Delaware Riverkeeper Network's Water Testing Protocols

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For real company and friendship, there is nothing outside of the animal kingdom that is comparable to a river.

- Henry Van Dyke, Little Rivers, 1895

Revised 3/09 with assistance from Lamotte Company

ROLE OF RIVER WATCH

The Delaware Riverkeeper Network (DRN) Monitoring Program, initiated in 1991, empowers local citizens by training them to be educated water quality monitors. Our monitors serve as a front-line of protection to streams in the Delaware Watershed that federal, state, and local agencies lack the resources to monitor. DRN has trained over 3,000 volunteers in water quality testing procedures. The majority of volunteer monitors collect data on dissolved oxygen, pH, nitrate, ortho-phosphate, temperature and physical parameters including riffle embededness and consolidation.

The goal of River Watch is to empower citizens with basic knowledge of water chemistry and parameters that affect their local streams. This knowledge and involvement gives citizens increased tools and credibility when speaking for their local streams during the decision-making process. Data collected by volunteer monitors is entered into a Delaware Watershed-wide database maintained by DRN and is used to advocate for better protection and restoration of the river and its tributary streams.

DRN's reputation of having a well-known and reliable Monitoring Program has earned it several unique partnerships. DRN's Monitoring Program is a service provider of the Consortium for Scientific Assistance to Watersheds (C-SAW). This state-wide partnership with others including USGS, Alliance for Aquatic Resource Monitoring, Stroud Water Research Center, Wilkes University, and EPCAMR is available to assist local watershed groups, municipalities and other water-based entities in Pennsylvania with a wide array of technical services. We are also part of New Jersey's Water Watch Advisory Council developed in 2003 and have been a partner with PA DEP's Citizens Volunteer Monitoring Program since 1999. In these roles, we have helped develop training workshops for volunteer monitors, make strides to improve the use and reliability of volunteer data through study design and standardization, and raise awareness of the power and necessity of volunteer-collected data.

Quality Assurance and Quality Control Measures

DRN has an agency-approved Quality Assurance and Quality Control Plan which is available for review upon request. To better validate our volunteer monitoring data, the measures listed below are followed with specific projects:

- All new volunteer monitors are required to attend a three-hour training session to review protocols and techniques.

- River Mentor Network – DRN has a regional River Mentor Network made up of reliable volunteer monitors who have been certified to understand and teach monitoring protocols to new volunteers entering the program. These River Mentors are also available to further assist new volunteers on a one-on-one basis.
- All active volunteer monitors who utilize our database, are required to attend annual QA/QC Sessions, which use split sampling and/or blind samples to text monitors' accuracy and techniques. At these sessions, datasheets are reviewed and monitoring issues are also discussed.
- Expired chemical reagents are disposed of and kit inspections are also performed regularly. New reagents are provided to the volunteer monitors if needed.
- Standardized step-by-step protocols, approved by kit manufacturers and tested by our volunteer monitors for usability, are provided to each volunteer monitor
- A set of standardized datasheets are provided to each volunteer monitor for data recording
- Data is sent to the DRN office, reviewed for completeness and accuracy by the DRN, and entered into a centralized database.

Kit Specifications and Equipment

The majority of volunteers use test kits manufactured by Lamotte Company. Below is a table outlining some of the kits that are used:

Parameter	*Measurement Range*[1]	*Accuracy*	*Manufacturer/Model/Cost*
Temperature	0-45 °C	+/- 0.5 °C	Lamotte 1066/$17.50
pH	3-10	+/- 1.0 pH unit	Lamotte 2117/$29.95
pH	0-14	+/-0.5 pH unit	ColorpHast indicator strips - EMD Chemicals
Nitrate-nitrogen	0.2-1.0 mg/L	+/- 0.2 mg/L	Lamotte 3119/$78.65
Nitrate-nitrogen	0 – 15 mg/L	+/- 2 mg/L	Lamotte 3354/$39.95
Ortho-phosphate	0.2-1.0 mg/L	+/- 0.2 mg/L	Lamotte 3119/$78.65
Dissolved oxygen	0-10 mg/L	+/- 0.2 mg/L	Lamotte 5850/$37.00

Kit ranges can be increased using standardized dilution measures recommended by kit manufacturer

BASICS ABOUT DISSOLVED OXYGEN

Almost all plants and animals, whether living on land or in water, require oxygen for their survival. In water, oxygen is present in a dissolved form and is measured as the oxygen gas present, in milligrams (mg), per liter (l) of water. Milligrams per liter (mg/l) can also be expressed as parts per million (ppm). Dissolved oxygen (DO) can also be measured as percent saturation which takes into account temperature. Saturation is the maximum level of DO that would be present in the water at a specific temperature, in the absence of other influences.

The amount of oxygen in water is a function of gas transfer from the atmosphere to surface waters. This transfer is assisted by the mixing action of wind, waves, riffles, and other turbulence that facilitates air mixing with water. When present, during the daylight hours, aquatic plants also add a substantial amount of oxygen to the stream via photosynthesis. At night, these same plants dramatically decrease DO resources due to respiration, as they use up oxygen. Since photosynthetic activities of floating and rooted aquatic plants are dependent on light, oxygen-producing processes occur nearer the water surface.

DO has a daily cycle, affected by temperature and photosynthesis. Water's ability to hold DO is decreased under warmer conditions. For example, streams near a cooling water discharge point or in an unshaded section of stream usually have higher temperatures and therefore, lower dissolved oxygen levels. Generally, the lowest DO is likely to occur during the hottest time of the year, at low flows, just before dawn (hottest temperatures and highest respiration of plants). Because the temperature of a stream varies daily, and even hourly, it is important to factor out the effect of temperature when analyzing dissolved oxygen levels in a sample of water. Percent saturation can be used to eliminate this variation due to temperature.

Increased organic pollution and nutrients (nitrogen and phosphorous) from fertilizers, manure piles, lawn waste dumped in or near streams, leaking septic systems and inadequate sewage treatment facilities indirectly decrease DO by stimulating the growth of algae and aerobic bacteria. When this algae uses up the excess nutrients, it dies, and the bacteria decay process robs the water column of DO. Increased sediment loads and stormwater runoff can also decrease dissolved oxygen. Dams may cause an oxygen deficit in the impoundment as water temperatures increase. When this water is released from the spillway, it may have less oxygen due to increased organic matter (in the form of plant and animal remains) that tends to accumulate in ponds below the surface (often, directly below the spillway, DO levels may be extremely high due to increased turbulence).

Many organisms begin to experience stress as dissolved oxygen levels drop, much like you would feel walking on a mountaintop at 8,000 feet. At DO levels of 5.0 milligrams per liter (mg/l) in warm-water fisheries (or 6.0 mg/l in cold-water fisheries), this stress is becoming large enough that scientists are able to see less diversity in our biological monitoring techniques, as the old, young and weak individuals die off. Some hardier species may be able to tolerate levels below 4.0 mg/l, but they are far from thriving and absolute lethal levels for these hardier species are reached at 2.0 mg/l.

In summary, oxygen is affected naturally by temperature, flow, aquatic plants and bacteria, altitude, and dissolved or suspended solids. Human activities affecting DO include removal of riparian vegetation, urban development, organic and nutrient inputs, and dams. Generally, oxygen supplies are lowest in the summer months, just before dawn, when waters are warmest, bacterial decomposition is at its highest, and photosynthesis is at its lowest.

TESTING PROCEDURES FOR DISSOLVED OXYGEN (LAMOTTE 5860)

This test (LaMotte 5860) uses the azide modification of the Winkler titration method to determine dissolved oxygen. Results are recorded as **mg O_2/L or ppm O_2** and the values should fall between 0.0-15.0. 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 a clean sample container to retrieve sample water. The DO procedure up to and including Step 6 should be followed *immediately* in order to obtain accurate results.

Step 1. To avoid contamination, thoroughly rinse the Water Sample Bottle (0688-DO) 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 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.

Step 4. Add 8 drops of Manganese Sulfate Solution (4167) followed by 8 drops of Alkaline Potassium Iodide (7166) to the sample. Be sure to hold the dropper-bottles of indicator solution vertically (not tilted) and at eye-level to dispense uniformly-sized drops. **Note** – chemicals will drop to the bottom and displace water from the sample bottle. This helps keep oxygen out of the sample.

Step 5. Carefully cap the bottle and mix by inverting 30 times. (Putting on the cap will also displace water, preventing oxygen from entering the sample.) A precipitate (floc) will form. Allow the precipitate to settle below the shoulders of the bottle.

Step 6. 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). Step 6 "*fixes*" the water sample and takes about 5 minutes. The solution will be clear yellow to orange if the sample contains dissolved oxygen. ***Note***: After performing this step, 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 and the sample should be refrigerated until testing. If sample is kept in the refrigerator, allow it to come to room temperature before proceeding with titration.

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. Continue the titration. 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 vigorously. 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 O_2/L or ppm O_2 in the water). Note both the whole and 0.2 unit graduations on side of the Titrator. Read results from the widest part of the titrator tip (see specific titrator directions published by Lamotte and note whether a plastic or glass titrator is in your kit – plastic titrators are used in the newer kits).

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 titrations 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 closest results on the Chemical Monitoring Data Sheet.

Step 15. Discard waste reagents by diluting and dumping 50 yards from the stream in a vegetated area. Rinse equipment with distilled water and replace in the kit. If using a glass titrator, store the titrator in two pieces to preserve the life of the syringe. A plastic titrator, used in updated kits, does not come apart for storage.

BASICS ABOUT pH

pH is a measure of how acidic or basic a solution. The pH test measures the concentration of hydrogen ions (H^+) and hydroxyl ions (OH^-) and is based on a scale of 0 to 14. A ph of 7.0 is neutral while lower numbers indicate an acid nature (more H^+ ions), and higher numbers indicate basic conditions (more OH^- ions). The pH scale is logarithmic which means that each incremental change is equal to a ten-fold increase or decrease in the acid or base.

Each stream tends to have a narrow range of pH values but most levels fall between 6 and 9. Natural unpolluted rain water has a pH as acidic as 5.6. As rainwater falls through the atmosphere, it absorbs carbon dioxide, forming carbonic acid. Where soils are alkaline (basic), like in limestone streams, pH levels may be greater than 7. These basic qualities often help buffer the stream, giving the stream an ability to maintain a constant pH even when large amounts of acid or base enter the stream. Aquatic plants, when present, also impact pH levels, causing pH to have a daily cycle. Photosynthesis causes increases in pH as plants remove carbon dioxide from the water during the day. Conversely, at night, pH decreases as plants give off carbon dioxide during respiration. As the pH changes, so do the chemical reactions in the water. For example, as pH increases, smaller amounts of ammonia are needed to reach a level that is toxic to fish. As pH decreases, the concentration of metals may increase because highly acidic water acts as a solvent, leaching metals from the sediments and substrate.

Aquatic organisms generally prefer a pH range of 6.5-8.0 (9.0 for marine) and deviations from this can have serious effects on the health of a stream. A pH of 4.0 or below can destroy aquatic eggs and larvae, and frequently results in fish kills and/or mutations. Low pH leaches metals from soils and rocks, resulting in poisoning and deformities. While the effects of high pH levels (above 9.0) are not as well documented, it is likely that these also cause mutations in aquatic organisms. In areas where coal mining is present, mine drainage often has acidic pH levels as low as 4.0, making streams inhospitable to wildlife. Acid rain also decreases pH levels (PA receives the most acid rain of any state). Acid rain is formed when nitrogen oxides and sulfur dioxides are released from our cars and fossil fuel burning power plants.

GENERAL PH TESTING PROCEDURES (LAMOTTE KIT 2117)

This test is performed using a wide-range (3.0-10.0) 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 3.0-10.0 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.
pH usually varies between

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. Remove cap and insert tube into comparator (2192). To obtain the pH, match the color of the test sample against the color standards and record the result on your datasheet.

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

Notes: 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.

If you are using Kit 2120, the procedure above applies. The only difference is that the comparator has a range from 5.0-10.0

General pH Testing Procedures (Using ColorpHast Indicator Strips)

Using four different indicators, the multi-colored pattern that results guarantees a high degree of precision. The ColorpHast 0-14 universal pH strips can be used with colored or turbid solutions and will not add impurities or bleed into test samples. Strips are bonded to a cellulose backing for easy use. The indicators used to express the pH of the samples are covalently bonded to the strip itself. Since these indicators are irreversibly bound, they will never bleed, form uneven color zones, or fade under intense light. This is an important and unique feature of colorpHast strips compared to most other pH methodologies. They are manufactured under ISO 9001 quality standards. Sensitivity of the test strips is (+/-) 0.5 pH unit. EMD color**pH**ast® test strips and indicator papers are optimized for accurate results if used with stream samples at 20°C (near room temperature-68°F).

Test strips should be stored between 10-25°C and out of direct light.

General Procedure for the Use of colorpHast® Test Strips (www.emdchemicals.com)

1. Remove the strip from the box and cap box to protect unused strips.
2. Immerse the reaction zone of the strip into the stream sample for one minute or until there is no further color change. Test strips are most accurate when sample water is at about 20°C (68°F) so best to check temperature before conducting test**
3. Remove the strip from the stream sample, wiping the strip along the edge of the vessel to remove excess liquid from the strip.
4. Compare the reaction zones to the color chart on the box holding the strip to the top of the box with the reaction zones to the bottom of the box. Read the result within 30 seconds to one minute.
5. Record the results of the closest matching color to the strip. You can record half units for this test. For example, if the colors are between a 6 and a 7, you can record your result as 6.5

** For weakly buffered solutions, an extended period of time may be necessary. In these cases, immerse strip until there is no further color change (1-10 minutes). Because the indicator is covalently bound to the colorpHast® strip, the test strip may be immersed in the solution for longer periods of time if necessary.

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BASICS ABOUT NITRATE

Nitrogen makes up about 80 percent of the air that we breathe and is an essential component of proteins. In aquatic systems, the inert gas Nitrogen is converted to useable forms by bacteria, which are then taken up by algae and other plants. Nitrogen occurs in natural waters in various forms including nitrate (NO_3), nitrite (NO_2), and ammonia (NH_3). Nitrate (or nitrate-nitrogen) is the most common form tested in water.

While nitrates are essential to plant growth, an overabundance can lead to eutrophication (increased plant growth), often blocking sunlight to the water column. When all nutrients are used up by algae, this excess algae dies and the natural decay process robs dissolved oxygen from the stream, potentially causing fish kills and other impairments to aquatic life. Nitrate is not usually the limiting growth factor for plant growth (phosphorus is the limiting factor).

Nitrogen levels are affected by ammonia in acid rain, freezing and thawing of soils, forest fires, and recycling by vegetation and retention by the soil's humus layer. High levels of nitrogen are generally the result of improperly treated sewage from treatment plants or leaky septic systems; runoff from over-fertilized agricultural fields, lawns, and golf courses; pet, livestock, and other animal waste; detergents; and industrial effluent. In addition to leading to eutrophication, high levels of nitrates also impact human health. The national drinking water standard is 10 mg/l nitrate-nitrogen and drinking water should not exceed this level. In many cases, rural communities where farms are in operation, have levels of nitrate-nitrogen higher than this in their wells. These elevated levels can cause blue baby syndrome or methemoglobinemia which is hazardous to infant animals and humans. This blood disorder is caused when nitrite interacts with the hemoglobin in red blood cells. Unlike hemoglobin, the methemoglobin formed in this interaction cannot carry sufficient oxygen to the body's cells and tissues.

When nitrate levels are greater than 1.0 mg/l as nitrate-nitrogen (or 4.4 mg/l as nitrate), you can suspect degraded water quality and unnatural sources of nitrogen entering the system. From the biological perspective, no nitrate limit is established in the water quality standards however; in a general sense, less is better.

NITRATE-N AND NITRATE TESTING PROCEDURES (LAMOTTE KIT 3119)

This test uses the cadmium reduction method to determine nitrate nitrogen which can be converted to nitrate by multiplying by 4.4. An axial reader and comparator are used to determine the results by comparing the sample with four color standards. The mirror on the axial reader should be aligned properly to ensure accurate results. Best results are obtained when all solutions are kept close to 20° Celsius (68° F).

Step 1. Fill one test tube (0820 or 0844) 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 a clean 0.1g spoon (0699) 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 30-40 times in one minute. Wait 10 minutes for the color to develop.

Step 6. While you're waiting for the color to develop, prepare two control "blanks" by adding unaltered sample water to the remaining two test tubes (be sure to fill each of these to the 5.0ml line). Put one "blank" in the rear of the comparator on either side of the test tube with 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).

Step 7. At ten minutes, mix test tube one last time, remove cap, and insert the test tube into the Axial Reader. Place the sample tube in the Axial Reader directly behind the clear window on the left-hand (Nitrate-N) side of the colorimeter and directly behind the ampoule.

Step 8. Holding the comparator near a clear light source or by a sunny window (if inside) OR with your back to direct sunlight but away from shade (if outside) slide the Axial Reader until you can closely match the color of the sample with the standards. Be sure to remove the test tube caps before determining the result and be sure you are aligning the axial reader mirror correctly. Numbers are expressed as mg Nitrate-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 mg Nitrate-N/L x 4.4 = 1.32 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 then 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.
- Always be sure to align the mirror on the axial reader behind the comparator standard you are comparing with the sample. The comparator unit should be moved carefully within the reading device to avoid spilling the samples.

***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 clean test tube.

Step 5. Continue with normal Nitrate-N testing procedure above, beginning with **Step 2.**

***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. If your sample station consistently requires a dilution, contact DRN for a high range comparator or different testing equipment.**

NITRATE-NITROGEN Testing Procedures (Lamotte Kit 3354 Zinc Reduction)

The Lamotte Kit 3354 is not as precise as the cadmium reduction method (Lamotte 3119) but this test does not use the heavy metal (cadmium). This test also allows for higher nitrogen measurements to be obtained without having to perform a dilution. The Lamotte 3354 Kit has varying degrees of error depending on the measurement. The higher the reading, the greater degree of error. Generally, the error can be as great as halfway between each color standard. For example, a reading of 2 has an accepted error of -.5/+1, a reading of 4 has an accepted error of +/-1, and a reading of 10 has an accepted error of +2.5/-1. You can estimate values between color standards if the sample does not match a color standard exactly.

Be sure to run a blank using distilled water first. This is part of the internal QA/QC for this test. Record your blank value on the datasheet. The value should be 0.

Step 1. Fill one of the test tubes (0124 or 0106) to the 5 mL line (middle line) with sample water.

Step 2. Add to the sample one **Nitrate #1** tablet (Item #2799).

Step 3. Cap the test tube with a small cap (provided) and mix vigorously until the tablet dissolves in the sample. (Tiny particles of the tablet may remain suspended in the sample and will not affect the accuracy of the test.)

Step 4. Uncap the test tube and add one **Nitrate #2** CTA tablet (item #NN-3703) to the sample.

Step 5. Recap the test tube and mix vigorously until the tablet is fully dissolved. (Tiny particles of the tablet may remain suspended in the sample and will not affect the accuracy of the test.) Once the tablet is dissolved, let the sample stand for five minutes. **Do not wait longer than 5 minutes to read the sample.

Step 6. While waiting 5 minutes for the sample to react, insert the Octa-Slide Bar (3494) into the Octa-Slide Viewer (1100).

Step 7. At the five-minute mark, insert the test tube into the opening in the top of the viewer (1100). Match the sample color with the color on the slide bar (nitrate standard). If outside when reading the result, be sure to read the value with your back to the sun or in indirect light. If inside, read result near a window. Record the value as ppm Nitrate Nitrogen.

Step 8. Rinse all test tubes with distilled water and keep the kit dry and clean and at room temperature for storage.

TO PREPARE A DILUTED NITRATE SAMPLE

If you run this test and get a value that is greater than 15 ppm, this means that the nitrate level is greater than 15 ppm and the test kit cannot read the actual value. You should prepare a diluted sample and run the test again.

. **To prepare a diluted sample**, fill the test tube with sample water to the 2.5 mL line. Add uncontaminated distilled water to the 5 mL line. Cap and mix the diluted sample. The diluted sample is now ready to run the test. Follow the directions above, starting from Step 2.

At Step 7, when recording the nitrate result, be sure to multiply the result by 2 to account for the dilution. For example, if you get a reading of 8 using the diluted sample, the actual reading you will record on your datasheet is 16 ppm of nitrate nitrogen.

BASICS ABOUT PHOSPHATE

Phosphorous usually occurs in nature as phosphate and is crucial for the formation of DNA and proteins. Phosphate (PO_4) is found in two forms in aquatic systems and both can either be dissolved in the water or suspended (attached to particles in the water). Organic phosphate is bound to plant or animal tissue. Inorganic phosphate (ortho-phosphate) is the form most readily available to plants, and thus the most useful indicator of immediate problems with excessive plant and algal growth. Ortho-phosphate is also the easiest form to measure.

In many natural waters, phosphate concentrations are normally low (less than 0.01 mg/l). When levels become greater than 0.1 mg/L, this often indicates pollution. Phosphate is usually the limiting nutrient for plant growth, meaning it is in short supply relative to nitrogen. Inputs from treated sewage and leaky septic systems; over fertilization of crops and lawns; commercial cleaning operations; and deforestation and draining of wetlands supply an abundance of phosphate that result in extremely high levels of plant productivity or eutrophication. When these plants die, the decay process robs dissolved oxygen from the stream, often causing impairments to aquatic life.

PHOSPHATE TESTING PROCEDURES (LAMOTTE 3119)

This test (Lamotte 3119) determines levels of Orthophosphates (those phosphates mostly attributed to land-related uses) by using the ascorbic acid method. An axial reader and comparator are used to determine the results by comparing the sample with four blue color standards. The mirror on the axial reader should be aligned properly to ensure accurate results. 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. Remove the test tube cap and place into the Axial Reader. Wait five minutes for the color to develop.

Step 4. 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 PO_4/L. Hold the comparator near a clear light source or by a sunny window (if inside) OR with your back to direct sun but away from shade (if outside). Be sure to remove the test tube caps and be sure you are aligning the axial reader mirror correctly to obtain accurate results. Record your result on your datasheet.

Step 6. After diluting and discarding the sample, rinse the sample vial with distilled water 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 PO_4/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 PO_4/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 Orthophosphate in the original sample and note that a dilution was carried out.**

ORTHO-PHOSPHATE USING HACH TEST KIT PO-19

This test is done by filling two small tubes with sample water and adding into them a phosphate reagent. The two tubes are placed into a color comparator for viewing. The color comparator has a measuring disc contained in it that allows the sampler to obtain a number that is then divided by 50 in order to obtain the orthophosphate reading in mg/L. Orthophosphates generally enter a water system through the use of fertilizers and insecticides. Low range testing is for phosphate levels ranging from 0-1 mg/L, mid-range testing is for levels ranging from 0-5 mg/L and high range testing is for levels ranging from 0-50 mg/L. This HACH test has a more sensitive detection limit than the Lamotte Test (3119).

Low Range Identification

Step 1. Insert the Long Path Viewing Adapter (small black piece) into the color comparator by opening the comparator and inserting the adapter much in the same way of loading film.

Step 2. Fill one viewing tube to the top line, which is designated by "No.1730", with sample water. This is the blank.

Step 3. Insert this tube into the top left opening (when looking at color comparator, the disc should be on the right) of the color comparator.

Step 4. Fill the square-mixing bottle (large bottle) to the 20-mL mark with sample water.

Step 5. Add the contents of one PhosVer® 3 Phosphate Reagent Powder Pillow into the mixing bottle.

Step 6. Swirl the mixture vigorously until all of the contents have dissolved. Be sure to wait eight minutes for full color to appear in the bottle. If phosphate is present, a blue-violet color will develop in the bottle.

Step 7. Using the solution in the mixture bottle, fill another small viewing tube to the line marked "No. 1730".

Step 8. Place the second viewing tube into the top right opening (when looking at comparator, the disc should be on your right) of the color comparator.

Step 9. View the color of the samples by holding the color comparator towards a light source and looking through the openings in the front of the comparator. Be careful not to spill the samples.

Step 10. While looking into the openings in the front of the comparator, rotate the color disc until the color matches between the two openings.

Step 11. When the colors in the two openings match, obtain the number from the scale window (directly below the two openings in the front) and divide the number by 50 to obtain the final reading in mg/L phosphate.

TURBIDITY

This test **(LaMotte Code 7519)** is made by comparing the turbidity of a measured amount of the sample with an identical amount of turbidity-free water containing a measured amount of standardized turbidity reagent. The readings are made by looking down through the column of liquid at a black dot. If turbidity is present, it will interfere with the passage of light through the column of liquid at a black dot. Small amounts of turbidity will cause a "blurring" of the black dot in the bottom of the tube. Large amounts of turbidity may provide sufficient "cloudiness" so that it is not possible to see the black dot when looking down through the column. Any color that may be present in the sample should be disregarded. This determination is concerned only with the haziness or cloudy nature of the sample.

Step 1. Fill one Turbidity Column (0835) to the 50 mL line with sample water. If the black dot on the bottom of the tube is not visible when looking down through the column of liquid, pour out a sufficient amount of the test sample so that the tube is filled to the 25 mL line.

Step 2. Fill the second Turbidity Column (0835) with an amount of distilled water that is equal to the amount of sample being measured (either 25 or 50 mL). This is the **"clear water" tube** or blank sample.

Step 3. Place the two tubes side by side and note the difference in clarity. If the black dot is equally clear in both tubes, the turbidity is zero. If the black dot in the sample tube is less clear, proceed to Step 4.

Step 4. Shake the Standard Turbidity Reagent (7520) **vigorously**. Add 0.5 mL to the "clear water" tube. Use the stirring rod (1114) to stir contents of both tubes to equally distribute turbid particles. Check for amount of turbidity by looking down through the solution at the black dot. If the turbidity of the sample water is greater than that of the "clear water", continue to add Standard Turbidity Reagent in 0.5 mL increments to the "clear water" tube, mixing after each addition until the turbidity equals that of the sample. Record the total amount of Turbidity Reagent added. **Please note:** When looking at the dot, it may be easier to look at the edges of the dot to determine cloudiness. Also, be sure to disregard any color.

Step 5. Record the number of additions and total reagent amount used on the datasheet. **Please note:** If you add reagent to the "clear water" sample and the sample becomes significantly cloudier than the creek

sample, record the number of additions prior to adding the final addition of reagent. Each 0.5 mL addition to the 50 mL size sample is equal to 5 Jackson Turbidity Unites (JTUs). If a 25 mL sample size is used, each 0.5 mL addition of the Standard Turbidity Reagent is equal to 10 Jackson Turbidity Unites (JTUs).

Step 6. Rinse both tubes thoroughly with distilled water after performing the test and let tubes dry before storing the kit. Waste reagents are not hazardous and can be disposed of 50 yards from the stream in a vegetated area.