



CHEMICAL ACTION TEAM: OFF-SITE PROCEDURES

Nitrate, Phosphate, Dissolved Oxygen Titration (after on-site fixation)

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Nitrate and Phosphate

The following sequence for the Nitrate and Phosphate tests is suggested for efficiency of time. Some steps require waiting several minutes for a reaction, so other steps are inserted there to allow work to continue uninterrupted. Each step below includes an indication of which test it is for: (N) Nitrate and (P) Phosphate. Stream sample should be at room temperature.

1. (N) Swish a tall test tube and cap with sample water, then fill it to the 2.5ml line. This is the **N-tube**. Carefully add *Mixed Acid Reagent* to the tube until it reaches the 5ml line. Cap and mix. Note current time on the back or side of the data sheet (you must wait at least 2 minutes to move on the next step of the Nitrate test.)
2. (P) Swish a tall test tube and cap with sample water, then fill it to the 10ml line. This is the **P-tube**. Using a 1ml pipette, add 1ml of *Phosphate Acid Reagent* to the tube. Cap and mix.
3. (P) Using the 0.1g spoon, add one level measure of *Phosphate Reducing Reagent* to the **P-tube**. Cap and mix until dissolved. Note current time on back or side of the data sheet (you must wait at least 5 minutes to move on to the next step of the Phosphate test.)
4. (N) Verify that at least 2 minutes have elapsed since the end of Step 1. Using a 0.1g spoon (different spoon from that used in the Phosphate test), add one level measure of *Nitrate Reducing Reagent* to the **N-tube**. Cap and invert tube once every two seconds for one minute. Note current time on back or side of data sheet (you must wait at least 10 minutes to move on to the next step of the Nitrate test.)
5. (P) Verify that at least 5 minutes have elapsed since the end of Step 3. Remove the cap from the **P-tube** and place it in the right side of the Axial Reader, behind the clear ampoule. Rinse and fill two separate test tubes with distilled water, then place in the slots on both sides of the **P-tube**. Verify that all 3 tubes are uncapped. Compare the color of the **P-tube** sample to the color standards in the Axial Reader. (Light should shine down from above through the top of the tubes for best results.) Record the results of the Phosphate test on the data sheet. Empty the **P-tube** into the poisons container and rinse with demineralized water. Save the two tubes of distilled water for the next step.
6. (N) Verify that at least 10 minutes have elapsed since the end of Step 4. Invert the **N-tube** several times, then uncap and place in the left side of the Axial Reader, behind the clear ampoule. Move two test tubes of distilled water to the slots on both sides of the **N-tube**. Verify that all 3 tubes are uncapped. Compare the color of the **N-tube** sample to the color standards in the Axial Reader. (Light should shine down from above through the top of the tubes for best results.) Record the results of the Nitrate test on the data sheet.

If the result is equal to 1ppm or less, the Nitrate test is complete. Empty all tubes into the poisons container and rinse with demineralized water. Store in kit uncapped.

If the results are greater than 1ppm, the sample water requires dilution and retesting. Empty the **N-tube** into the poisons container and rinse with demineralized water. Save the two tubes of distilled water for the next step. Move to Step 7.

7. (N) This step is only required if the initial nitrate result from Step 6 was greater than 1ppm.
 - 7a. Rinse a fresh 10ml test tube and cap with demineralized water, then sample water. Fill to the 2.5ml line with the sample water, then add demineralized water to bring the tube to the 10ml line. Cap and mix thoroughly.
 - 7b. After having emptied and rinsed the **N-tube** from Step 6 with demineralized water, rinse again with the diluted sample water from Step 7a. Using a 1ml pipette, transfer 2.5ml of the diluted sample water to the **N-tube**. Then carefully add *Mixed Acid Reagent* to the tube until it reaches the 5ml line. Cap, mix, and wait 2 minutes.
 - 7c. Wait two minutes until the end of Step 7b, then go back and complete Steps 4 and 6 above with the diluted water sample. After measuring the result on the Axial Reader, multiply the reading by 4 to obtain the Nitrate result of the original sample. Record the result on the data sheet.
 - 7d. Empty all tubes into the poisons container and rinse with demineralized water. Store in kit uncapped.

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Dissolved Oxygen (DO)

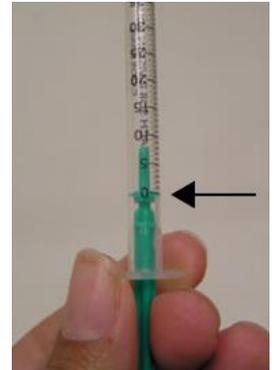
Fixed DO samples must be no older than 8 hours.

1. Rinse titration tube with demineralized water and fill to the 20ml line with the fixed sample liquid from one of the DO bottles.
2. Add 8 drops of *Starch Indicator Solution* to the titration tube. Cap and swirl (sample should turn blue.)
3. Fill the titrator to the 0 mark with *Sodium Thiosulfate*.
(Insert titrator into plastic fitting on *Sodium Thiosulfate* bottle, invert, slowly withdraw plunger until the solution reaches the 0 mark as shown by the green edge of the plunger, then revert bottle and remove titrator from bottle. If an air bubble is introduced into the titrator, eject reagent back into the bottle and try again.)
4. Insert titrator into the center hole of the titration tube cap. Add one single drop at a time. Swirl tube between drops to disperse titrant. (This requires patience!) Continue adding drops until the liquid inside the titration tube becomes completely clear. Do not add more drops after the liquid clears. (Holding up a white sheet of paper behind the tube will help with an accurate reading.) Read the test result from the side of the titrator, the number where the green edge of the plunger ends up. (Each small division equals 0.2ppm.) Record this result in ppm on the data sheet.

If you empty the titrator without clearing the blue color in the titration tube, your result is greater than 10ppm. Fill the titrator again (according to Step 3), and continue titrating until the liquid in the tube becomes completely clear. Read the result from the titrator and be sure to add the 10ppm from the first titration to calculate your final result.
5. Discard the liquid from the titration tube, the liquid from the opened DO sample bottle, and any remaining reagent in the titrator into the poisons container. Rinse with demineralized water.
6. Repeat Steps 1-5 using the fixed sample from a second DO bottle.

If the results are within 0.6ppm of each other, record both results and average them on the data sheet.

If the two results are greater than 0.6ppm apart, repeat Steps 1-5 using the fixed sample from the third DO bottle. Average only the two closest results on the data sheet.
7. After rinsing all equipment with demineralized water, store in kit. Store the DO bottles uncapped.



Post-Testing Procedures

1. Submit your data to the StreamWatch Coordinator within one week of your sample. You may scan and email, fax, or mail your original data sheet. There are also fillable PDF forms and Word documents on the StreamWatch webpage if you would prefer to complete it electronically.
2. Check the expiration dates on all reagents. If any have expired or will expire the following month, contact the StreamWatch Coordinator to replace them prior to the next month's test.
3. Check your poisons container. If it is full, contact the StreamWatch Coordinator to empty it or obtain a new container.
4. Refill your demineralizer bottle with tap water and shake for 30 seconds. It should be stored full.
5. **Every other month, please perform the Kit Cleaning procedures as listed below:**
 1. Fill a bucket or bowl with hot, soapy water. Use only detergents/soaps that are phosphate free, such as Ivory Liquid Detergent.
 2. Submerge all glass test tubes, bottles, spoons, and droppers. Be sure that the top part of the dropper is removed from the tube and allowed to soak separately. Let sit for several minutes.
 3. Gently scrub the inside and outside of your labware until all deposits have been removed. (You may use a tube brush, pipe cleaner, Q-Tip, baby toothbrush, or whatever you have on hand.) Toothpicks can be used to loosen and remove deposits from spoons.
 4. Rinse tubes in fresh warm tap water until soap residue is gone. Then rinse again with distilled water.
 5. Let air dry overnight and store in CAT kit with bottles and tubes uncapped.