

WATER SAMPLING

Before chemical analysis can begin, it is necessary to complete the following tasks in the specified order:

First - Empty the rain gauge 5 days before sampling occurs. On the fifth day, before leaving to sample, record the amount of precipitation for the period.

Second - On arrival at your sampling site, record current weather conditions, noting conditions from the previous 24 hours, as well as wildlife and other observations.

Third - Record both air and water temperatures, allowing 3-5 minutes for the thermometer to equilibrate. Air temperatures should be taken first with the thermometer in indirect sunlight and away from paved surfaces, while water temperature is best read with the thermometer suspended on a string in the middle of the water column. The reading should be recorded in degrees Celsius to the nearest half (0.5) degree on the Monitoring Data Sheet.

Fourth - Record water depth (in meters), flow and clarity. In shallower streams (less than one meter) where the bottom is visible, this can be accomplished with the meter stick. However, deeper streams (greater than one meter) or waters where the bottom is not visible may necessitate use of the Secchi disk.

STREAM BOTTOM SURVEY

Step 1. Estimate the relative proportions of each type of substrate material in your sampling area. Record this as **Substrate Size Percentage** on your data sheet.

Step 2. Remove several cobble sized stones from area just downstream of your site and estimate how much of each one was embedded in the finer sediments. There is generally a line or discoloration that indicates where the sediment line was. Take the average from the stones selected and record this as **Embeddedness**.

Step 3. Finally determine how consolidated the material is by kicking some rocks downstream of your site. The relative ease or difficulty should be recorded as **Consolidation**.

SUBSTRATE SIZE CATEGORIES

Extremely fine	Silt, clay, mud
Less than 0.25 cm	Sand
0.25 cm to 5 cm	Gravel
5 cm to 25 cm	Cobble
Greater than 25 cm	Boulder
Massive, unbroken	Bedrock

STREAM DEPTH

Step 1. Using your tape measure, determine the total width of the stream.

Step 2. Using the meter stick, measure stream depth at one foot intervals beginning 6 inches from the bank. Record each depth on the data sheet as **Depth₁**, **Depth₂**, etc. Divide the sum of the depths by the number of measurements taken + 1 (Sum of the depths/Number of measures + 1). This is the **Average Depth** of the stream.

SECCHI DISK

Step 1. Lower the disk into the water until it just disappears. Record the depth marked on the Secchi disk line as **disappears** on the data sheet.

Step 2. Lower the disk until it cannot be seen and slowly raise it until it reappears. Record this as **reappears** on the data sheet.

Step 3. Average the two depth values. This is the **Secchi disk transparency**.

Step 4. To ascertain depth, lower the disk until it touches bottom. Record the reading as **water depth**.

Note: In areas with strong currents, the disk may need to be weighted to ensure an accurate reading.

DISSOLVED OXYGEN TESTING PROCEDURES

The DO test consists of Winkler-type titration's, which rely on color indicators to yield results. Up to 3 tests may be done with a single sample bottle. It is a good idea to do periodic Quality Control checks on your kits by running duplicate DO samples (two separate sample bottles) at least once every 3 months. Results are recorded as **mg O₂/L** and the values should fall between 0.0-15.0.

It is *critical* for this test that in be preformed at approximately the same time of day for all sampling efforts, with mid-day preferred (10am to 2pm). Oxygen levels fluctuate widely throughout the day as demand from various organisms increases or decreases.

To ensure accuracy, the Water Sampling Bottle (0688-DO) should be filled *directly* from the body of water being sampled. There may however be times when you do not have direct access to a site. In this case it is permissible to use the bucket to retrieve sample waters. The DO procedure up to and including Step 6 should be followed *immediately*.

Step 1. To avoid contamination, thoroughly rinse the Water Sampling Bottle (0688-DO) 2 to 3 times with the water to be sampled.

Step 2. With the sample bottle pointed downstream, slowly tilt it while

submerging it slightly, and allow the water to fill the bottle. It is important to avoid a lot of bubbling as the water enters, since this can artificially increase your readings. Once the bottle has filled, keep it submerged and return it to a vertical position. Gently tap the side to remove any stray air bubbles and then cap the bottle while it is still under water.

Step 3. Lift the bottle out of the water, turn it upside down and look carefully to make sure that no air bubbles are trapped inside. Once a satisfactory sample has been collected, proceed immediately with Steps 4 through 6. *Note:* Be careful not to introduce air into the sample while adding the reagents in Steps 4 to 6. Physically put the dropper just above the surface of the sample bottle while adding the reagents, then cap it and mix gently.

Step 4. Add 8 drops of Manganese Sulfate Solution (4167) to the sample. Be sure to hold the dropper-bottle of indicator solution vertically (not tilted) and at eye-level to dispense uniformly-sized drops.

Step 5. Add 8 drops of Alkaline Potassium Iodide Solution (7166) to the sample. Carefully cap the bottle and mix by inverting gently 20-30 times. A precipitate (floc) will form. Allow the precipitate to settle below the shoulders of the bottle. Invert the bottle again and allow the precipitate to settle totally (note the color change). The clear-yellow to brown-orange color that develops is a result of the iodine in the reagent.

Step 6. Gently add 8 drops of Sulfuric Acid (6141) to the sampling bottle. Cap the bottle and gently mix until both the reagent and the precipitate have dissolved (some suspended material may remain). *Note:* **Step 6** "fixes" the water sample and takes about 5 minutes. Exposure of the sample to the atmosphere will no longer affect the test results. It is not necessary to perform the rest of the procedure (the actual test) immediately. Samples fixed in the field can be carried back to a testing station, laboratory or other sheltered area for testing. Titration (Step 9) should be completed no longer than 8 hours after fixing.

Step 7. Rinse the Titration Tube (0299) with distilled water, then pour the fixed DO sample into the tube filling it so that the bottom of the meniscus is level with the white (20ml) line.

Step 8. Fill the syringe-like Titrator (0377) to the "0" mark with Sodium Thiosulfate Solution (4169) making sure no air bubbles are in the Titrator. The next step is called titration.

Step 9. Titrate the sample using the following guidelines: Insert the Titrator into the hole in the cap of the Titrator Tube. Add 2 drops of Sodium

Thiosulfate Solution to the Titration Tube and gently swirl to mix. Keep adding Sodium Thiosulfate Solution 2 drops at a time and swirling until the yellow-brown color of the solution begins to fade (iodine reduction is occurring). Stop when the solution is a pale yellow (straw-colored). Remove the Titrator and store in its protective sleeve in DO kit (*do not* remove the remaining Sodium Thiosulfate Solution!).

Step 10. Add 8 drops of Starch Solution (4170) to the Titration Tube. Swirl the tube to mix. The solution should turn from light yellow to dark blue (this indicates that the iodine has been neutralized).

Step 11. Remove the Titrator from the kit, insert into the Titrator Tube (with the scale facing you) and inject 1 drop of Sodium Thiosulfate Solution and swirl. Continue this process until the solution turns from blue to clear.

Step 12. Using the scale on the side of the Titrator, record the total number of units of Sodium Thiosulfate used in titration (this amount equals the **mg O₂/L** in the water). Note both the whole and 0.2 unit graduations on side of the Titrator. Read the results from the tip of the stopper.

Step 13. Empty the Titration Tube and rinse it with distilled water. *Return to Step 7 and perform a second titration with the existing fixed sample.*

Step 14. Record the two acceptable readings on the data sheet, then record the average of those tests. This is your DO reading for the period. *Note:* At least two titration's are required for accurate DO measurements. If the amount of DO in the second titration varies from the DO from the first titration by greater than 0.6 mg/L, you must do a third titration. Record the average of the two lowest results on the Chemical Monitoring Data Sheet. It is a good idea to do periodic Quality Control checks on your kits by running duplicate DO samples (two separate sample bottles) at least once every 3 months.

Step 15. Discard the contents of the Water Sample Bottle, Titrator and Titrator tube. Rinse them with distilled water and replace in the kit, making sure to leave the plunger on the Titrator retracted slightly to preserve the life of the titrator. *Note:* Disposal may be accomplished by diluting with untreated sample water in your bucket and emptying the bucket either on the ground or in deeper water.

GENERAL PH TESTING PROCEDURES

This test is performed using a wide-range (5.0-10.0) pH field test kit which utilizes an Octet Comparator, much like those used for swimming pools or aquariums. The comparator contains eight permanent color standards, ranging from 5.0-10.0 pH units. A test sample is inserted into one of the openings in the top of the comparator and then compared to four color standards at once. If the test sample color matches one of the standard colors, the value of the standard is read directly on the face of the comparator. For optimum color comparison, the comparator should be positioned between the operator and a light source, so that light enters through the special light-diffusing screen in the back of the comparator. Avoid irregular or colored light sources.

WIDE RANGE TEST

Step 1. Rinse the small test tube (0230) with sample water, then refill the tube to the 5ml line with sample.

Step 2. Add ten (10) drops of wide-range indicator solution (2218) to the sample in the tube. Be sure to hold the dropper-bottle of indicator solution vertically (not tilted) and at eye-level to dispense uniformly-sized drops.

Step 3. Cap the tube and invert 10 times to mix the contents.

Step 4. Insert tube into comparator and remove the cap. To obtain the pH, match the color of the test sample against the color standards.

Step 5. After discarding the test sample, rinse the test tube with distilled water and replace all materials in the test kit.

Note: pH readings can only be read in whole and half (0.5) units. Thus, pH readings of 6.0 or 5.5 would be acceptable, while a reading of 7.25 would not.

ACID MINE PH (3.0 - 10.0)

Step 1. Thoroughly rinse two viewing tubes with the water to be tested. Fill the tubes to the 5 mL mark with the water sample.

Step 2. Add six drops of Wide Range 4 pH Indicator Solution to one of the tubes and swirl to mix. Be sure to hold the dropper-bottle of indicator solution vertically (not tilted) and at eye-level to dispense uniformly-sized drops.

Step 3. Insert the prepared sample into the right top opening of the color comparator.

Step 4. Insert the tube of untreated sample into the left top opening of the comparator.

Step 5. Hold the comparator up to a light source such as the sky, a window or lamp and view through the openings in front. Rotate the disc to obtain a color match. Read the pH directly through the scale window. Avoid colored or irregular light sources.

Step 6. Rinse the vials with distilled water and return them to the kit.

NITRATE-N AND NITRATE TESTING PROCEDURES

Best results are obtained when all solutions are kept close to 20° Celsius (68° F).

Step 1. Fill one test tube (0820) with sample water so that the bottom of the meniscus is level with the first line (2.5 ml).

Step 2. Add mixed Acid Reagent (6278) to the second line (5.0 ml total).

Step 3. Cap and mix. Wait two minutes before proceeding to the next step.

Step 4. Using the 0.1g spoon marked "N", add one level measure of Nitrate Reducing Reagent (6279) to the tube, being careful to avoid moisture and recapping the reagent bottle immediately.

Step 5. Cap and gently invert the tube 50-60 times (or for one minute).

Step 6. Wait 10 minutes and mix one last time before inserting the tube into the Axial Reader. While you're waiting for the color to develop prepare 2 control "blanks" by adding unaltered sample water to the remaining 2 test tubes (be sure to fill each of these above the 5.0ml line). Put one "blank" in the rear of the comparator on either side of the sample. Then, insert the ampoule of distilled water (included in your kit) in the opening in the front of the comparator (directly in front of the sample).

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Step 7. Place the sample tube in the Axial Reader directly behind the clear window on the left-hand (Nitrate-N) side and remove the cap.

Step 8. Holding the Comparator in front of a clear light source, slide the Axial Reader until you can closely match the color of the sample with the standards. Numbers are expressed as mg Nitrogen/L.

Step 9. Record the level of Nitrate-Nitrogen on the Monitoring Data Sheet.

Step 10. Multiply the Nitrate-Nitrogen reading by 4.4. This multiplier will convert Nitrate-N to Nitrate. E.g.: $0.3 \text{ mg Nitrate-N/L} \times 4.4 = 1.32 \text{ mg Nitrate}$.

Step 11. Due to traces of cadmium in Nitrate waste, collect your Nitrate waste in a bottle for proper disposal. After discarding the sample, rinse the test tube with distilled water and replace it in the kit.

Helpful hints for working with the Axial Reader:

- If the color of the test sample is less than the color of the lowest value (0.2 mg/L), the result should be recorded as "less than (<) 0.2 mg/L". Conversely, if it is greater than the highest value (1.0 mg/L) it should be recorded as "greater than (>) 1.0 mg/L".
- If the color of the test sample matches one of the color standards in a quadrant, the result is recorded as the value of that color standard.
- If the color of the test sample falls between these two values, it is recorded as the *average* of these two values.
- If the color of the test sample is darker than the color of the second color standard, the comparator is moved to a position where the bottom of the Axial Reader is level with the bottom of the comparator. This movement aligns the mirror with the bottom row of windows in the comparator. The comparator unit should be moved carefully within the reading device to avoid spilling the samples. The comparison of the unknown sample is made with the standards in the lower left-hand quadrant of the Octet comparator.

TO PREPARE A DILUTED NITRATE-N SAMPLE: When Nitrate-N readings are consistently out of range for the Comparator (greater than 1.0 mg N/L), it may be necessary to dilute the sample so that it can be read with the Axial Reader. To perform this test, you *must* use *uncontaminated* distilled water. A dilute sample should be run under the following conditions and should be so *noted on the Data Sheet!*:

- If the amount of Nitrate is repeatedly above the Axial Reader's detection limit (greater than 1.0 mg N/L); or
- you cannot distinguish the difference in color in the 0.6-1.0 range on the Axial Reader.

Step 1. Fill one of the test tubes so that the bottom of the meniscus is level with the first line (2.5 ml) with water from the sample bottle.

Step 2. Add distilled water to the second line (5.0 ml total).

Step 3. Cap and mix thoroughly. The dilute sample can now be used for testing.

Step 4. Transfer 2.5 ml of the diluted sample to the first line of a test tube.

Step 5. Continue with normal Nitrate-N testing procedure above, beginning with **Step 3**.

DO NOT FORGET to multiply the diluted test result by 2.0 to obtain the amount of Nitrate-N in the original sample and note that a dilution was carried out.

PHOSPHATE TESTING PROCEDURES

This test determines levels of Orthophosphates (those phosphates mostly attributed to land-related uses) and should be run on clear samples only. The best results are obtained when solution temperatures are close to 23° C (73° F).

Step 1. Fill a test tube with sample water so that the bottom of the meniscus is level with the third line (10 ml).

Step 2. Use the 1.0 ml pipette to add 1.0 ml of Phosphate Acid Reagent (6282) to the sample, cap and mix.

Step 3. Use the 0.1g measuring spoon (0699) marked "P" to add one level measure of Phosphate Reducing Reagent (6283). Cap and mix until dissolved. Then place in the Axial Reader.

Step 4. Wait 5 to 7 minutes for the color to develop. While you're waiting for the color to develop, prepare 2 control "blanks" by adding unaltered sample water to the remaining 2 test tubes (be sure to fill each of these above the 10.0ml line). Put one "blank" in the rear of the comparator on either side of the sample. Then, insert the ampoule of distilled water (included in your kit) in the opening in the front of the comparator (directly in front of the sample).

Note: The tubes that were used as blanks for the nitrogen test may be reused, simply shift them to the position for phosphorous.

Step 5. Remove the cap from your sample tube and read the result in mg PO₄/L.

Step 6. After diluting and discarding the sample, rinse the sample vial with dilute acid from the red wash bottle and replace it in the test kit. Rinse the control blank vials with distilled water and replace them in the test kit.

TO PREPARE A DILUTED PHOSPHOROUS SAMPLE: When phosphorous readings are consistently out of range for the Comparator (greater than 1.0 mg PO₄/L), it may be necessary to dilute the sample so that it can be read with the Axial Reader. To perform this test, you must use *uncontaminated* distilled water. A dilute sample should be run under the following conditions and should be so noted on the *Data Sheet!*:

- If the amount of Phosphorous is repeatedly above the Axial Reader's detection limit (greater than 1.0 mg PO₄/L); or
- you cannot distinguish the difference in color in the 0.6-1.0 range on the Axial Reader.

Step 1. Fill one of the test tubes so that the bottom of the meniscus is level with the second line (5.0 ml) with water from the sample bottle.

Step 2. Add distilled water to the third line (10.0 ml total).

Step 3. Cap and mix thoroughly. The dilute sample can now be used for testing.

Step 4. Continue with normal Phosphate testing procedures beginning at **Step 2.**

DO NOT FORGET to multiply the diluted test result by 2.0 to obtain the amount of Phosphorous in the original sample and note that a dilution was carried out.

TOTAL ACIDITY

Step 1. Fill the plastic measuring tube level full of the water to be tested. Pour the contents of the tube into the mixing bottle.

Step 2. Open one Phenolphthalein Indicator Powder Pillow and add the contents of the pillow to the mixing bottle. Swirl gently to mix.

Step 3. If the sample turns pink the total acidity is zero.

Step 4. If the sample remains colorless, use the dropper to add Sodium Hydroxide Standard Solution drop by drop to the mixing bottle. Hold the dropper vertically above the bottle, at eye level and swirl the bottle after each drop is added. Count the total drops used until the sample maintains a pink color for 30 seconds.

Step 5. The total acidity of the water, in mg calcium carbonate per Liter (mg CaCO₃/L), is equal to multiplying by 17.1 the number of drops of Sodium Hydroxide Standard Solution needed to bring about the color change in Step 4.

Step 6. Rinse the measuring tube with distilled water and return it to the kit.

ALKALINITY

High Range

Step 1. Fill the plastic measuring tube full to the top with the water to be tested. Pour the contents of the tube into the mixing bottle.

Step 2. Open a Phenolphthalein Indicator Powder Pillow and add the contents of the pillow to the mixing bottle. Swirl gently to mix.

Step 3. If the water remains colorless after the addition of the phenolphthalein powder, the phenolphthalein alkalinity is zero. If this is the case, proceed to Step 6.

Step 4. If the water becomes pink upon addition of phenolphthalein powder, use the dropper to add Sulfuric Acid Standard Solution drop by drop to the mixing

bottle. Swirl to mix after each drop is added. Count the drops used, continuing to add drops until the water becomes colorless.

Step 5. The phenolphthalein alkalinity of the water, in mg of calcium carbonate per Liter (mg CaCO₃/L), is equal to multiplying by 17.1 the number of drops of Sulfuric Acid Standard Solution used to bring about the color change in Step 4.

Step 6. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the mixing bottle. Swirl to mix.

Step 7. Add Sulfuric Acid Standard Solution drop by drop to the mixing bottle. Count the drops added and swirl to mix after each drop is added. Continue to add drops until the color changes from blue-green to pink.

Step 8. The total (methyl orange) alkalinity, in mg of calcium carbonate per Liter (mg CaCO₃/L), is equal to multiplying by 17.1 the sum of the drops of Sulfuric Acid Standard Solution used in Steps 4 through 7.

Step 9. Rinse the mixing vial with distilled water and return it to the kit.

Low Range

Step 1. Fill the mixing bottle to the 15 mL mark with the water to be tested.

Step 2. Open a Phenolphthalein Indicator Powder Pillow and add the contents of the pillow to the mixing bottle. Swirl gently to mix.

Step 3. If the water remains colorless after the addition of the phenolphthalein powder, the phenolphthalein alkalinity is zero. If this is the case proceed to Step 6.

Step 4. If the water becomes pink upon addition of phenolphthalein powder, use the dropper to add Sulfuric Acid Standard Solution drop by drop to the mixing bottle. Swirl to mix after each drop is added. Count the drops used, continuing to add drops until the water becomes colorless.

Step 5. The phenolphthalein alkalinity of the water, mg of calcium carbonate per Liter (mg CaCO₃/L), is found by multiplying by 6.84 the number of drops of Sulfuric Acid Standard Solution used to bring about the color change in Step 4.

Step 6. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the mixing bottle and swirl gently to mix.

Step 7. Add Sulfuric Acid Standard Solution drop by drop to the mixing bottle. Count the drops added and swirl to mix after each drop is added. Continue to add drops until the color changes from blue-green to pink.

Step 8. The total (methyl orange) alkalinity, mg of calcium carbonate per Liter (mg CaCO₃/L), is found by multiplying by 6.84 the drops of Sulfuric Acid Standard Solution used in Steps 4 through 7.

Step 9. Rinse the mixing vial with distilled water and return it to the kit.

IRON

Step 1. Open one FerroVer Iron Reagent Pillow and add the contents of the pillow to the sample, stopper the vial and shake to mix. An orange color will develop if iron is present. Wait at least two minutes for full color development before proceeding.

Step 2. Place the tube of prepared sample in the right top opening of the color comparator.

Step 3. Fill the other viewing tube with an untreated water sample and place the tube in the left top opening of the comparator.

Step 4. Hold the comparator up to a light source such as a window, the sky or a lamp and view through the openings in front. Rotate the disc to obtain a color match and read the mg/L iron (Fe) directly through the scale window. Be careful to avoid colored or irregular light sources.

Step 5. Rinse both vials with distilled water and return them to the kit.

SUSPENDED SOLIDS

Fill the labeled sample bottle from the middle of the water column and cap tightly. Make arraignments to get the bottle to your Volunteer Coordinator within 48 hours.

