu.

8. Use the keypad to enter a filename up to 8 characters in length. Refer to Section *2.9 Keypad Use*.

9. Press the **Enter** key to enter the new filename.

Press the **Escape** key repeatedly to return to the main menu screen.

10.8 TDS Constant

This selection allows you to set the constant used to calculate Total Dissolved Solids (TDS). TDS in g/L is calculated by multiplying this constant times the specific conductance in mS/cm.

10.8.1 Changing the TDS Constant

1. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

2. Use the arrow keys to highlight the **TDS Constant** selection. See Figure 10.1 Main Menu.

3. Use the keypad to enter a value. Refer to Section *2.9 Keypad Use*. The default value is 0.65.

4. Press the **Enter** key to enter the correct TDS constant.

5. Press the **Escape** key repeatedly to return to the main menu screen.

10.9 Barometer Units

The following information is only for instruments with the barometer option.

1. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

2. Use the arrow keys to highlight the **Barometer units** selection on the system setup screen. See Figure 10.2 System Setup Screen.

3. Press the **Enter** key. The Barometer units screen will appear.

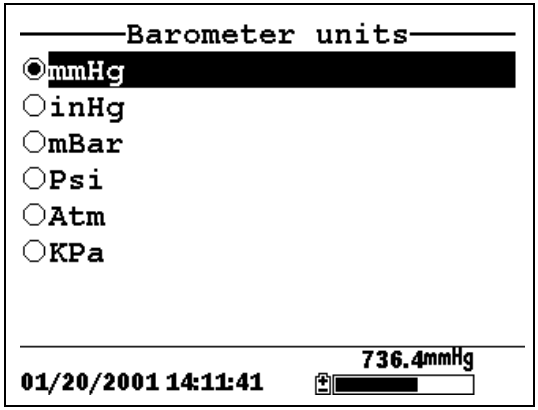


Figure 10.5 Barometer Units Screen

A black dot indicates the currently selected units.

4. Use the arrow keys to highlight your desired barometric unit.

5. Press the **Enter** key to select your choice. A black dot will appear in the circle next to your selected units.

6. Press the **Escape** key repeatedly to return to the main menu screen.

10.10 Calibrate Barometer

The optional barometer has been factory calibrated to provide accurate readings. However, some sensor drift may occur over time, requiring occasional calibration by the user, as follows:

1. Determine your local barometric pressure from an independent laboratory barometer or from your local weather service.

2. If the barometric pressure (BP) reading is from your local weather station, reverse the equation that corrects it to sea level.

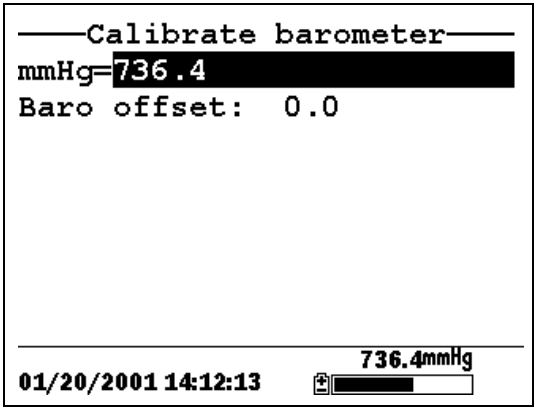
   NOTE: For this equation to be accurate, the barometric pressure units must be in mm Hg.

   True BP = (Corrected BP) – [2.5 * (Local Altitude/100)]

3. Go to the system setup screen as described in Section *10.1 Accessing the System Setup Screen*.

4. Use the arrow keys to highlight the **Calibrate barometer** selection on the system setup screen. See Figure 10.2 System Setup Screen.

5. Press the **Enter** key. The Calibrate Barometer screen is displayed.



6. Use the keypad to input the known barometric pressure value as determined in Step 2.

7. Press the **Enter** key. The new barometer reading is displayed as well as the approximate offset from the factory reading.

   NOTE: To return the sensor to the factory setting, subtract the offset amount from the current setting and repeat Steps 5 to 7.

8. Press the **Escape** key repeatedly to return to the main menu screen.

11. Maintenance

11.1 Sensor Care and Maintenance

Once the sensors have been properly installed, remember that periodic cleaning and DO membrane changes are required.

11.1.1 DO Sensor

For best results, we recommend that the KCl solution and the membrane cap be changed at least once every 30 days.

1. It is important to recognize that oxygen dissolved in the sample is consumed during sensor operation. It is therefore essential that the sample be continuously stirred at the sensor tip. If stagnation occurs, your readings will be artificially low. Stirring may be accomplished by mechanically moving the sample around the sensor tip, or by rapidly moving the sensor through the sample. The rate of stirring should be at least 1 foot per second.

2. Membrane life depends on usage. Membranes will last a long time if installed properly and treated with care. Erratic readings are a result of loose, wrinkled, damaged, or fouled membranes, or from large (more than 1/8" diameter) bubbles in the electrolyte reservoir. If erratic readings or evidence of membrane damage occurs, you should replace the membrane and the electrolyte solution. The average replacement interval is two to four weeks.

3. If the membrane is coated with oxygen consuming (e.g. bacteria) or oxygen producing organisms (e.g. algae), erroneous readings may occur.

4. Chlorine, sulfur dioxide, nitric oxide, and nitrous oxide can affect readings by behaving like oxygen at the sensor. If you suspect erroneous readings, it may be necessary to determine if these gases are the cause.

5. Avoid any environment that contains substances that may attack the probe module and sensor materials. Some of these substances are concentrated acids, caustics, and strong

solvents. The sensor materials that come in contact with the sample include FEP Teflon, acrylic plastic, EPR rubber, stainless steel, epoxy, polyetherimide and the PVC cable covering.

6. It is possible for the silver anode, which is the entire silver body of the sensor, to become contaminated. This will prevent successful calibration. To restore the anode, refer to Section *11.1.1 DO Sensor, Silver Anode Cleaning.*

7. For correct sensor operation, the gold cathode must always be bright. If it is tarnished (which can result from contact with certain gases), or plated with silver (which can result from extended use with a loose or wrinkled membrane), the gold surface must be restored. To restore the cathode, refer to Section *11.1.1 DO Sensor, Gold Cathode Cleaning.*

8. To keep the electrolyte from drying out, store the sensor in the transport/calibration cup with at least 1/8″ of water.

Silver Anode Cleaning

After extended use, a thick layer of AgCl builds up on the silver anode reducing the sensitivity of the sensor. The anode must be cleaned to remove this layer and restore proper performance. The cleaning can be chemical or mechanical:

Chemical Cleaning: Remove the membrane cap and soak the entire anode section in a 14% ammonium hydroxide solution for 2 to 3 minutes, followed by a thorough rinsing with distilled or deionized water. The anode should then be thoroughly wiped with a wet paper towel to remove the residual layer from the anode.

Mechanical Cleaning: Sand off the dark layer from the silver anode with 400 grit wet/dry sandpaper. Wrap the sandpaper around the anode and twist the sensor. Rinse the anode with clean water after sanding, followed by wiping thoroughly with a wet paper towel.

NOTE: After cleaning, a new membrane cap must be installed. Refer to Section *3.4.3 Membrane Cap Installation***.**

Turn the instrument on and allow the system to stabilize for at least 30 minutes. If, after several hours, you are still unable to calibrate, contact your dealer or YSI Customer Service. Refer to *Appendix E Customer Service*.

Gold Cathode Cleaning

For correct sensor operation, the gold cathode must be textured properly. It can become tarnished or plated with silver after extended use. The gold cathode can be cleaned by using the adhesive backed sanding disc and tool provided in the YSI 5238 Probe Reconditioning Kit.

Using the sanding paper provided in the YSI 5238 Probe Reconditioning Kit, wet sand the gold with a twisting motion about 3 times or until all silver deposits are removed and the gold appears to have a matte finish. Rinse the cathode with clean water after sanding, followed by wiping thoroughly with a wet paper towel. If the cathode remains tarnished, contact your dealer or YSI Customer Service. Refer to *Appendix E Customer Service*.

NOTE: After cleaning, a new membrane cap must be installed. Refer to Section *3.4.3 Membrane Cap Installation***.**

11.1.2 DO Sensor Replacement

1. Remove the probe sensor guard.

⚠ **CAUTION:** Thoroughly dry the sensor so that no water enters the probe module sensor port when the sensor is removed.

2. Insert the long end of the hex key wrench into the small hole in the side of the probe module bulkhead. Turn the wrench counterclockwise and remove the screw. (You do not have to remove the screw all the way to release the sensor.)

3. Pull the old DO sensor module straight out of the probe module body.

   NOTE: The DO sensor is not threaded, it is keyed, so it cannot be removed by twisting.

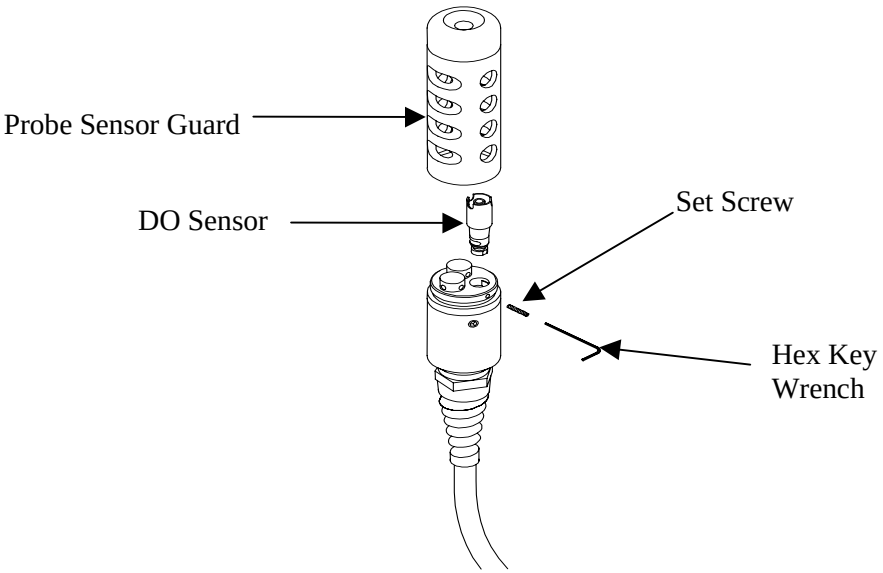


Figure 11.1 DO Sensor Replacement

4. Insert the new DO sensor module. Make sure that the inside of the probe module sensor port and the o-ring on the sensor are clean, with no contaminants, such as grease, dirt, or hair. The DO sensor is keyed, or has a flat side, so that it cannot be aligned improperly.

NOTE: Make sure the DO sensor bottoms out before the set screw is inserted.

5. Insert the set screw into the small hole in the side of the probe module bulkhead, and turn clockwise to rethread.

⚠ **CAUTION:** Make sure that you do not cross-thread the set screw. Use the hex key wrench to tighten the screw in properly, making sure that the screw does not stick out of the side of the probe module bulkhead. The probe sensor guard will not thread

on properly and damage may result if the screw is allowed to stick out.

NOTE: The YSI 5563 DO sensor is shipped dry. A shipping membrane was installed to protect the electrode. A new membrane cap must be installed before the first use. Refer to Section *3.4.1 Sensor Installation.*

11.1.3 YSI 5564 pH and 5565 Combination pH/ORP Sensor Cleaning

Cleaning is required whenever deposits or contaminants appear on the glass and/or platinum surfaces of these sensors or when the response of the sensor becomes slow.

1. Remove the sensor from the probe module.

2. Initially, simply use clean water and a soft clean cloth, lens cleaning tissue, or cotton swab to remove all foreign material from the glass bulb (YSI 5564 and YSI 5565) and platinum button (YSI 5565). Then use a moistened cotton swab to carefully remove any material that may be blocking the reference electrode junction of the sensor.

⚠ **CAUTION:** When using a cotton swab with the YSI 5564 or YSI 5565, be careful NOT to wedge the swab tip between the guard and the glass sensor. If necessary, remove cotton from the swab tip, so that the cotton can reach all parts of the sensor tip without stress.

NOTE: If good pH and/or ORP response is not restored by the above procedure, perform the following additional procedure:

1. Soak the sensor for 10-15 minutes in clean water containing a few drops of commercial dishwashing liquid.

2. GENTLY clean the glass bulb and platinum button by rubbing with a cotton swab soaked in the cleaning solution.

3. Rinse the sensor in clean water, wipe with a cotton swab saturated with clean water, and then re-rinse with clean water.

NOTE: If good pH and/or ORP response is still not restored by the above procedure, perform the following additional procedure:

1. Soak the sensor for 30-60 minutes in one molar (1 M) hydrochloric acid (HCl). This reagent can be purchased from most distributors. Be sure to follow the safety instructions included with the acid.

2. GENTLY clean the glass bulb and platinum button by rubbing with a cotton swab soaked in the acid.

3. Rinse the sensor in clean water, wipe with a cotton swab saturated with clean water, and then re-rinse with clean water. To be certain that all traces of the acid are removed from the sensor crevices, soak the sensor in clean water for about an hour with occasional stirring.

NOTE: If biological contamination of the reference junction is suspected or if good response is not restored by the above procedures, perform the following additional cleaning step:

1. Soak the sensor for approximately 1 hour in a 1 to 1 dilution of commercially available chlorine bleach.

2. Rinse the sensor with clean water and then soak for at least 1 hour in clean water with occasional stirring to remove residual bleach from the junction. (If possible, soak the sensor for period of time longer than 1 hour in order to be certain that all traces of chlorine bleach are removed.) Then re-rinse the sensor with clean water and retest.`

11.1.4 Temperature/Conductivity Sensor Cleaning

The single most important requirement for accurate and reproducible results in conductivity measurement is a clean cell. A dirty cell will change the conductivity of a solution by contaminating it. The small cleaning brush included in the YSI 5511 Maintenance Kit is ideal for this purpose.

To clean the conductivity cell:

1. Dip the brush in clean water and insert it into each hole 15-20 times.

2. Rinse the cell thoroughly in deionized or clean tap water.

NOTE: In the event that deposits have formed on the electrodes, perform the following additional procedure:

1. Use a mild detergent solution in combination with the brush. Dip the brush in the solution and insert it into each hole 15-20 times.

2. Rinse the cell thoroughly in deionized or clean tap water.

   NOTE: After cleaning, check the response and accuracy of the conductivity cell with a calibration standard.

   NOTE: If this procedure is unsuccessful, or if sensor performance is impaired, it may be necessary to return the sensor to a YSI authorized service center for service, Refer to *Appendix E Customer Service*.

   The temperature portion of the sensor requires no maintenance.

11.2 Upgrading YSI 556 MPS Software

1. Access the YSI Environmental Software Downloads page as described in *Appendix G EcoWatch* Step 1 through 3.

2. Click on the **YSI Instruments Software Updates** link (or scroll down until you see YSI 556 MPS).

3. Click on the file icon to the right of the **YSI 556 MPS** listing and save the file to a temporary directory on your computer.

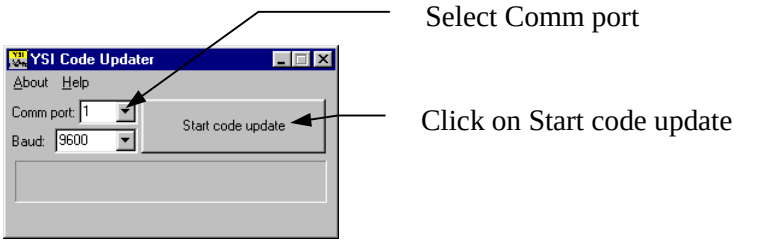
4. After the download is complete, run the file (that you just downloaded) and follow the on screen instructions to install the YSI Code Updater on your computer. If you encounter difficulties, contact YSI customer service for advice. Refer to *Appendix E Customer Service*.

5. If necessary, disconnect the YSI 5563 Probe Module from the YSI 556 MPS instrument.

6. Connect the YSI 556 MPS to a serial port of your computer via the 655173 PC interface cable. See Figure 8.6 Computer/Instrument Interface.

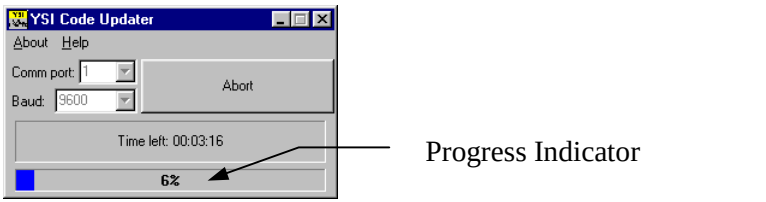
7. Press the **On/off** key on the YSI 556 MPS to display the run screen.

8. Run the YSI Code Updater software that you just installed on your computer. The following window will be displayed:

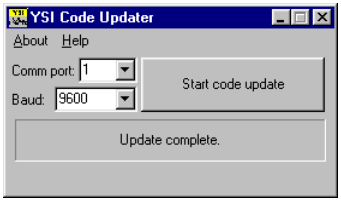


9. Set the Comm port number to match the port that you connected the 655173 PC Interface Cable to, then click on the **Start Code Update button**.

The YSI 556 MPS screen will blank out and a progress indicator will be displayed on the PC.



When the update is finished (indicated on the PC screen), the YSI 556 MPS will return to the Run screen. See Figure 7.1 Run Screen.



10. Close the YSI Code Updater window (on the PC) by clicking on the "X" in the upper right corner of the window.

11. Disconnect the YSI 556 MPS from the 655173 PC interface cable and reconnect it to the YSI 5563 Probe Module. Refer to Section *3.6 Instrument/Cable Connection*.

12. Storage

Proper storage between periods of usage will not only extend the life of the sensors, but will also ensure that the unit will be ready to use as quickly as possible in your next application.

12.1 General Recommendations for Short Term Storage

No matter what sensors are installed in the instrument, it is important to keep them moist without actually immersing them in liquid. Immersing them could cause some of them to drift or result in a shorter lifetime.

YSI recommends that short term storage of all multi-parameter instruments be done by placing approximately 1/2 inch of tap water in the transport/calibration cup that was supplied with the instrument, and by placing the probe module with all of the sensors installed into the cup. The use of a moist sponge instead of a 1/2 inch of tap water is also acceptable, as long as its presence does not compromise the attachment of the cup to the probe module. The transport/calibration cup should be sealed to prevent evaporation.

NOTE: Ensure that an o-ring is installed in the o-ring groove on the threaded end of the probe module body. See Figure 3.7 Transport/Calibration Cup Installation.

⚠ **CAUTION:** The water level has to be low enough so that none of the sensors are actually under water. Check the transport/calibration cup periodically to make certain that the water is still present or the sponge is still moist.

NOTE: If the storage water (tap water) is accidentally lost during field use, environmental water can be used.

12.2 General Recommendations for Long Term Storage

12.2.1 Probe Module Storage

1. Remove the pH or pH/ORP sensor from the probe module and store according to the individual sensor storage instructions found in Section *12.2.2 Sensor Storage*.

2. Seal the empty port with the provided port plug.

NOTE: Leave the conductivity/temperature sensor and dissolved oxygen sensor, with membrane cap still on, in the probe module.

3. Place 1/2″ of water, deionized, distilled or tap, in the transport/calibration cup.

⚠ **CAUTION:** The water level has to be low enough so that none of the sensors are actually under water. Check the transport/calibration cup periodically to make certain that the water is still present or the sponge is still moist.

4. Insert the probe module into the cup.

NOTE: Ensure that an o-ring is installed in the o-ring groove on the threaded end of the probe module body. See Figure 3.7 Transport/Calibration Cup Installation.

12.2.2 Sensor Storage

Temperature/Conductivity Sensor

No special precautions are required. Sensor can be stored dry or wet, as long as solutions in contact with the thermistor and conductivity electrodes are not corrosive (for example, chlorine bleach). However, it is recommended that the sensor be cleaned with the provided brush prior to long term storage. Refer to Section *11.1.4 Temperature/Conductivity Sensor Cleaning.*

pH and Combination pH/ORP Sensor

The key to sensor storage is to make certain that the reference electrode junction does not dry out. Junctions which have been allowed to dry out due to improper storage procedures can usually be rehydrated by soaking the sensor for several hours (overnight is recommended) in a solution which is 2 molar in potassium chloride. If potassium chloride solution is not available, soaking the sensor in tap water or commercial pH buffers may restore sensor function. However in some cases the sensor may have been irreparably damaged by the dehydration and will require replacement.

⚠ **CAUTION:** Do not store the sensor in distilled or deionized water as the glass sensor may be damaged by exposure to this medium.

1. Remove the pH or pH/ORP sensor from the probe module.

2. Seal the empty port with the provided port plug.

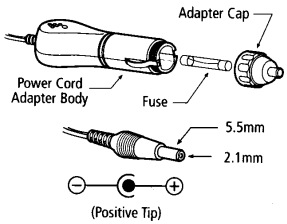
3. Place the sensor in the storage vessel (plastic boot or bottle) which was on the sensor at delivery. The vessel should contain a solution which is 2 molar in potassium chloride.

   NOTE: Make certain that the vessel is sealed to prevent evaporation of the storage solution.

13. Troubleshooting

The following sections describe problems you may encounter when using the YSI 556 MPS and provides suggestions to overcome the symptom.

PROBLEM	POSSIBLE SOLUTION
Display Problems	
No display is visible after pressing the on/off key.	If C cells are used, make certain that they are installed properly with regard to polarity and that good batteries are used. If a rechargeable battery pack is used, place the pack in the instrument and charge for 30 minutes.
Instrument software appears to be locked up as evidenced by no response to keypad entries or display not changing.	First, attempt to reset the instrument by simply turning off and then on again. If this fails, remove battery power from the instrument for 30 seconds and then reapply power. When using C cells, remove the battery lid and one of the batteries; when using the rechargeable battery pack, remove the pack completely from the instrument. After 30 seconds replace the battery or battery pack and check for instrument function.
The 556 display flashes and the instrument speaker makes a continuous clicking sound.	The battery voltage is low. Change to new C cells or recharge the 6117 battery pack.
Water Damage to Instrument	
Leakage detected in battery compartment when using C cells	Dispose of batteries properly. Dry the battery compartment using compressed air if possible. If corrosion is present on battery terminals, contact YSI Customer Service.
Water has contacted rechargeable battery pack	Remove battery pack immediately. Send battery pack to YSI Product Service for evaluation. **CAUTION**: DO NOT REUSE BATTERY PACK UNTIL YSI PRODUCT SERVICE HAS EVALUATED IT.
Leakage suspected into the main cavity of the instrument case	Remove the batteries immediately. Return the instrument to YSI Product Service.

PROBLEM	POSSIBLE SOLUTION
Optional Cigarette Lighter Charger	
Power cord fuse blown 	1. Unscrew adapter's cap, remove tip and pull out fuse. 2. Replace fuse with a new 2-amp fast-blow fuse from an electronics store such as Radio Shack. 3. Reassemble the adapter and securely screw the cap back onto the adapter body.
File Problems	
Upload of files from YSI 556 MPS to PC fails	1. Make sure that cable is connected properly to both 556 and PC. 2. Make certain that the proper Comm port is selected in EcoWatch for Windows.
Barometer data is not stored with sensor data file.	Make sure **Store barometer** is active in the 556 **Logging setup** menu.
Site Descriptions in the **Site List** are "grayed-out" and not available for appending files with additional data.	There is a parameter mismatch between the current 556 setup and that initially used. Change the current logging and sensor setup to match the setup that was initially used to create the file.
Sensor Problems	
Dissolved Oxygen reading unstable or inaccurate. Out of Range message appears during calibration.	Sensor not properly calibrated. Follow DO cal procedures.
	Membrane not properly installed or may be punctured. Replace membrane cap.
	DO sensor electrodes require cleaning. Follow DO cleaning procedure. Use 5511 Maintenance kit.
	Water in sensor connector. Dry connector; reinstall sensor.
	Algae or other contaminant clinging to DO sensor. Rinse DO sensor with clean water.
	Barometric pressure entry is incorrect. Repeat DO cal procedure.
	Calibrated at extreme temperature. Recalibrate at (or near) sample temperature.
	DO sensor has been damaged. Replace sensor.
	Internal failure. Return probe module for service.

PROBLEM	POSSIBLE SOLUTION
Sensor Problems	
pH or ORP readings are unstable or inaccurate. Out of Range message appears during calibration.	Sensor requires cleaning. Follow sensor cleaning procedure.
	Sensor requires calibration. Follow cal procedures.
	pH sensor reference junction has dried out from improper storage. Soak sensor in tap water or buffer until readings become stable.
	Water in sensor connector. Dry connector; reinstall sensor.
	Sensor has been damaged. Replace sensor.
	Calibration solutions out of spec or contaminated with other solution. Use new calibration solutions.
	ORP fails Zobell check. Take into account temperature dependence of Zobell solution readings.
	Internal failure. Return probe module for service.
Conductivity unstable or inaccurate. Out of Range message appears during calibration.	Conductivity improperly calibrated. Follow calibration procedure.
	Conductivity sensor requires cleaning. Follow cleaning procedure.
	Conductivity sensor damaged. Replace sensor.
	Calibration solution out of spec or contaminated. Use new calibration solution.
	Internal failure. Return probe module for service.
	Calibration solution or sample does not cover entire sensor. Immerse sensor fully.
Temperature, unstable or inaccurate	Water in connector. Dry connector; reinstall sensor.
	Sensor has been damaged. Replace the 5560 sensor.
Installed sensor has no reading	The sensor has been disabled. Enable sensor.
	Water in sensor connector. Dry connector; reinstall sensor.
	Sensor has been damaged. Replace the sensor.
	Report output improperly set up. Set up report output.
	Internal failure. Return probe module for service.

If these guidelines and tips fail to correct your problem or if any other symptoms occur, contact YSI Customer Service for Advice. Refer to *Appendix E Customer Service*.

14. Appendix A YSI 556 MPS Specifications

14.1 Sensor Specifications

Dissolved Oxygen	
Sensor Type	Steady state polarographic
Range: *% air sat'n* *mg/L*	▪ 0 to 500% air saturation ▪ 0 to 50 mg/L
Accuracy: *% air sat'n* *mg/L*	▪ 0 to 200% air saturation: ±2% of the reading or 2% air saturation; whichever is greater ▪ 200 to 500% air saturation: ±6% of the reading ▪ 0 to 20 mg/L: ±2% of the reading or 0.2 mg/L; whichever is greater ▪ 20 to 50 mg/L: ±6% of the reading
Resolution: *% air sat'n* *mg/L*	▪ 0.1% air saturation ▪ 0.01 mg/L
Temperature	
Sensor Type:	YSI Precision™ thermistor
Range:	-5 to 45°C
Accuracy:	±0.15°C
Resolution:	0.01°C
Conductivity	
Sensor Type:	4-electrode cell with auto-ranging
Range:	0 to 200 mS/cm
Accuracy:	±0.5% of reading or ±0.001 mS/cm; whichever is greater–4 meter cable ±1.0% of reading or ±0.001 mS/cm; whichever is greater–20 meter cable
Resolution:	0.001 mS/cm to 0.1 mS/cm (range-dependent)
Salinity	
Sensor Type:	Calculated from conductivity and temperature
Range:	0 to 70 ppt
Accuracy:	±1.0% of reading or 0.1 ppt; whichever is greater
Resolution:	0.01 ppt

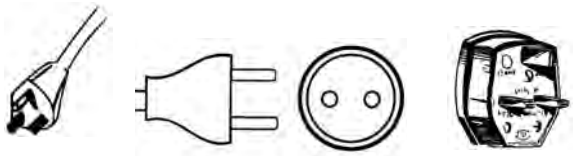
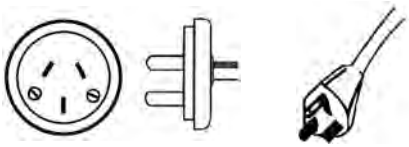
pH (optional)	
Sensor Type:	Glass combination electrode
Range:	0 to 14 units
Accuracy:	±0.2 units
Resolution:	0.01 units
ORP (optional)	
ORP Sensor Type:	Platinum button
Range:	-999 to +999 mV
Accuracy:	±20 mV
Resolution:	0.1 mV

Barometer (optional)	
Range:	500 to 800 mm Hg
Accuracy:	±3 mm Hg within ±15°C temperature range from calibration point
Resolution:	0.1 mm Hg

14.2 Instrument Specifications

Memory Size:	1.5 MB Flash Memory 49,000 data sets (@ 6 parameters per set plus time stamp) 100 Sites
Size:	11.9 cm width x 22.9 cm length (4.7 in. x 9 in.)
Weight with batteries:	0.92 kg (2.1 lbs)
Power:	4 alkaline C-cells; optional rechargeable pack
Cables:	4, 10, and 20 m (13.1, 32.8, 65.6 ft.) lengths
Warranty:	3-Years for the instrument; 1-Year for the probe modules and cable

15. Appendix B Instrument Accessories

ITEM #	ACCESSORY
5563-4	4m Cable with DO/temp/conductivity
5563-10	10m Cable with DO/temp/conductivity
5563-20	20m Cable with DO/temp/conductivity
5564	pH Kit
5565	pH/ORP Kit
6118	Rechargeable Battery Pack Kit for use in US
5094	Rechargeable Battery Pack Kit with universal charger and three adapter cables for use in international applications 
5095	Rechargeable Battery Pack Kit with universal charger and two adapter cables for use in international applications 
5083	Flow Cell – probe module is secured in the flow cell and groundwater is pumped through it
616	Charger, Cigarette Lighter – used to power up the instrument from a car's cigarette lighter
4654	Tripod
614	Ultra Clamp, C Clamp –used to clamp the instrument to a table top or car dashboard
6081	Large Carrying Case, Hard-sided
5085	Hands-free Harness
5065	Carrying Case, Form-fitted, for use in the field – has a clear vinyl window, shoulder strap, belt loop strap and hand strap

16. Appendix C Required Federal Communications Notice

The Federal Communications Commission defines this product as a computing device and requires the following notice.

This equipment generates and uses radio frequency energy and if not installed and used properly, may cause interference to radio and television reception. It has been type tested and found to comply with the limits for a Class A or Class B computing device in accordance with the specification in Subpart J of Part 15 of FCC Rules, which are designed to provide reasonable protection against such interference in a residential installation. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient the receiving antenna
- Relocate the computer with respect to the receiver
- Move the computer away from the receiver
- Plug the computer into a different outlet so that the computer and receiver are on different branch circuits.

If necessary, the user should consult the dealer or an experienced radio/television technician for additional suggestions. The user may find the following booklet, prepared by the Federal Communications Commission, helpful: "How to Identify and Resolve Radio-TV Interference Problems". This booklet is available from the U.S. Government Printing Office, Washington, D.C. 20402, Stock No.0004-000-00345-4.

17. Appendix D Health and Safety

YSI Conductivity solutions: 3161, 3163, 3165, 3167, 3168, 3169

INGREDIENTS:

- ❑ Iodine
- ❑ Potassium Chloride
- ❑ Water

WARNING: INHALATION MAY BE FATAL.

⚠ ***CAUTION*: AVOID INHALATION, SKIN CONTACT, EYE CONTACT OR INGESTION. MAY EVOLVE TOXIC FUMES IN FIRE.**

Harmful if ingested or inhaled. Skin or eye contact may cause irritation. Has a corrosive effect on the gastro-intestinal tract, causing abdominal pain, vomiting, and diarrhea. Hyper-sensitivity may cause conjunctivitis, bronchitis, skin rashes etc. Evidence of reproductive effects.

FIRST AID:
INHALATION: Remove victim from exposure area. Keep victim warm and at rest. In severe cases seek medical attention.
SKIN CONTACT: Remove contaminated clothing immediately. Wash affected area thoroughly with large amounts of water. In severe cases seek medical attention.
EYE CONTACT: Wash eyes immediately with large amounts of water, (approx. 10 minutes). Seek medical attention immediately.
INGESTION: Wash out mouth thoroughly with large amounts of water and give plenty of water to drink. Seek medical attention immediately.

YSI pH 4.00, 7.00, and 10.00 Buffer Solutions: 3821, 3822, 3823

pH 4 INGREDIENTS:

- ❑ Potassium Hydrogen Phthalate
- ❑ Formaldehyde
- ❑ Water

pH 7 INGREDIENTS:

- ❑ Sodium Phosphate, Dibasic
- ❑ Potassium Phosphate, Monobasic
- ❑ Water

pH 10 INGREDIENTS:

- ❑ Potassium Borate, Tetra
- ❑ Potassium Carbonate
- ❑ Potassium Hydroxide
- ❑ Sodium (di) Ethylenediamine Tetraacetate
- ❑ Water

⚠ ***CAUTION* - AVOID INHALATION, SKIN CONTACT, EYE CONTACT OR INGESTION. MAY AFFECT MUCOUS MEMBRANES.**

Inhalation may cause severe irritation and be harmful. Skin contact may cause irritation; prolonged or repeated exposure may cause Dermatitis. Eye contact may cause irritation or conjunctivitis. Ingestion may cause nausea, vomiting and diarrhea.

FIRST AID:

INHALATION - Remove victim from exposure area to fresh air immediately. If breathing has stopped, give artificial respiration. Keep victim warm and at rest. Seek medical attention immediately.

SKIN CONTACT - Remove contaminated clothing immediately. Wash affected area with soap or mild detergent and large amounts of water (approx. 15-20 minutes). Seek medical attention immediately.

EYE CONTACT - Wash eyes immediately with large amounts of water (approx. 15-20 minutes), occasionally lifting upper and lower lids. Seek medical attention immediately.

INGESTION - If victim is conscious, immediately give 2 to 4 glasses of water and induce vomiting by touching finger to back of throat. Seek medical attention immediately.

YSI Zobell Solution: 3682

INGREDIENTS:

- ❑ Potassium Chloride
- ❑ Potassium Ferrocyanide Trihydrate
- ❑ Potassium Ferricyanide

⚠ ***CAUTION* - AVOID INHALATION, SKIN CONTACT, EYE CONTACT OR INGESTION. MAY AFFECT MUCOUS MEMBRANES.**

May be harmful by inhalation, ingestion, or skin absorption. Causes eye and skin irritation. Material is irritating to mucous membranes and upper respiratory tract. The chemical, physical, and toxicological properties have not been thoroughly investigated.

Ingestion of large quantities can cause weakness, gastrointestinal irritation and circulatory disturbances.

FIRST AID:

INHALATION - Remove victim from exposure area to fresh air immediately. If breathing has stopped, give artificial respiration. Keep victim warm and at rest. Seek medical attention immediately.

SKIN CONTACT - Remove contaminated clothing immediately. Wash affected area with soap or mild detergent and large amounts of water (approx. 15-20 minutes). Seek medical attention immediately.

EYE CONTACT - Wash eyes immediately with large amounts of water (approx. 15-20 minutes), occasionally lifting upper and lower lids. Seek medical attention immediately.

INGESTION - If victim is conscious, immediately give 2 to 4 glasses of water and induce vomiting by touching finger to back of throat. Seek medical attention immediately.

18. Appendix E Customer Service

For information on Customer Service Centers, refer to *Authorized Service Centers* in this appendix.

Equipment exposed to biological, radioactive, or toxic materials must be cleaned and disinfected before being returned or presented for service. A cleaning certificate must accompany the equipment. Refer to *18.2 Cleaning Instructions* in this appendix.

18.1 YSI Environmental Authorized Service Centers

International Service Centers

YSI Incorporated • Repair Center • 1725 Brannum Lane
Yellow Springs, Ohio • 45387 • Phone: (937) 767-7241
E-Mail: support@ysi.com

Hydrodata Services (UK) Ltd. • Unit 8 • Business Centre West
Avenue One • Letchworth • Herts • SG6 2HB
Phone: (44-1462) 673581 • Fax: (44-1462) 673582
Email: hydrodatauk@cs.com

YSI Nanotech • Kaizuka 1-15-4, Kawasaki-Ku • Kawaskaki
City • Japan • 210-0014 • Phone: 011-814-4222-0009
Fax: 011-81-44-221102 • E-mail: Nanotech@ysi.com

Nortech GSI • 1131 Derry Road East • Mississauga, ONT
L5T 1P3 • Canada • Phone: 800-263-3427 • Fax: 905-564-4700

US Service Centers for Water Quality and 6-Series Instruments

Ohio
YSI Incorporated • Repair Center • 1725 Brannum Lane
Yellow Springs, Ohio • 45387 • Phone: (800) 765-4974
(937) 767-7241• E-Mail: info@ysi.com

California
EQUIPCO Sales and Service • 1110 Burnett Avenue, Suite D
Concord, CA • 94520 • Phone: (800)550-5875
Fax: (510)674-8655

Colorado
Ted D. Miller Associates, Inc. • 2525 S. Wadsworth Blvd., Suite 300 • Lakewood, CO • 80227 • Phone: (303) 989-7737 Fax: (303) 989-8875 • E:mail: tdma@earthlink.net

Mississippi
C.C. Lynch & Associates, Inc. • P.O. Box 456 • 300 Davis Street • Pass Christian, MS • 39571 Phone: (800) 333-2252 (228) 452-4612 • Fax (228) 452-2563

US Service Centers for Water Quality Instruments Only

Florida
Aquatic Eco Systems, Inc. • 1767 Benbow Court • Apopka, Florida • Phone: (407) 886-3939 • Fax: (407) 886-6787

Maine
Q.C. Services • P.O. Box 68 • Harrison, Maine • 04040 Phone: (207) 583-2980

Mississippi
Aquacenter • 166 Seven Oaks Road • Leland, Mississippi 38756 • Phone: (601) 378-2861 • Fax: (601) 378-2862

Oregon
Q.C. Services • P.O. Box 14831 • Portland, Oregon • 97293 Phone: (503) 236-2712

Wisconsin
North Central Labs • 400 Lyons Road • Birnamwood, Wisconsin • Phone: (800) 648-7836 • Fax: (715) 449-2454

18.2 Cleaning Instructions

Equipment exposed to biological, radioactive, or toxic materials must be cleaned and disinfected before being serviced. Biological contamination is presumed for any instrument, probe, or other device that has been used with body fluids or tissues, or with wastewater. Radioactive contamination is presumed for any instrument, probe or other device that has been used near any radioactive source.

If an instrument, probe, or other part is returned or presented for service without a Cleaning Certificate, and if in our opinion it represents a potential biological or radioactive hazard, our service personnel reserve the right to withhold service until appropriate cleaning, decontamination, and certification has been completed. We will contact the sender for instructions as to the disposition of the equipment. Disposition costs will be the responsibility of the sender.

When service is required, either at the user's facility or at a YSI Service Center, the following steps must be taken to ensure the safety of service personnel.

- In a manner appropriate to each device, decontaminate all exposed surfaces, including any containers. 70% isopropyl alcohol or a solution of 1/4-cup bleach to 1-gallon tap water is suitable for most disinfecting. Instruments used with wastewater may be disinfected with .5% Lysol if this is more convenient to the user.
- The user shall take normal precautions to prevent radioactive contamination and must use appropriate decontamination procedures should exposure occur.
- If exposure has occurred, the customer must certify that decontamination has been accomplished and that no radioactivity is detectable by survey equipment.
- Any product being returned to the YSI Repair Center should be packed securely to prevent damage.
- Cleaning must be completed and certified on any product before returning it to YSI.

18.3 Packing Procedure

- Clean and decontaminate items to ensure the safety of the handler.
- Complete and include the Cleaning Certificate.
- Place the product in a plastic bag to keep out dirt and packing material.
- Use a large carton, preferably the original, and surround the product completely with packing material.
- Insure for the replacement value of the product.

18.4 Cleaning Certificate

Organization _____

Department _____

Address _____

City _____ State __ Zip _____

Country _____ Phone _____

Model No. of Device __ Lot Number _____

Contaminant (if known) _____

Cleaning Agent(s) used _____

Radioactive Decontamination Certified?

(Answer only if there has been radioactive exposure)

_____ Yes _____ No

Cleaning Certified By _____

Name Date

18.5 Warranty

The instrument is warranted for three years against defects in workmanship and materials when used for its intended purposes and maintained according to instructions. The probe module and cables are warranted for one year. The dissolved oxygen, temperature/conductivity, pH, and pH/ORP combination sensors are warranted for one year. Damage due to accidents, misuse, tampering, or failure to perform prescribed maintenance is not covered. The warranty period for chemicals and reagents is determined by the expiration date printed on their labels. Within the warranty period, YSI will repair or replace, at its sole discretion, free of charge, any product that YSI determines to be covered by this warranty.

To exercise this warranty, write or call your local YSI representative, or contact YSI Customer Service in Yellow Springs, Ohio. Send the product and proof of purchase, transportation prepaid, to the Authorized Service Center selected by YSI. Repair or replacement will be made and the product returned transportation prepaid. Repaired or replaced products are warranted for the balance of the original warranty period, or at least 90 days from date of repair or replacement.

Limitation of Warranty

This Warranty does not apply to any YSI product damage or failure caused by (i) failure to install, operate or use the product in accordance with YSI's written instructions, (ii) abuse or misuse of the product, (iii) failure to maintain the product in accordance with YSI's written instructions or standard industry procedure, (iv) any improper repairs to the product, (v) use by you of defective or improper components or parts in servicing or repairing the product, or (vi) modification of the product in any way not expressly authorized by YSI.

THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. YSI'S LIABILITY UNDER THIS WARRANTY IS LIMITED TO REPAIR OR REPLACEMENT OF THE PRODUCT, AND THIS SHALL BE YOUR SOLE AND EXCLUSIVE REMEDY FOR ANY DEFECTIVE PRODUCT COVERED BY THIS WARRANTY. IN NO EVENT SHALL YSI BE LIABLE FOR ANY SPECIAL, INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES RESULTING FROM ANY DEFECTIVE PRODUCT COVERED BY THIS WARRANTY.

19. Appendix F Ferrite Bead Installation

⚠ **WARNING:** If you are using your YSI 556 in a European Community (CE) country or in Australia or New Zealand, you must attach a ferrite bead to the 655173 PC Interface Cable and the YSI 6117 Charger Adapter Cable in order to comply with the Residential, Commercial and Light Industrial Class B Limits for radio-frequency emissions specified in EN55011 (CISPR11) for Industrial, Scientific and Medical laboratory equipment. These ferrite assemblies are supplied as part of cable kits.

1. Make a small loop (approximately 5 cm in diameter) in the cable near the YSI 556 MS-19 connector.

2. Lay the open ferrite bead assembly under the loop with the cable cross-over position within the cylinder of the ferrite bead.

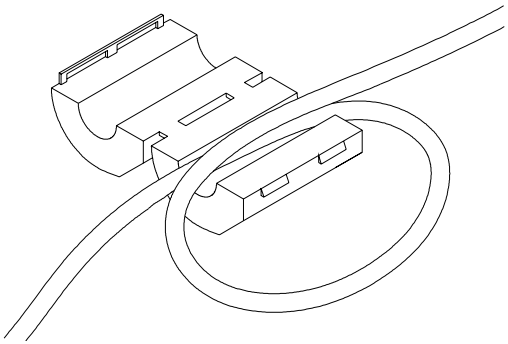


Figure 19.1 Ferrite Bead Installation

3. Snap the two pieces of the bead together making certain that the tabs lock securely.

4. When the installation is complete, the 655173 and YSI 6117 cables should resemble the following drawings.

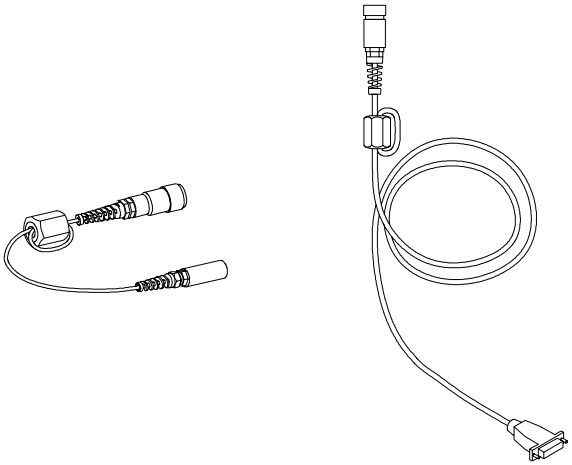


Figure 19.2 Cables with Ferrite Beads

20. Appendix G EcoWatch

EcoWatch™ for Windows™ must be used as the PC software interface to the YSI 556 MPS. EcoWatch is a powerful tool that can also be used with YSI 6-series sondes. Many features of the software will only be utilized by advanced users or are not relevant to the 556 MPS at all. This section is designed in tutorial format to familiarize you with the commonly used features of EcoWatch so that it will be possible to:

- Upload data from a 556 MPS to a PC
- Assemble plots and reports of your data
- Zoom in on certain segments of the plots of your data to facilitate analysis
- Show statistical data for your studies
- Export data in spreadsheet-compatible formats
- Print plots and reports

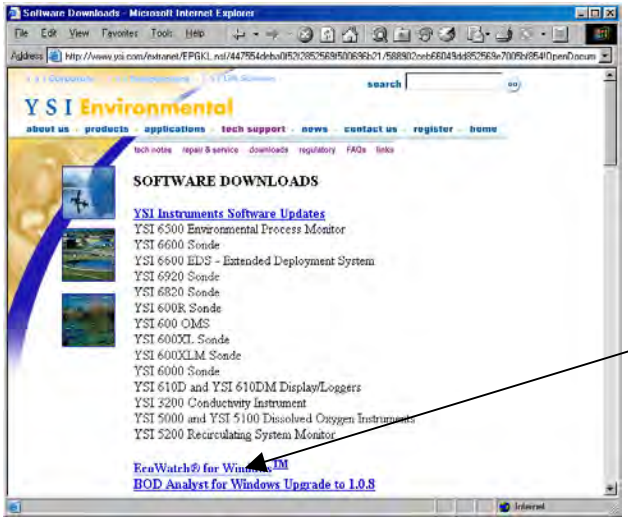
The advanced features of EcoWatch can be explored by downloading a 6-series manual from the YSI Web Site (www.ysi.com), purchasing a hard copy of the manual through YSI Customer Service (Item # 069300), or utilizing the on-line help feature of the software.

20.1 Installing EcoWatch for Windows

EcoWatch for Windows is available at no cost via a download from the YSI Web Site.

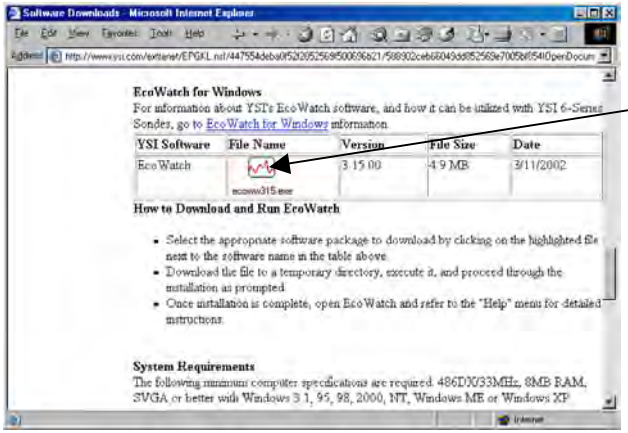
1. Access the YSI Environmental Web Site at www.ysi.com/edownloads.

2. Click on the **EcoWatch for Windows** link (or scroll down until you see the EcoWatch for Windows icon).



EcoWatch Link

3. Click on the EcoWatch for Windows icon and save the file to a temporary directory on your computer.



EcoWatch Icon

4. After the download is complete, run the EcoWatch file (that you just downloaded) and follow the on screen instructions to install the software on your computer.

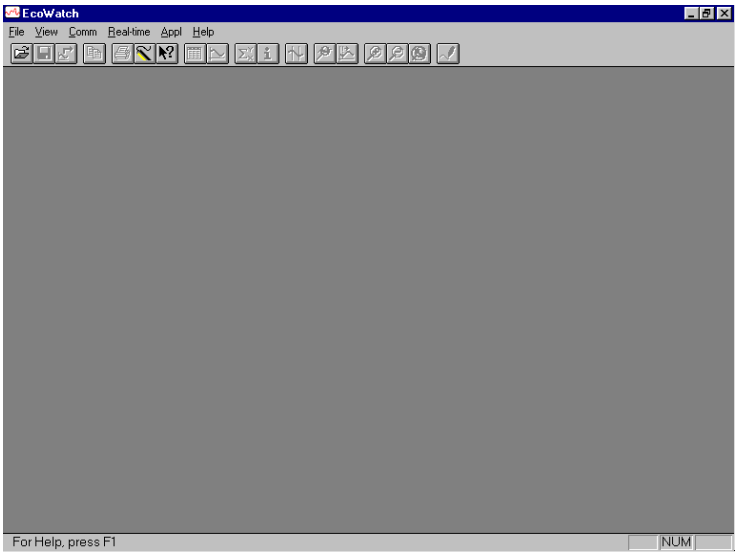
If you encounter difficulties in the download procedure, contact YSI Customer Service. Refer to ***Appendix E Customer Service***. Alternatively, you may purchase the software on CD ROM (Item #006075) by contacting YSI Customer Service.

20.2 EcoWatch Tutorial

This EcoWatch tutorial is designed to teach you the commonly used operations associated with the software when used with your 556 MPS.

After you have uploaded a file, Refer to Section 8.4 Upload to PC, you will see two files in the C:\ECOWWIN\DATA directory; the file you transferred and a file supplied by YSI designated SAMPLE.DAT. This SAMPLE.DAT file is referred to in the remainder of this tutorial section. After following the instructions below for the analysis of SAMPLE.DAT, you apply the same analysis to the data file which was uploaded from your 556 MPS to assure that you are familiar with the basic features and capabilities of EcoWatch for Windows.

To start the analysis of the SAMPLE.DAT file, note that a shortened menu bar is visible and many of the tools in the toolbar appear dimmed or “grayed out” before any file is opened (see below).



Full activation of EcoWatch features will be realized after a file is opened.

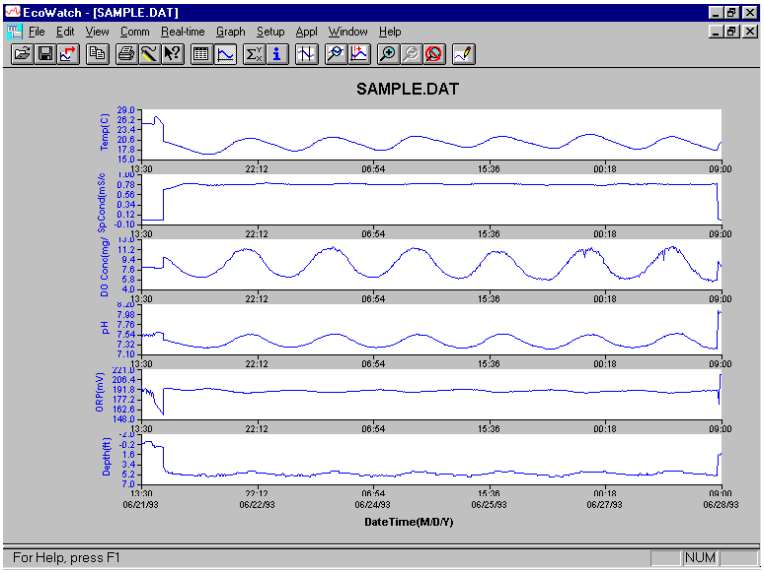
To open the sample data file:

5. 1. Click the **File** menu button in the toolbar.

6. 2. Select the **SAMPLE.DAT** file.

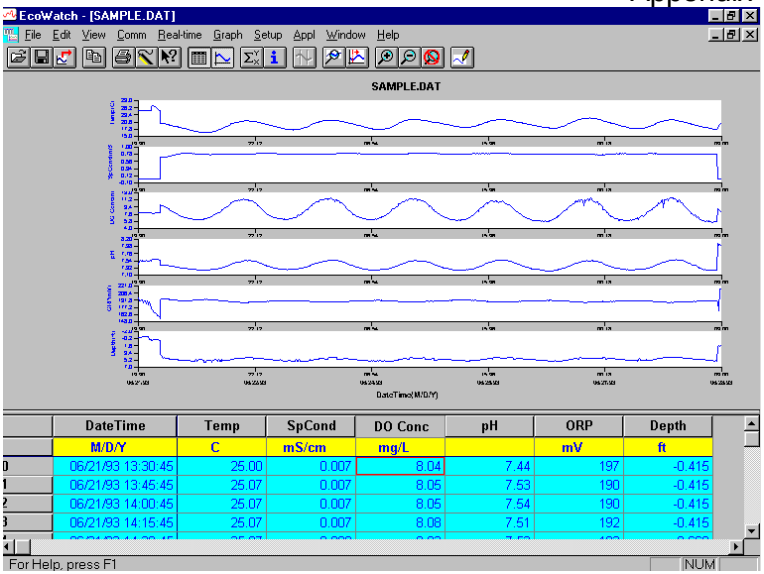
7. 3. Click **OK** to open the file.

The following display will appear:



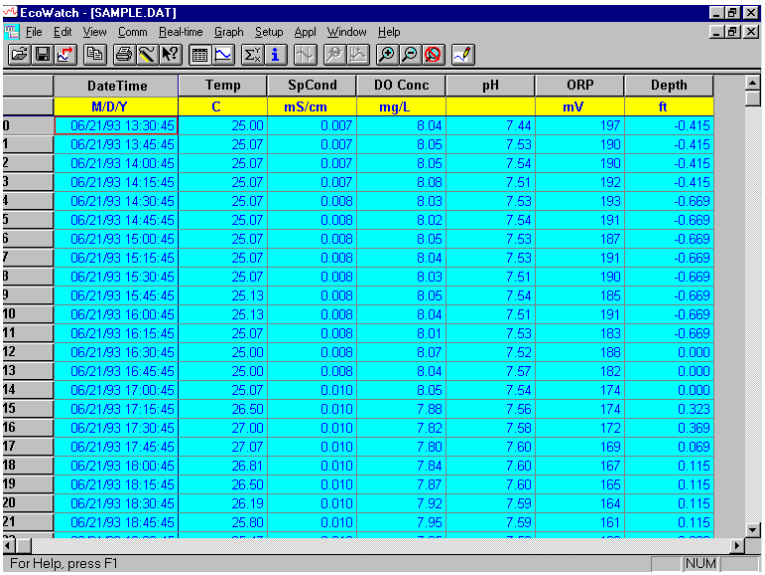
Note that the data in this file appears as a graph of temperature, specific conductance, dissolved oxygen, pH, ORP, and depth, all versus time. The graphs are scaled automatically so that all data fits comfortably on the computer screen. Note also that this data file was obtained with a 6-series sonde for which a depth sensor is available. Depth is NOT a current parameter for the 556 MPS.

The **Table** and **Graph** buttons on the toolbar are on/off switches that are used to display or hide the graph and table pages respectively. When displaying a graph and a table at the same time, you can control the relative size of the two pages by placing the cursor over the small bar that separates them and then dragging it to the desired location. Click the **Table** button to generate the following dual display of data.



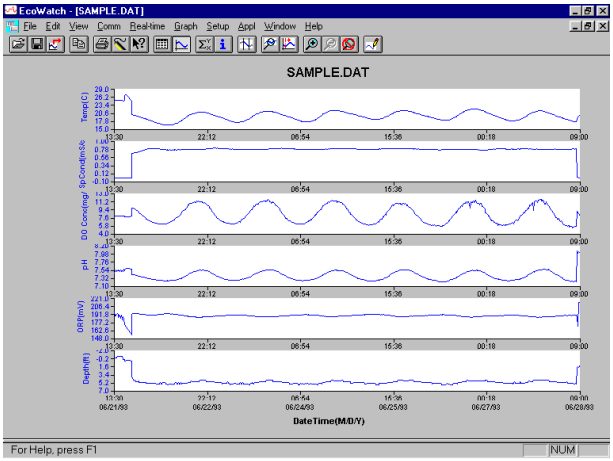
	DateTime	Temp	SpCond	DO Conc	pH	ORP	Depth
	M/D/Y	C	mS/cm	mg/L		mV	ft
0	06/21/93 13:30:45	25.00	0.007	8.04	7.44	197	-0.415
1	06/21/93 13:45:45	25.07	0.007	8.05	7.53	190	-0.415
2	06/21/93 14:00:45	25.07	0.007	8.05	7.54	190	-0.415
3	06/21/93 14:15:45	25.07	0.007	8.08	7.51	192	-0.415

Now click the **Graph** button (turn it off) to display only a report of your data as shown below. Note that the size of the report can be varied by clicking on the and buttons in the Toolbar.

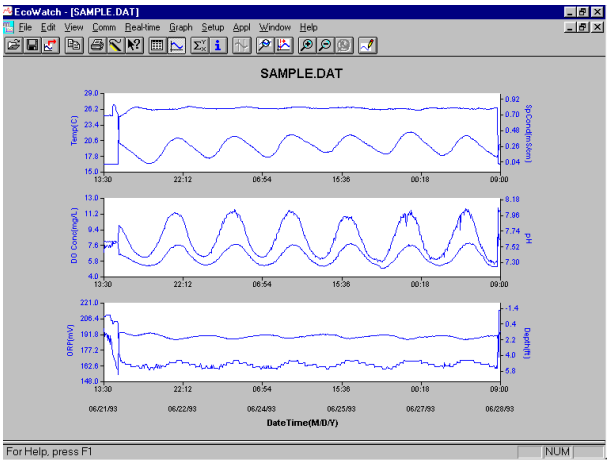


	DateTime	Temp	SpCond	DO Conc	pH	ORP	Depth
	M/D/Y	C	mS/cm	mg/L		mV	ft
0	06/21/93 13:30:45	25.00	0.007	8.04	7.44	197	-0.415
1	06/21/93 13:45:45	25.07	0.007	8.05	7.53	190	-0.415
2	06/21/93 14:00:45	25.07	0.007	8.05	7.54	190	-0.415
3	06/21/93 14:15:45	25.07	0.007	8.08	7.51	192	-0.415
4	06/21/93 14:30:45	25.07	0.008	8.03	7.53	193	-0.669
5	06/21/93 14:45:45	25.07	0.008	8.02	7.54	191	-0.669
6	06/21/93 15:00:45	25.07	0.008	8.05	7.53	187	-0.669
7	06/21/93 15:15:45	25.07	0.008	8.04	7.53	191	-0.669
8	06/21/93 15:30:45	25.07	0.008	8.03	7.51	190	-0.669
9	06/21/93 15:45:45	25.13	0.008	8.05	7.54	185	-0.669
10	06/21/93 16:00:45	25.13	0.008	8.04	7.51	191	-0.669
11	06/21/93 16:15:45	25.07	0.008	8.01	7.53	183	-0.669
12	06/21/93 16:30:45	25.00	0.008	8.07	7.52	188	0.000
13	06/21/93 16:45:45	25.00	0.008	8.04	7.57	182	0.000
14	06/21/93 17:00:45	25.07	0.010	8.05	7.54	174	0.000
15	06/21/93 17:15:45	26.50	0.010	7.88	7.56	174	0.323
16	06/21/93 17:30:45	27.00	0.010	7.82	7.58	172	0.369
17	06/21/93 17:45:45	27.07	0.010	7.80	7.60	169	0.069
18	06/21/93 18:00:45	26.81	0.010	7.84	7.60	167	0.115
19	06/21/93 18:15:45	26.50	0.010	7.87	7.60	165	0.115
20	06/21/93 18:30:45	26.19	0.010	7.92	7.59	164	0.115
21	06/21/93 18:45:45	25.80	0.010	7.95	7.59	161	0.115

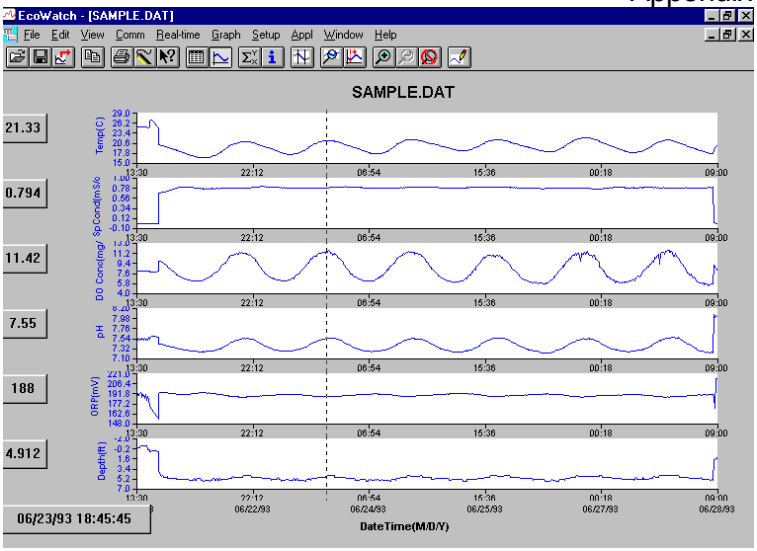
Now return to the original graphic display by toggling the **Table** button “off” and **Graph** button “on”.



From the **Setup** menu, click **Graph**. Click **2 Traces per Graph** and notice that the parameters are now graphed in pairs for easy comparison of parameters.

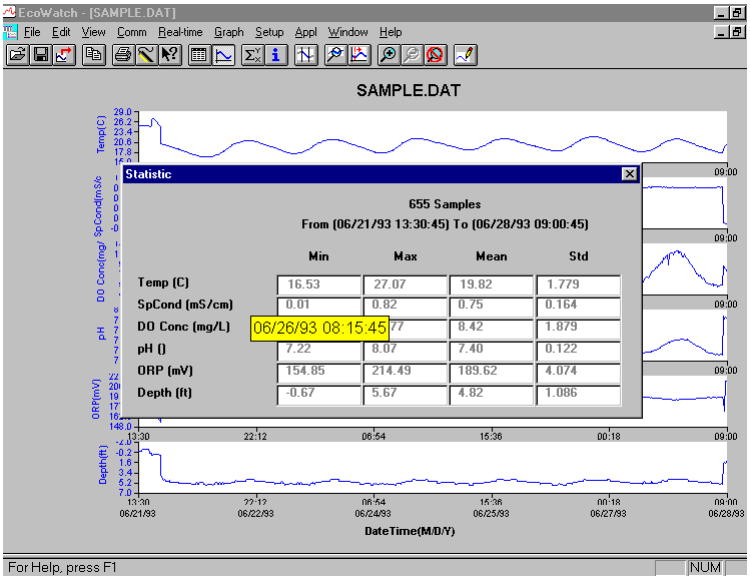


Click **1 Trace per Graph** to return the display to the original setting. Move the cursor to any position in the graph, then click and hold the right mouse button.



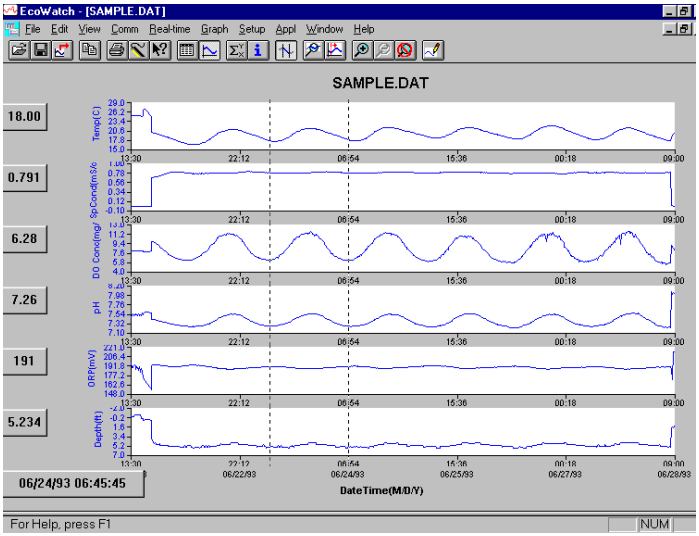
Note that the exact measurements for this point in time are displayed to the left of the graph. While holding down the right mouse button, move to another area on the graph. Notice how the measurements change as you move. When you release the mouse button, the display returns to normal.

To view statistical information for the study, click the **Statistics** button on the toolbar. On the statistics window, click on any min or max value to display the time when it occurred.

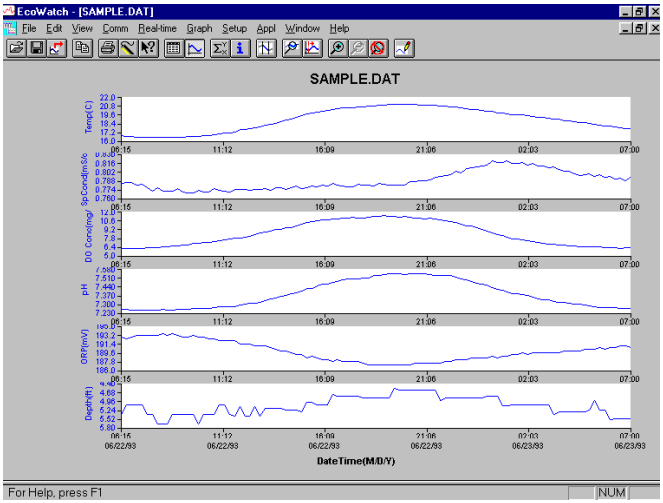


After viewing statistics, click the “x” at the upper right to close the window and return to the normal display.

Now click on the delimiter icon in the toolbar and then move the displayed icon to the graph. Click at the two points shown by dotted lines in the display below, being sure that the first click is to the left of the second.

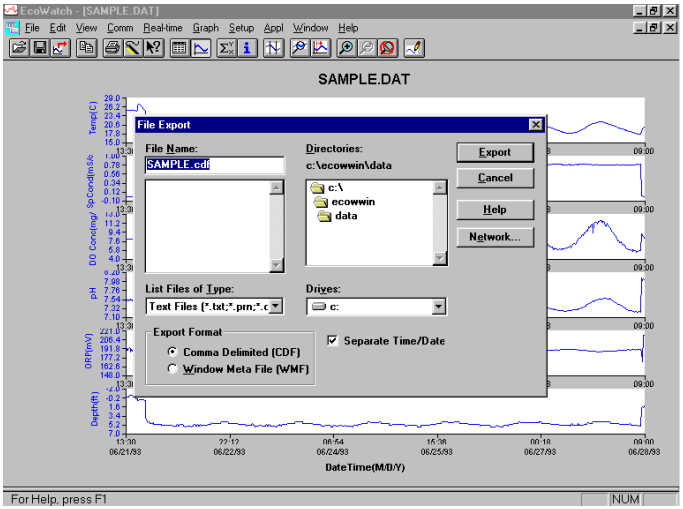


The data between the two selected points will then be graphed in higher resolution as shown below.

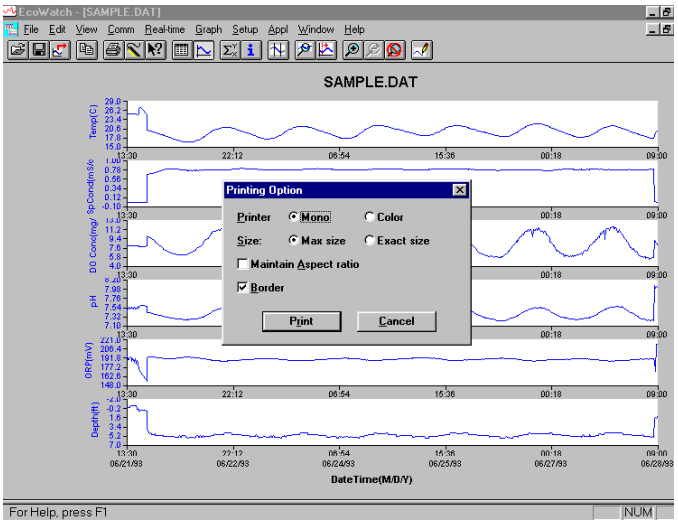


To return to the complete data set, select **Graph** from the toolbar and then click **Cancel Limits**.

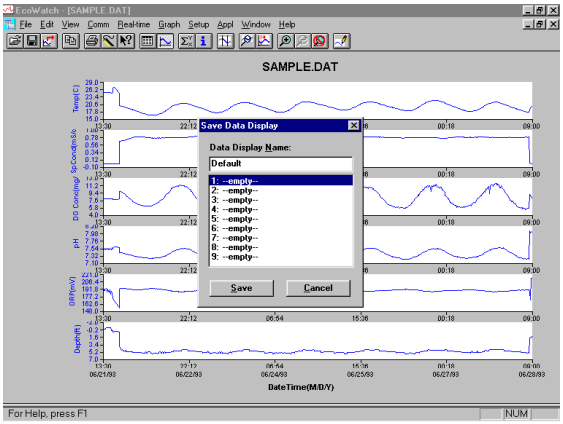
Now select the icon from the Toolbar to create a new data file which will allow your data to be imported into spreadsheets. Select the default export settings for a Comma Delimited File (.CDF) and click OK. A new spreadsheet-importable file (SAMPLE.CDF) is now present in the same folder as the SAMPLE.DAT file.



Now select the icon from the toolbar to print the plot. Accept the default settings and click OK to complete the printing operation.



Finally, end the tutorial by saving the **Data Display** in the format shown. From the **File** menu, click **Save Data Display**.



Then type “Default” for the file name and click **Save**. The parameters, colors, format, and x-axis time interval associated with the current display are now saved and can be accessed any time in the future. Nine different data displays may be saved for any data file. You can easily switch between various displays of the data. The data files can be accessed by clicking **Load Data Display** from the file menu and then selecting the desired presentation.

20.2.1 Summary of Toolbar Capability

The EcoWatch toolbar includes buttons for some of the most common commands in EcoWatch, such as **File Open**. To display or hide the toolbar, open the **View** menu and click on the **Toolbar** command. A check mark appears next to the menu item when the toolbar is displayed.

The toolbar is displayed across the top of the application window, below the menu bar.

Click To:

Open an existing data file (.DAT). EcoWatch displays the **Open** dialog box, in which you can locate and open the desired file.

Save the working Data Display of the active data file. EcoWatch displays the **Save Data Display** dialog box in which you can overwrite existing Data Display or save to a new one.

Export data as a graph in Window Meta File (.WMF) format or as data in Comma Delimited (.CDF) format.

Copy the whole graph page or data from the selection on the table to the clipboard.

Print the active graph page or table page depending on which one is currently active.

Open a new terminal window to communicate with the sonde.

Access context sensitive help (Shift+F1).

Toggle table window during file processing.

Toggle graph window during file processing.

Display study statistics.

Display study info.

Limit the data to be processed in a study.

Enlarge a selective portion of graph.

Center the graph under the cursor.

Enlarge graph or table 20%.

Reduce graph or table 20%.

Return graph or table to its normal state (unzoom)

Redraw the graph.

20.2.2 Other Capabilities

The above tutorial and function list for the toolbar provide basic information to allow you to view and analyze the field data which was stored in your 556 MPS. Some of the other commonly used capabilities of EcoWatch which the user may want to explore are listed below:

- Customize the units for each parameter, e.g., report uS/cm instead of mS/cm for conductivity.
- Customize the order of parameters in each plot or report.
- Customize the colors and fonts of each data display.
- Manually scale the y-axis sensitivity for each parameter.
- Merging of two or more data files with compatible parameter formats
- View information about the study such as number of points, instrument serial number, etc. which was stored in the 556 with the data.
- Print data reports in different statistical formats.
- Create plots of parameter vs. parameter rather than parameter vs. time.

These additional features of EcoWatch for Windows are explained in detail in the YSI 6-series manual (which can be downloaded at no cost from the YSI Web Site as described above) and the Help selection in the EcoWatch menubar. To purchase a hard copy of the 6-series manual, contact YSI Customer Service using the contact information in *Appendix E Customer Service*.

21. Appendix H Calibration Record Information

When your YSI 556 MPS sensors are initially calibrated, relevant information about the sensors will be stored in a separate file in the YSI 556 MPS memory.

NOTE: This file, by default, will have the name "556 Circuit Board Serial Number.glp." The circuit board serial number is assigned at the factory and has a hexadecimal format such as 000080A4. Thus the default calibration record file would be designated 00080A4.glp. Refer to Section *10.7 GLP Filename* to change the filename.

The information in the calibration record will track the sensor performance of your instrument and should be particularly useful for programs operating under Good Laboratory Practices (GLP) protocols.

21.1 Viewing the Calibration Record (.glp) File

NOTE: Make certain that you have performed a calibration on at least one of the sensors associated with your YSI 556 MPS.

1. Follow the procedures outlined in Section *8.3 View File*.

21.2 Uploading the Calibration Record (.glp) File

NOTE: Make certain that you have performed a calibration on at least one of the sensors associated with your YSI 556 MPS.

1. Follow the procedures outlined in Section *8.4 Upload to PC*.

21.3 Understanding the Calibration Record (.glp) File

1. Open a calibration record file. Refer to Section *8.3 View File*.

2. Use the arrow keys to scroll horizontally and/or vertically to view all the data.

00008003.glp

m/d/y	hh:mm:ss	S/N
01/24/2001	08:17:51	00008003
01/24/2001	08:17:51	00008003
01/24/2001	08:17:51	00008003
01/24/2001	08:17:51	00008003
01/24/2001	08:17:51	00008003
01/24/2001	08:17:51	00008003
01/24/2001	08:17:51	00008003
01/24/2001	08:17:51	00008003
01/24/2001	08:25:40	00008003
01/24/2001	08:25:40	00008003

735.9mmHg

01/24/2001 08:39:53

Figure 21.1 Calibration Record Screen 1

00008003.glp

Type	Value
Conductivity gain	1.000000
DO gain	1.000000
pH gain (pH-7)*K/mV	-5.05833
pH offest (pH-7)*K	0.000000
ORP offset mV	0.000000
TDS constant	0.650000
Barometer offset PSI	0.000000
DO gain	1.110250
pH gain (pH-7)*K/mV	-5.05833
pH offest (pH-7)*K	-12.2899

735.9mmHg

01/24/2001 08:39:19

Figure 21.2 Calibration Record Screen 2

NOTE: Each sensor (not parameter) is characterized by either 1 line (Conductivity, Dissolved Oxygen, ORP, TDS, or Barometer (Optional)) or 2 lines (pH) of calibration documentation.

The left hand portion of each calibration entry shows the date and time that a calibration of a particular sensor was performed. In addition, each calibration entry is characterized by the instrument serial number, as defined by YSI. See Figure 21.1 Calibration Record Screen 1. The right hand portion shows the YSI designation of the calibration constants and their values after their calibration has been performed. A more detailed description of the calibration constants is provided below:

- **Conductivity Gain** – A relative number which describes the sensitivity of the sensor. Basically, the value represents the calculated cell constant divided by the typical value of the cell constant (5 cm^{-1}).

- **DO Gain** – A relative number which describes the sensitivity of the sensor. Basically, the value represents the sensor current at the time of calibration divided by the typical value of the sensor current (15 uA).

- **pH Gain** – A number which basically represents the sensitivity of the pH sensor. To remove the effect of temperature on the slope of the relationship of probe output in mv versus pH, the value of pH/mv is multiplied by the temperature in degrees Kelvin (K).

- **pH Offset** – A number which basically represents the offset (or intercept) of the relationship of probe output in mv versus pH, the value of pH is multiplied by the temperature in degrees Kelvin (K).

Anytime you perform a calibration, information concerning the calibration constants will be logged to the Calibration Record file (.glp file). However, if the **Delete All Files** command is used, Refer to Section *8.6 Delete All Files*, the Calibration Record file will also be lost. It is critical that this file should be uploaded to your PC prior to issuing a **Delete All Files** command. Refer to Section *8.4 Upload to PC*.

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sampling variability. A set equals 10 or fewer samples; thus 8 samples would be considered 1 set and 1 field duplicate would be collected, whereas 11 samples would be considered 2 sets and 2 field duplicates would be collected. The site where the field duplicates are collected will be pre-determined before the sampling run using a random number table or generator. If there is not adequate flow to sample at the pre-determined site, the field duplicate will be sampled at the next site in the sampling run. Variability of field duplicates will be analyzed against pre-determined data quality objectives. Results exceeding objectives will be flagged and qualified or rejected.

Table 1. Summary of typical analytical methods for fecal coliform analysis.

Parameter	Description	Method	Sample Container	Preservation	Holding Time	Precision/ Quantification Limits
Fecal coliform bacteria	Membrane filtration	SM9222D[1]	125mL or 250mL, sterile	10°C, dark	8 hours, maximum 24 (delivered to lab w/i 6 hrs, 2 hrs until sample processed)	20% RSD[2] 1cfu/100mL

[1]APHA et al. 2005. Standard Methods for the Examination of Water and Wastewater, 21st Edition.
[2] RSD- Relative standard deviation, divided by the mean

8.0 Safety

- Field staff will typically work in a team of two and carry a cell phone. In some circumstances, field staff will collect samples independently. In this case, it is essential that a cell phone is carried.
- High-visibility safety vests will be worn, particularly when working from or near a roadway.
- Field staff should use caution when approaching sampling sites. Sites may have slippery or unstable conditions. If field staff have concerns regarding accessing a site safely due to environmental conditions, construction, or other factors, the sample collection for that site should be aborted. The reason for not collecting a sample should be noted on the field sheet. If the condition is permanent, the site may need to be relocated.
- Gloves should be worn or hands washed after sampling surfaces waters.

9.0 References

City of Portland (Oregon). 2003. *Field Operations Standard Operating Procedures: Direct Grab Sample Collection with Sample Bottle.* Bureau of Environmental Services, Environmental Investigations Division.

Environmental Protection Agency (EPA). 1992. *NPDES Storm Water Sampling Guidance Document.* USEPA Office of Water. EPA 833-8-92-001.

Washington State Department of Ecology. 2007. *Standard Operating Procedures for the Collection of Fecal Coliform Bacteria Samples: Version 1.2.* Environmental Assessment Program.

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