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| Measurement of Dissolved Oxygen in Water Samples |                   |                   |  |
| Revision: #9                                     | Replaces: 2/19/14 | Effective: 9/3/19 |  |

1. **Purpose and Scope**: Sampling of any water body which supports aquatic life must be accompanied by a measurement of the dissolved oxygen (DO) content. These measurements help to evaluate the capacity of the water to support life. All visible aquatic species require a minimum DO to survive, which varies with the species. When DO decreases, species become stressed and mortality results at extremely low DO concentrations. This SOP describes how to collect, handle, and measure DO from surface water samples using the Hach AccuVac DO test kit. If you have previously used a DO meter or a Hach Powder Pillow test kit to measure DO and are proficient in their use, you may continue to use one of these two methods, if available. This method can be used in place of previously implemented methods to measure DO. Any instructions on surface water sampling and documentation found in the Environmental Monitoring Plan (EMP) supersedes instructions contained in this SOP.

**2. Supplies Required**: To request sampling supplies, contact the monitoring supplies coordinators, Lisa Mosser (305) 278 4902, or Richard King (305) 278 2905, Center for Plant Health Science and Technology, Miami, or email the Environmental Monitoring Supplies Checklist to lisa.k.mosser@usda.gov or richard.a.king@usda.gov.

- 2.1 "grab sampler" (a wide-mouth plastic container firmly attached to a 6-foot pole)
- 2.2 AccuVac Dissolved Oxygen Test Kit Hach Company
  - 2.2.1 AccuVac test ampules for high range concentrations of DO (0-15 mg/l)
  - 2.2.2 AccuVac color comparator
  - 2.2.3 40 ml plastic beaker (supplied in kit)
  - 2.2.4 Zeroing vial
  - 2.2.5 Plastic ampule breaker
  - 2.2.6 Blue ampule cap
- 2.3 field log book
- 2.4 environmental monitoring forms (APHIS Form 2060)
- 2.5 thermometer

**3. Measuring DO in a Surface Water Sample with a Hach AccuVac DO test kit.** Directions for using the test kit are supplied with each test kit, and should be carefully followed. Directions are reiterated in this document as well. The chemicals needed for measuring DO have been individually packaged in a sealed (under vacuum), glass vial.

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- 3.1 Collect a "grab" water sample with the wide-mouth container affixed to the end of a pole. Minimize the agitation of water during the collection process so as not to introduce oxygen via air bubbles.
- 3.2 After collecting water, dispense 40 ml into the small plastic beaker provided in the test kit and 10 ml into the zeroing vial provided.
- 3.3 The sample in the zeroing vial serves as a blank control. Place this vial in the left top opening of the AccuVac color comparator.
- 3.4 Use the ampules provided in the kit (dissolved oxygen range between 1-15 mg/l). Place the ampule breaker into the beaker containing the 40 ml sample. Place the AccuVac ampule in the ampule breaker and push down on the ampule with firm, steady pressure to break the tip. The ampule has been sealed under a vacuum, so once the glass tip is broken the ampule automatically fills with the water sample. The ampule can also be filled by simply breaking the glass tip against the side of the beaker well below the water surface.
- 3.5 Once the ampule is filled with the water sample, and without inverting the ampule, immediately place the blue ampule cap over the tip of the ampule. Capping the ampule tip prevents contamination of the sample with atmospheric oxygen. If atmospheric oxygen is accidentally introduced into the sample it could result in an erroneously high DO measurement.
- 3.6 Shake the ampule for 30 seconds. Allow two minutes for color development inverting the ampule occasionally to ensure thorough mixing.
- 3.7 Shake the ampule again for 10-15 seconds and place the ampule in the right top opening of the AccuVac color comparator.
- 3.8 Rotate the color disc until the color matches in the two openings. Read the dissolved oxygen concentration in mg/l through the scale window. Record the results in the field book.
- 3.9 Collect another grab sample and measure the temperature of this water with a thermometer. Do not use the water initially collected for the DO since the temperature may have changed to match that of the air.
- 3.10 The water may be discarded on the ground. The test ampule should be disposed of in the same manner as broken glass. Rinse out all equipment with pond water or distilled water. Upon return to the office, glassware should be washed, rinsed with tap water and allowed to dry.