**STANDARD OPERATING PROCEDURE**
**MAINTENANCE/CALIBRATION CHECKS OF DISSOLVED OXYGEN PROBES**

## INTRODUCTION

Streamkeepers may use a variety of meters to measure the dissolved oxygen content of water. With most meters, when measuring dissolved oxygen, a barometer is also used to measure atmospheric pressure. Streamkeepers generally maintains and calibrates dissolved oxygen meters per the following chart, subject to particular needs of a monitoring activity. Normally, maintenance-calibration periods revolve around month-long Streamkeepers quarterly monitoring sessions (see below); however, in some cases maintenance/calibration activity might occur in the middle of a Streamkeepers monitoring month (if a problem is noted), and in other cases sampling periods may differ for monitoring done for other projects that extend beyond Streamkeepers' normal monitoring sessions.

| **Instrument** | **Maintenance activity** | **Calibration check interval** | **Calibration interval** |
|---|---|---|---|
| YSI-85 | Quarterly electrode cleaning & membrane replacement | Check vs. Winkler titration after installing & before removing membrane | Calibrate in the field prior to each reading |
| Hydrolab Quanta | Quarterly electrode cleaning & membrane replacement; membrane integrity check | Quarterly check vs. Winkler titration | Calibrate immediately prior to sampling day |
| YSI ProDSS | Clean sensor cap, replace when reading stability and response time are unacceptable | Quarterly check vs. Winkler titration | Quarterly |
| Barometer | Handle with care, check battery | Check before recalibrating | Calibrate before each sampling period* |

**PRE-QUARTERLY DO MEMBRANE PROBE MAINTENANCE/MEMBRANE REPLACEMENT**

Streamkeepers perform quarterly water-quality sampling during the following month-long sampling windows: January; April; August; and Sept. 15 - Oct. 15. (There is some leeway on either side of these periods, depending on weather conditions.) In addition, the meters are used for other monitoring projects throughout the year. Prior to each quarterly monitoring session and before and after any field data collection, the YSI-85 meters need to be maintained as follows. Similar procedures are used for other membrane-electrode meters, such as the Hydrolab Quanta.

***NOTE: Prior to removing any DO membrane, make sure that a successful post-check of membrane integrity has been performed subsequent to all sampling events with this probe; if not, perform a membrane integrity post-check and confirm that it passes QC criteria prior to installing a new membrane. (To confirm that the post-check has passed, you will have to enter the post-check data into the database and run the pass/fail report BEFORE removing the old membranes, in case the post-check fails and you need to re-check.)**

**First, gather the equipment you will need to take with you:**

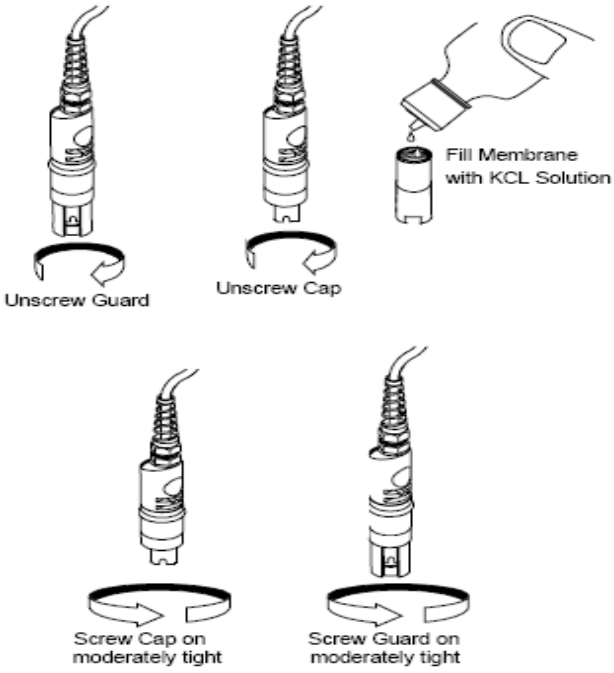
1. "DO/Barometer Calibration Protocol & Data Sheets" notebook
2. YSI -85 meters located in each field kit, OPI meter if in office
3. YSI 5906 Membrane Cap Kit containing:
    a. new plastic DO membranes
    b. small nylon scrub brush
    c. quarter-sized sanding disk
    d. oxygen probe electrolyte solution
4. purified water
5. bucket
6. lint-free tissues
7. commercial ammonia cleaner

**Cleaning Procedure:**

1. **Clean the DO probe first.**
    a. Unscrew the sensor guard at the end of the probe.
    b. Unscrew and discard the old DO membrane cap.
    c. Gently wet-sand the silver anode sides and gold cathode top, using the two different marked sanding disks in the Membrane Cap kit. Avoid intermingling the metals. Stop when darkening deposits have been wiped off – don't overdo it. Rinse with purified water and pat dry using lint-free tissues.
    d. In the event the probe is stubbornly dirty and unshiny, you can soak it in ammonia overnight with the meter off and the membrane removed. The next day, rinse for 5 minutes in running water and repeat the sanding procedure.

2. **Installing the new DO membrane cap:**
    a. Prepare a bottle of electrolyte solution according to the instructions on the bottle in the Membrane Cap Kit.
    b. Rinse the inside of the new cap with the electrolyte solution, to get rid of any foreign material.
    c. Holding the new cap upside-down, put in 15 drops of the electrolyte solution.
    d. With the cap still upside-down, screw the cap onto the probe until it is snug but not over tight. Some solution may leak out, indicating the membrane cap is indeed filled.
    e. Examine the new membrane. It should not be loose, wrinkled, damaged or dirty. <u>There should be NO bubbles under it</u>. If there are problems, rinse, refill and reattach the membrane, or install a new one.
    f. Check the sponge inside the calibration chamber. If it's in tatters or seriously discolored, replace it. It should be wet but not too wet. Dampen with a few drops of purified water, or tip chamber to drain off excess water, as needed.
    g. Store the probe back inside the chamber.
    h. Record the date, your initials and maintenance information on the YSI Maintenance form in the maintenance/calibration notebook.

**DRAWING OF YSI-85 PROBE FOR CLARIFICATION**



**DO METER ZERO-CHECK:**

Before each quarter, the following procedure should be performed on the YSI-85 meters to assure that they are reading accurately in near-zero dissolved oxygen conditions, so that their internal "zero-dissolved-oxygen" point is being set properly:

1. Make a solution using 2 gm sodium sulfite/1000 mL DI water. (the sodium sulfite is in the lab) Stir to dissolve and allow to sit for at least 15 min.
2. Calibrate the DO instrument
3. Place DO probe in the sodium sulfite solution.
4. DO% reading should be 10% or less within 28 seconds and 2% or less within 5 minutes (the faster the better).
5. Record results on the data sheet.
6. If the reading fails to get to 2%, recondition electrodes and retest after allowing for a 1 hour warm up period.
7. Rinse the probe thoroughly in tap water before placing it back in the calibration chamber - this is important or it could mess up future calibrations.

***From Tim Grooms, Product Development Manager, YSI, 7/29/08**

**DISSOLVED OXYGEN CALIBRATION CHECK**

DO calibration periodicities differ according to the instrument (see chart above), but at the end of each sampling session, we test the validity of the instrument's calibration by doing a side-by-side Winkler titration. Proof of the validity of the meter's DO calibration is essential to acceptance of our data by the State.

**Equipment needed:**

1. 1 or 2 large plastic bucket(s) - depends on method used below
2. DI water from lab
3. "DO/Barometer Calibration Protocol & Data Sheets" notebook
4. Blank DO calibration sheet
5. DO meters
6. Calibration foam block for each meter
7. Hydrolab meters, if being done with ours
8. Purified water, lint-free tissues for cleaning
9. Spare batteries for meters
10. A calibrated barometer
11. Aquarium air pump and aeration stone

**First prepare the 100% oxygenated water in the lab:**

**Method 1**

1. Fill one of the buckets with DI water in the lab
2. Pour this water into the other bucket and repeat 20 times
3. Let this water sit with a lid on it until the following day. If the temperature does not change, it will stabilize and remain at 100% oxygenation

**Method 2 (preferred)**

1. Fill a bucket with DI water in the lab
2. Place the aeration stone on the bottom of the bucket and turn on pump
3. Loosely cover the bucket and set aside for approximately 24 hours

**Steps to calibrate (YSI-85) or stabilize (Hydrolab, YSI ProDSS) the meters:**

1. Turn on meters. Erase previous data. (YSI-85: Depress the **MODE** button repeatedly until the 85 displays **ErAS**. Then depress and hold the **DOWN** arrow and **ENTER** buttons simultaneously for ~5 sec. **DONE** will flash on the display for 1-2 sec. The instrument will automatically change to normal operation after completion.) (Hydrolabs: Press the left arrow button which moves to Review at the bottom of the screen, press Enter, press Enter and you will see "Clear" and "Clear All" at the bottom, choose one and press Enter to erase.
2. Soak the calibration foam blocks in tap water and lightly ring out, should be a little drippy
3. Take the probes out of the meter chambers, check for bubbles under or dirt on the membranes, clean if dirty.

**YSI-85:**

Place probe in foam block leaving space between end of probe and bottom of foam to prevent the foam from touching the membrane

**Hydrolab:**

A. Install calibration cup and fill with the water near to room temp, up to the bottom of the DO membrane O-ring. Set upright in the meter tote box.
B. Place black chamber cover loosely upside down over calibration chamber

**YSI ProDSS:** Simply place the probe in the water.

4. While stabilizing the YSI-85s, check the sponges inside the chambers; if dry, add a few drops of purified water. If soaking wet, dump out excess water.
5. Get barometer and data sheet and begin to record information (date, samplers, site, estimated speed of water current, barometric pressure, membranes replaced, etc.).
6. Wait for the DO and temperature readings to stabilize on all meters, approximately 15-20 min.:
   (a) YSI-85: for 2 minutes, both DO% and Temp. (°C) remain within ± 0.1.
   (b) Hydrolab: Temp. & DO mg/L as stable as possible; note how long the mg/L remain stable within ± 0.01.
7. Calibrate the YSI-85s:
   (a) Use two fingers to press and release both the UP and DOWN arrow buttons simultaneously.
   (b) The LCD will blink with "ALT X100". Adjust to ZERO if it's not already there, then press ENTER once. (We don't use altitude since we use barometric pressure.)
   (c) You should now see CAL in the lower left, the calibration value should be displayed in the lower right and the current % (before calibration) should be on the main display. Press ENTER. The display should read SAVE and then should return to the normal operation mode.

8. Calibration Check for the Hydrolabs:
    a. Turn on meter. It will take about 30 seconds for the meter to run through self-checks, then the screen will change to one with "Temp" at upper-left and "Screen" flashing at bottom-center.
    b. Hit ENTER key ( ↵ ) to check and record voltage. Generally, you should replace batteries if <3.5 V, or if <3.2 V if you won't be using the stirrer. The machine should take readings properly if the working voltage is ≥3 V and does not show the low-battery icon in the lower right-hand corner. However, operating the stirrer reduces the working voltage by about 0.3 V, and at working voltages <3.5 V, it's possible that the display will show dashes instead of digits or be missing parts of the display; if you notice these things happening you should replace the batteries, though calibrations should be fine if they were performed at ≥3 V.
    c. Remove the storage cup so that it will hold the water when removed. Set aside with the water still in it.
    d. Rotate the black turbidity sensor ring out so you can see the sensors beneath. The DO membrane is the stretched plastic membrane with a black O-ring around the sides. Gently shake all droplets off this membrane.
    e. Check membrane for wrinkles, bubbles, etc. and note as needed on data sheet. (If membrane needs replacement, make sure that a post-sampling Winkler test does not have to be performed to verify the data already gathered with that membrane.)
    f. Rotate black turbidity ring back to center position. Attach the calibration cup, the one with an open top. Turn upside down, pour in the water from the storage cup and top off as needed with room-temp de-ionized water or tap water (conductivity <0.5 mS/cm) to bring the water level up to the O-ring.
    g. Re-check for droplets on membrane and carefully remove with tissue or by blowing.
    h. Turn the black calibration cup cover upside down (concave upward) and lay it over the top of the calibration cup. Set probe upright in a secure spot, without kinking the cable.
    i. Hit ENTER key ( ↵ ) to go to screen with DO mg/L (Screen 1), and wait for stabilization:
        *** ± 0.01 mg/L for 2 minutes (similar to criterion for stabilization of YSI-85) ***
    j. When stabilization has been achieved, record the mg/L reading.
    k. Record the temperature reading at stabilization.
    l. Navigate to CALIB screen using the left/right arrow key, and then hit ENTER
    m. Choose "DO %" (down arrows + ENTER).
    n. Record barometric pressure in mmHg from a calibrated barometer.
        i. If barometer reads inHg, convert to mmHg by multiplying by 25.4. If barometer reads in mbar, convert to mmHg by multiplying by 0.75.
    o. On the data sheet, record barometric pressure and information about the barometer you're using.
    p. On the Hydrolab DO% calibration screen, adjust barometric pressure as needed using up or down arrows, then hit ENTER.

q. Hit "ESC" to get to Screen 1; record the post-calibration DO mg/L reading.
r. Record the expected DO mg/L reading from the DO solubility chart (attached). Your post-cal DO should be within 2% of the expected reading. If it's not, allow to sit and stabilize a while longer, then recheck. Record the DO mg/l and temperature on the field sheet.

## Side-by-side meter vs. Winkler trials

Basic procedure is Winkler samples, Meter samples, and Meter Drift checks

**Preparing Winkler samples:**

a. Put on safety equipment: goggles, gloves, apron
b. Take out BOD bottle #1 and fill it with the air saturated water previously prepared - fill to neck, a clean beaker can be used to transfer water from the sample bucket you prepared earlier
c. Put on the glass stopper.
d. The BOD bottles need to have the DO stabilized as they are collected immediately by adding two chemicals as follows:
    a. Take the 1st pipette out of the manganous sulfate solution. Check and make sure it is filled with approximately 2 mL of solution and then add it to the BOD #1 bottle by immersing the tip of the pipette into the sample before injecting the solution. Make sure when you add solution you do not squeeze any air bubbles into the sample. Keep squeezing the top of the pipette when you replace it in the sulfate solution so that it will be filled for the next time.
    b. Take the second 2nd pipette out of the alkaline-azide solution,check and make sure it has approximately 2 mL of solution and add it to the BOD #1 bottle using the same procedure as before.
    c. Adding these solutions turns the water brown and chemically converts the free oxygen into a manganese precipitate which falls to the bottom of the bottle. Put the glass stopper in the BOD bottle and invert slowly a couple of times to mix the contents. The solution is now stable.
    d. Repeat all the sampling steps for the other two BOD bottles.
    e. Record the time the samples were taken on the data sheet.

**WINKLER TITRATIONS IN THE LABORATORY**

**Equipment for completing Winkler titrations:**

1. Graduated cylinder burette with stopcock at bottom, 25 mL/ with 3-way stopcock
2. Volumetric burette, 10 mL with 3-way stopcock or 10 mL pipette with bulb on the end
3. 3 Erlenmeyer flasks, 250 mL (number them-sample 1, 2, & 3)
4. Volumetric pipette 10 mL stored in "Streamkeepers" cardboard tube at back of counter
5. 3 BOD bottles and glass stoppers
6. Plastic caps for each BOD bottle
7. Manganous sulfate solution with its eyedropper (2 mL pipette)
8. Alkali-iodate-azide reagent with its eyedropper (2 mL pipette)
9. Pipette suction/dispenser bulb
10. Magnetic stirrer
11. Stirring bars (one for each sample)
12. 203 mL Volumetric flask (plastic flask cut to hold exactly 203 mL when completely filled above the top; a rubber gasket around the top helps to deliver this flask's contents into the Erlenmeyer flasks)
13. Concentrated sulfuric acid
14. 2 mL disposable pipette stored in glass jar, for acid transfer
15. Aqueous starch solution preserved with salicylic acid
16. Squirt bottle (250 mL) with starch solution
17. Sodium thiosulfate, 0.025 M (Check pull date – shelf life critical)
18. Potassium bi-iodate, 0.025 M (Check date – good 18mos from mfr.)
19. Rubber apron
20. Nitrile gloves
21. Acid face shield
22. **Ensure all liquids are within the expiration date and there is enough quantity to complete all 3 samples.**

**Titration Steps:**

*NOTE: dilute the chemicals going into the sink during the process with a continuous stream of tap water to prevent damage to plumbing.

1. Put on the plastic apron, face shield and Nitrile/Latex gloves.
2. Pour off the water seal and invert the bottle several times to mix the floc.
3. Remove the glass stoppers and put sample bottles in sink.
4. Using a pipette, draw and add 2 mL of sulfuric acid to each sample bottle. Keep the tap water running.
5. Rinse the pipette and put the cap securely on the acid bottle.
6. Put the stoppers back on the bottles and invert them several times over the sink until the precipitate has completely dissolved.
7. To transfer the liquid to the 203 mL volumetric flask, place the flask on top of BOD bottle number 1 and invert over sink. Once the flask is full, quickly remove the BOD bottle. The liquid should appear slightly convex on the top of the flask. Transfer the sample to the #1 Erlenmeyer flask. Repeat for samples # 2 and 3.
8. Carefully uncover the sodium thiosulfate burette (a purple cover on it for protection and storage.)

9. Ensure there is enough thiosulfate solution to complete the three trials in the burette. Add solution to the reservoir if necessary.
10. Empty any of the sodium thiosulfate left in the burette.
11. Turn the stopcock valve so the handle faces the burette body (directly away from you toward the wall).
12. Tighten the valve on the rubber bulb and pressurize the reservoir. Pump the rubber bulb gently to raise the solution, drain, refill and drain twice more to rinse, then fill the burette until the sodium thiosulfate escapes from the top nipple.
13. Turn the stopcock 90 degrees and loosen the valve on the bulb to allow the excess solution to return to the reservoir.
14. Drop a stir bar in the first sample flask and place it centered on the magnetic stirrer. Place the stirrer and flask centered under the burette valve to ensure drips fall cleanly into flask.
15. Turn the stirrer on the lowest setting.
16. Turn the stopcock valve handle toward you (there is a guide mark) and begin adding the sodium thiosulfate drops. You can add drops somewhat quickly at first but slow down when the sample **starts** turning a pale yellow.
17. Now, SLOWLY add one drop at a time until the sample turns very pale yellow and stop titrating. (It helps to place white paper between the burette and sample bottle.)
18. Add 1 to 2 mL of the starch solution into the flask using the squirt bottle until the sample turns a dark purple.
19. Continue to titrate the sample, adding drops VERY SLOWLY until the purple color just disappears and the sample is clear. Record the volume of solution used for each sample, to the nearest 0.1 mL. If ANY doubt about the end point, you can check the titration of the sample.
20. Check the end point by adding a drop of potassium bi-iodate into the flask with a dropper that delivers 20 drops per mL, identical to the burette. If the end point is correct, the purple color should reappear. If more than one drop is required, then the end point was overrun.
21. If the end point was overrun, back-titrate the sample with the bi-iodate standard (1 drop = 0.05 mg/L using a disposable 2 mL pipette) and correct the final value and data sheet. (For values halfway between the 0.1 mL marks, round down even numbers and round up odd numbers.)
22. **Sodium Thiosulfate Normality Check:** When the sample has been titrated to its end point, add 10 mL of the bi-iodate standard to the sample using the pipette in the cardboard box. This is the "nominal value" that you'll record on the data sheet. Then record the correction factor for that pipette (i.e., how many actual mL are delivered for 10 mL nominal value on the pipette, as measured by repeated tests with purified water, an accurate balance, and factoring in air temperature). Refill the burette with sodium thiosulfate and re-titrate. Record the volume of the "sodium thiosulfate normality check" volume needed to back-titrate to clear.
23. Titrate the other two samples following the same steps as the first. Make sure you refill the burette and dab any excess fluid from the burette tip between each sample.
24. Wash the glassware and put away all liquids and equipment. **Check the dates and amounts of all solutions used to ensure there's enough unexpired liquids for the next calibration activity. If not, inform the office staff immediately.**

**Meter Samples**

**For YSI-85**

1. Place the bucket of prepared DI water in the sink
2. Record the time and take water temp, DO, and conductivity readings from all meters in the bucket as follows:
3. Set the probe screens to DO% saturation.
4. Place a probe in the bucket of DI water and allow the temperature to stabilize
5. Holding the probe by the cable just above the connector, swirl the probe as fast as you comfortably can without introducing bubbles or banging into the sides of the bucket
6. Hold this swirling action until the readings are stabilized: DO sat ± .5% & Temp ± 0.1° for 30 sec. for the YSI-85, then press **ENTER** and hold for 2 seconds to save the readings. You can record the readings at this time, or, wait until you have completed the other instruments.
7. Shake off probes and place back into the foam block.
8. Repeat this procedure with the rest of the YSI-85's.

**For Hydrolabs:**

1. Place probe in bucket of DI water and allow temperature to stabilize
2. Hold cable just above connector and swirl probe as fast as comfortable without introducing bubbles or banging into the sides of the bucket
3. Wait for readings stabilize: ± 0.01 mg/L for 2 minutes

**For YSI ProDSS:** Let reading stabilize.

**Post Winkler Calibration Check**

1. Record time on data sheet under "Post-sampling cal-✓".
2. For YSI-85's place probes back into foam blocks and allow meters to stabilize: for 2 minutes, both DO% and Temp. (°C) remain within ± 0.1 of their initial readings. Record the time, temp, and post-cal DO%.
3. For Hydrolabs, replace the calibration cup, invert probe and fill with water to the o-ring, set in tote and place cover lightly over cup
4. Save or record readings at this time
5. You can now pack everything up and record your sampling data from the meters back at the lab.

**Recording Data from Meter Sampling**

1. Record sampling trials from stored data under site 1 and 2. You do this by pressing **MODE** repeatedly until "**rcl**" is displayed on the screen. The number below "**rcl**" should read site 1 first and then site 2 (use the last two sites if previous info was not erased.)
2. Once on the right site, press **ENTER** successively to get the readings in the following order:
    s. Temperature - record to the nearest 0.1% C
    t. DO Saturation %
    u. DO Concentration – record to the nearest 0.1 mg/L
    v. Conductivity
2. Record this on the data sheet for both trials 1 and 2.
3. Note the range in the conductivity readings. We don't record these in the database, but a disagreement of >5% should be cause for concern.
4. After recording data, erase the readings from the meter
    a. Press **MODE** button until "**ErAS**" appears on the screen
    b. Press **DOWN** arrow and **ENTER** simultaneously for approximately 5 seconds.
    c. When "**DONE**" flashes on the screen for 1-2 seconds, the data has been erased and the meter will return to normal operation.

**Once you have finished calibrating the equipment, YOU are responsible for entering the new data into the SK database. Once the data has been entered, (steps to enter data on following page) the database will automatically tell you if the instruments have passed their quality control criteria. Print this report and staple it to the front of the data sheet, 3-hole punch these pages and place them in the back of the calibration notebook behind the "completed data sheet" tab. Put the most recent data sheet on top.**