Collecting the Sample

Remember that photosynthesis and respiration will continue after a sample is collected, so water can gain or lose oxygen while sitting in the sample bottle. Therefore, you should begin D.O. testing immediately upon reaching the shore after you have collected the sample.

You should measure water temperature at the same time and location that you collect the D.O. sample.

Think Like A Scientist!

Follow the directions VERY CAREFULLY!
Accuracy is a must for valid data comparisons.

1) Use bottle with the stopper included in the Hach or LaMotte kit.
2) Collect your sample in roughly one-foot deep, normally moving water.
3) Facing upstream, slowly lower the bottle so opening of the bottle faces away from you and water current is entering the bottle.
4) Allow the bottle to fill with water gradually, turning it to allow air bubbles to float out.
5) Cap bottle while still submerged and leave extra water in the neck of the bottle.
6) When lifting the bottle out of water, look for bubbles. If you see any, take another sample using the same procedure.

Testing for Dissolved Oxygen

using the Hach Model 146900

Note: If you see any air bubbles trapped in the sample bottle during steps 2-4 below, discard the sample and start over.

1) Put on protective gloves and safety goggles. If your skin comes in contact with any powder or titrant, rinse the area liberally with water.
2) Remove the stopper and add the contents of D.O. powder pillow #1 (manganous sulfate powder) and D.O. powder pillow #2 (alkaline iodide azide powder) to the sample.
3) Insert the stopper, being careful not to trap an air bubble and shake vigorously, holding on to the top. If oxygen is present, a brownish-orange floc will form.
4) Allow the sample to stand until the floc settles halfway. Shake the bottle a second time and allow the floc to settle halfway again.
5) Remove the stopper and slowly add the contents of D.O. powder pillow #3 (sulfamic acid).
6) Stopper and shake vigorously until the acid is dissolved. The yellow color is from iodine. This is called the prepared sample. Prepared samples can be stored in the dark for a short time if it is more convenient or comfortable to return to your home/school to complete the analysis.
7) Transfer two plastic measuring tubes full of prepared sample to the square glass mixing bottle (your Hach kit instructions probably say one measuring tube full). Using two measuring tubes allows you to determine D.O. to the nearest 0.5 mg/L instead of 1 mg/L .
8) ***a.)*** Holding the dropper vertically, add one drop at a time of sodium thiosulfate standard solution titrant to the square mixing bottle, and count each drop. ***b.)*** Swirl the solution after each drop. ***c.)*** Continue adding sodium thiosulfate drops until the sample is a very light yellow. ***d.)*** Add 3 to 4 drops of starch solution. The prepared sample will turn blue from the added starch solution. If you do not have starch solution, proceed with the next step but be aware that your sample will turn from yellow to colorless instead of blue to colorless. ***e.)*** Continue adding drops, mixing and counting until the prepared sample turns from blue (or yellow) to colorless (the end point). Often this is just one or two more drops, so be careful.
9) The dissolved oxygen content of the water in mg/L is the total number of drops of titrant used to get to the endpoint divided by two if two measuring tubes of prepared sample were used. If only one measuring tube of prepared sample was used, the dissolved oxygen content is equal to the number of drops of titrant. Example: If you used two tubes of sample, you need to divide by two (13 drops divided by two tubes = 6.5 mg/L). If you only used one tube of sample, it's the actual number of drops of titrant used (6 drops with one tube = 6 mg/L).
10) Report dissolved oxygen (mg/L) and temperature on the recording form.
11) Use instructions and chart below to convert D.O. to % saturation. Report % saturation on the recording form.

Testing for Dissolved Oxygen
using the LaMotte Test Kit Model 7414 or 5860

FIX YOUR SAMPLE

1) Put on protective gloves and safety goggles. If your skin comes in contact with any powder or titrant rinse the area liberally with water.
2) Holding the reagent bottle completely upside down, add 8 drops of Manganous Sulfate solution (labeled "1" on bottle).
3) Holding the reagent bottle completely upside down, add 8 drops of Alkaline Potassium Iodide Azide (labeled "2" on bottle).
4) Cap and shake the bottle for 30 seconds. A white to brownish orange floc will cloud the sample bottle. Let the floc settle until the top half of the bottle is clear.
5) Shake the bottle again. Allow the floc to settle again.
6) Add 8 drops of Sulfuric Acid 1:1 (red cap on bottle) and shake for 30 seconds. The solution will turn from cloudy to clear in color (If you still see some dark "pepper specks" in the solution add 1 more drop). Your sample is now "fixed".
7) Pour your fixed sample into the graduated cylinder to the 20 ml mark and then pour it into the titration vial (glass vial labeled code 0299).

PREPARE TO TITRATE

1) Pick up the plastic titrator syringe (labeled code 1649) and push in the plunger to expel air.
2) Put the tip of the titrator syringe into the hole in the top of the titrating solution (bottle labeled Sodium Thiosulfate 0.025N). Fill the syringe by turning the bottle upside down and slowly pull back on the syringe plunger until the tip on the bottom of the plunger is well past the zero mark on the scale on the titrator. You may have to push the plunger in and out a few times to get rid of any air bubbles in the syringe.
3) Turn everything right side up.
4) Slowly push the plunger until the large ring on the plunger of the plastic titrator syringe is right at the zero mark on the titrator.
5) Remove the titrator from the sodium thiosulfate bottle.

TITRATE THE SAMPLE

1) Put the tip of the titrator into the opening on the plastic cap of the titration vial (code 0299) that contains your fixed sample.
2) Add the titrating solution one drop at a time by gently pushing the plunger. Swirl the solution between drops until the sample has turned pale yellow. If your solution is already pale yellow skip this step. If your solution is colorless you have zero mg/l dissolved oxygen (if this is the case you can proceed to step 24 for confirmation, if you like).
3) Pop off the plastic cap from the titration vial with the titrator still in the hole without moving the plunger in the syringe.
4) Add 8 drops of starch indicator solution to the pale yellow sample in the titration vial. The sample should now turn deep blue or black.
5) Put the cap back on the titration vial.
6) Swirl to mix the contents.
7) Continue to add sodium thiosulfate one drop at a time, swirling the solution between each drop Observe the color change from dark blue to light blue.
8) Stop when the solution turns from pale blue to colorless. (If no color change occurs by the time the plunger tip reaches the bottom of the scale on the titrator, refill the titrator by filling with titrant to the zero mark and continue the titration. Include both titration amounts in the final test results.)
9) Read the test result directly from where the scale intersects the ring of the plunger for plastic titrator. The titrator is marked at 0.2 ppm increments. So if the titrator ring is touching the third line below the line marked "7" the result would be 7.6 mg/l dissolved oxygen. (If the titrator has been refilled once before, the result would be 17.6 mg/l dissolved oxygen.)
10) Report dissolved oxygen (mg/L) and temperature on the recording form.
11) Use instructions and chart below to convert D.O. to % saturation. Report % saturation on the recording form.

– Modified from URI Watershed Watch