Washington State Department of Ecology

Environmental Assessment Program

Water Quality Studies Unit

Standard Operating Procedure for Measuring Dissolved Oxygen in Surface Water

Version 1.1

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EAP035

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Signatures on File

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
4/16/07		Editorial Review	All	Bill Kammin
6/27/07		Editorial Review	All	Karol Erickson
6/28/07	1.1	Minor editorial changes	All	Nuri Mathieu
7/25/2007	1.1	Final SOP revisions	All	Bill Kammin

Environmental Assessment Program

Standard Operating Procedure for Sampling Bacteria

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Directed Studies Unit Standard Operating Procedure (SOP) for Measuring Dissolved Oxygen in freshwater and marine waterbodies.

2.0 Applicability

2.1 This SOP focuses on the collection of dissolved oxygen samples for determination by the azide-modified Winkler titration method. The proper titration method is covered by the provisional SOP EAP023: Winkler Determination of Dissolved Oxygen.

3.0 Definitions

3.1 Dissolved Oxygen (DO): The amount of oxygen gas dissolved in a volume of water; usually expressed in milligrams per liter or percent of saturation.

3.2 Ecology : Washington State Department of Ecology.

3.3 EAP: Environmental Assessment Program.

3.4 Thalweg**:** The deepest part of the active channel of a water course.

4.0 Personnel Qualifications/Responsibilities

4.1 All field staff must comply with the requirements of the EA Safety Manual (EA Program, 2006). A full working knowledge of the procedures in Chapter 1 'General Field Work', especially the sections 'Working in Rivers and Streams', 'Working near Traffic and from Bridges', and 'Fall Protection' is expected. If they are sampled, Chapter 5 'Inspecting Wastewater Treatment Facilities' is required reading. Sampling from an Ecology boat requires one person onboard to be a qualified boat operator as described in Interim Ecology Policy 11-60; all persons onboard must be familiar with Chapter 3 of the EA Safety Manual, 'Boating.'.

4.2 The Field Lead directing sample collection must be knowledgeable of all aspects of project's Quality Assurance Project Plan (QAPP) to ensure that credible and useable data are collected. All field staff should be briefed by the Field Lead or Project Manager on the sampling goals and objectives prior to arriving to the site.

5.0 Equipment, Reagents, and Supplies

5.1 General Equipment and Supplies

5.1.1 DO transport box

5.1.2 300 ML glass biological oxygen demand (BOD) bottles and caps(Figure 1)

5.1.3 Plastic BOD bottle water seal caps

5.1.4 Rubber gloves

5.1.5 2 mL pipettes

5.1.6 Deionized water squirt polyethylene (PE) bottle

5.1.7 Deionized water (Type 1, Resistivity of greater than 10 megaohm-cm).

5.1.8 1 Liter or larger PE bottle or funnel with bottom removed and tubing attached to mouth (Figure 2d).

5.1.9 Van Dorn, Kemmerer sampler, or other appropriate sampler for sampling lakes, from bridges, or at greater depths (Figures 2a-c).

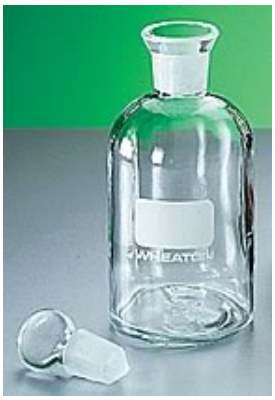

Figure 1. 300mL glass BOD bottle and cap

5.2 Reagents

5.2.1 Manganous sulfate solution in 125 mL clear polyethylene (PE) bottle.

5.2.2 ***Caution* This chemical is a skin and eye irritant and may be harmful if inhaled or swallowed.** For more information see MSDS @: X:\EA PROGRAM\ECYEAPSOP\Approved SOPs\Winkler SOP Attachments\ Magnanese Sulfate.pdf

5.2.3 Alkali-iodide-azide reagent in 125 mL clear polyethylene (PE) bottle. ***Danger* This chemical is corrosive and may cause eye or skin burns or internal damage if inhaled or swallowed.** For more information see MSDS @: X:\EA PROGRAM\ECYEAPSOP\Approved SOPs\Winkler SOP Attachments\ Alkaline Iodine Azide.pdf

5.3 Sample Containers

5.3.1 The normal container for dissolved oxygen samples is a 300 mL glass biological oxygen demand (BOD) bottle, narrow and flared mouth, tapered and pointed ground glass stopper.

5.3.2 Containers should be cleaned as needed with soap and water and well rinsed with tap water. Store bottles upside down on a perforated mat to promote drying and to keep out dust. Store glass stoppers in a manner that prevents dust and other contaminants from adhering to them.

5.3.3 Check DO bottle numbers before sampling to ensure that all numbers are different (no duplications) to avoid sample confusion.

6.0 Summary of Procedure

6.1 Sampling Preparation

6.1.1 EAP Wet Lab Preparation

6.1.1.1 The wet labs located at the EAP Western Operations Center (OC), the Eastern Regional Office Operations Center (EROC), and the Central Regional Office (CRO) are maintained by all lab users. Please do your part to keep it clean, tidy and safe. Titration chemicals and equipment are maintained by the current lab stewards at each facility. Bottles and preservatives for each vehicle, or for each project, are maintained by the vehicle custodian and/or project officer.

6.1.1.2 The appropriate EAP lab steward should be contacted immediately if lab supplies or chemicals or running low or might be contaminated or expired so that fresh supplies/chemicals can be ordered. The project officer should check all equipment and reagents before sampling and notify others involved in the project of the status of the DO lab and other equipment. The project officer is also responsible to coordinate with lab stewards for the replacement/repair of DO equipment and/or chemicals.

6.1.1.3 The following procedures were compiled from a variety of references including the personal experiences of EAP personnel.

6.1.2 Prepare Preservatives and Dispensers

6.1.2.1 Ensure that preservatives are fresh and that the dispensing equipment is clean. Small, plastic, bulb-type pipettes are commonly used. Automatic pipettes have not worked well due to infrequent use and difficulty of maintenance.

6.1.2.2 The alkali-iodide-azide solution is chemically unstable.. This supersaturated solution will form precipitate with the introduction of dirt or other impurities and be difficult to pipette. Discard azide solution if precipitate forms and replace with fresh solution. Standard Methods indicates that the white turbidity in the azide solution is acceptable. Stir turbid solution before using and discard annually (APHA, 1998).

6.1.2.3 Transport only small amounts of the preservatives into the field. 125 mL clear PE bottles are recommended to transport preservatives. The use of clear bottles makes it easier to determine if the reagent has spoiled. Only 2 mL of each preservative is needed per sample.

6.1.2.4 Label reagents and pipettes to avoid cross contamination. Have extra pipettes on hand. Store pipettes in a manner that will prevent their coming into contact with contaminants (e.g. bucket bottoms, van drawers, etc.).

6.2 Sample Collection

6.2.1 Rinse bottle and stopper with sample (2-3 times) before filling.

6.2.2 Collect sample just under the surface and in the upper 1/2 meter of the water body (unless collecting at predetermined depths). Avoid collecting the surface film. Avoid entraining or dissolving atmospheric oxygen. Prevent turbulence and bubble formation.

6.2.3 Selecting an Appropriate Intermediate Sampling Device.

6.2.3.1 The American Public Health Association (APHA) type DO sampler (Figure 2a) is used to collect samples from a bridge or from the stream bank through the use of a rope.

6.2.3.2 The Kemmerer (Figure 2b) and Van Dorn (Figure 2c) samplers are often used to collect samples at depth in lakes or deeper rivers.

6.2.3.3 The PE bottle with tube (Figure 2d) is typically used to collect samples in shallow, wadeable streams.

Figure 2a. APHA type DO sampler

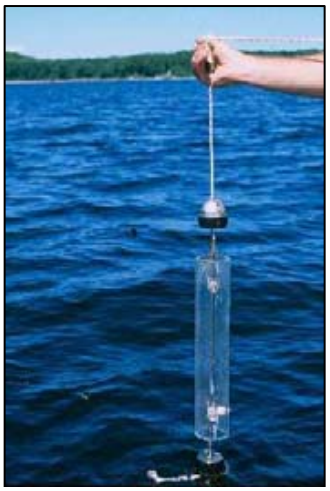

Figure 2b. Kemmerer Sampler

Figure 2c. Van Dorn sampler

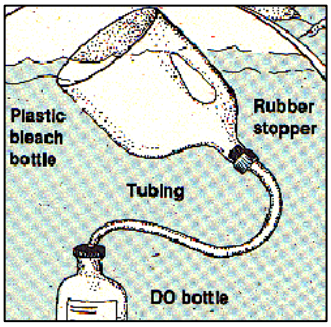

Figure 2d. Example of a PE bottle and tube surface sampler

6.2.4 Sample collection with APHA type DO sampler.

6.2.4.1 Rinse the sampler, top, and filler tubes with stream, tap water, or DI water.

6.2.4.2 Place the BOD bottle into the sampler. Orient the lid of the sampler to insure the filler tube is inserted into the BOD bottle and the lid is secure.

6.2.4.3 Attach the sampling rope to sampler.

6.2.4.4 Move to a well mixed location such as the main part of the channel where a representative sample may be collected.

6.2.4.5 Carefully lower the sampler to the water surface, taking care to not dislodge any bridge debris onto it. Raise the sampler off the water for a few moments to allow any debris from the bottom of the sampler to drop off and float away, and rapidly lower the sampler about 0.5 meters to submerged it. This minimizes the sampling of surface film and any debris from the bottom of the sampler.

6.2.4.6 Retrieve the sampler when the bubbles from the vent tube stop (bottle is full). If a swift current carries the sampler downstream (before it can completely fill) then pull the

sampler from the water, allow it to swing upstream, and then drop it back into the water. This action may need to be repeated a few times until the BOD bottle fills.

6.2.4.7 Retrieve the sampler, taking care not to dislodge bridge debris into it.

6.2.4.8 Set the sampler down and carefully remove the top.

6.2.4.9 Remove the BOD bottle.

6.2.5 Sample collection using PE bottle with tube.

6.2.5.1 Rinse the funnel in the stream.

6.2.5.2 Invert the funnel or orient the open end of the funnel upstream and slowly submerge it until it and the funnel tubing completely fills avoiding any entrainment of air bubbles.Pinch the end of the funnel tubing and remove the funnel (top end first) from the water.

6.2.5.3 Insert the end of the funnel tubing into the bottom of a BOD bottle, allow the funnel to overfill the bottle 2-3 times until it is nearly empty, and then quickly withdraw the tubing (do not use any samples that were aerated by the final discharge from the funnel).

6.2.6 Sample Collection using Van Dorn or Kemmerer sampler.

6.2.6.1 Rinse the sampler with water, attach the sampling rope, and set the sampler trigger mechanism.

6.2.6.2 Lower the sampler to the desired depth and trip the trigger.

6.2.6.3 Insert the end of the sampler tubing into the bottom of a BOD bottle, open the top of the sampler or the air inlet valve, and over fill the bottle with 2-3 times its volume prior to quickly removing the tubing (do not use any samples that were aerated by the final discharge from the sampler).

6.2.7 Sample Collection by hand.

6.2.7.1 ** Warning: Using this method may bias the sample toward saturation, particularly when dissolved oxygen of the water is significantly below saturation. This method does not allow for the Standard Methods guidance that the sample overflow 2-3 times its volume. It is recommended that this method only be used if the equipment for the methods listed above (Section 6.2.1) is unavailable. Record in the field notes if this method is used.*

6.2.7.2 Invert bottle and submerge to appropriate depth. Slowly turn the bottle upright until the bottle is completely full and contains no visible air bubbles.

6.2.8 Stopper the Sample

6.2.8.1 Ensure that no air bubbles remain in bottle after filling. If bubbles adhere to the sides of the bottle, dislodge them by tapping bottle with the top part of the glass stopper. If bubbles prove difficult to remove, collect another sample and/or use another bottle (bubble trapping bottles probably need cleaning).

6.2.8.2 Stopper immediately. Don't let sample remain in contact with air after collection. Avoid trapping air as the stopper is inserted.

6.2.8.3 Once stoppered, dump the excess sample from the top of the bottle. This ensures that the sample volume used to determine DO is correct; adding preservatives to an unstoppered and full-to-the-lip bottle may result in over-estimating the DO content of the sample.

6.3 Sample Preservation and Storage

6.3.1 Sample Preservation

6.3.1.1 References indicate that sample preservation include addition of three preservatives after the sample is collected (manganous sulfate, alkali-iodide-azide, and sulfuric acid). EAP deviates from this by adding the acid just prior to titration rather than adding acid to the freshly collected sample. This eliminates the need to transport and handle the extremely hazardous sulfuric acid in the field.

6.3.1.2 Samples that have an appreciable oxygen or iodine demand should have reagents added immediately. (Iodine demand occurs in waters experiencing intense phytoplankton growth - see references for preservation of, and subsequent correction for, iodine demanding samples).

6.3.1.3 To Preserve Sample:

6.3.1.3.1 Remove stopper from bottle (holding the upper, flat part between fingers with stopper pointing away from the back of your hand works for many people), avoid agitating the sample.

6.3.1.3.2 Add 2 mL manganous sulfate to the sample first. **(NOTE:** The order that preservatives are added is critical). Hold pipette tip just above the surface of the sample and dispense reagent. Avoid introducing air into the sample. Do not submerge the tip of the dispenser into the sample, air might inadvertently be introduced into the sample. **If** the pipette is dipped into the sample, rinse with Deionized water or discard the pipette to prevent contamination of the reagent stock.

6.3.1.3.3 Add 2 mL alkali-iodide-azide solution to the sample in the same manner as the manganous sulfate (avoid contact with skin). A brown precipitate will begin to form. Samples with very low DO may exhibit a milky-white floc.

6.3.1.3.4 (Standard Methods 20th Ed. states to add 1 mL**,** rather than 2 mL, of each of the two fixing reagents. We add the reagents to "excess," and this is accounted for during the transfer of prepared sample to the titrating flask; the volume used is 203 mL rather than 201 mL)

6.3.1.3.5 Inspect the bottle for the presence of air bubbles entrained during the fixing process. If needed, dislodge bubbles by tapping the bottle with the glass stopper.

6.3.1.3.6 Stopper carefully to exclude air and twist stopper gently to ensure a good seal. Mix sample by inverting the bottle several times. The brown precipitate will begin to settle. Rinse bottle of chemical residue with stream water or DI.

6.3.1.3.7 Add DI or stream water to bottle/stopper top to provide a water seal. Cap bottle with plastic cap to prevent water seal from evaporating or spilling. Record sample bottle number, time, location, etc.

6.3.2 Sample Storage

6.3.2.1 It is preferable to store samples in the dark and at the temperature of the water source or at 10-20 degrees Celsius. Samples should be titrated as soon as possible. For iodine or oxygen demanding samples, see Standard Methods 20th Edition.

6.3.2.2 (Most references state that samples should be titrated within a few hours or within 8 hours - this is coincident with field acidification of the sample as well. Our practices vary and samples have been stored for several days with 2 preservatives before adding acid and titrating. We recommend titrating as soon as possible, at the end of the day (within 8 hours). If samples are held longer before titrating, remarks indicating the longer holding time should be made in the methods section of the report).

6.4 Sample Titration

6.4.1 Refer to Stream Sampling Protocols for the Environmental Monitoring and Trends Section available online at: http://www.ecy.wa.gov/biblio/0103036.html

6.5 Measurement by Dissolved Oxygen Meter

6.5.1 Refer to EAP's Standard Operating Procedures for Hydrolab® DataSonde® and MiniSonde® Multiprobes located on the web at: http://www.ecy.wa.gov/programs/eap/qa/docs/ECY_EAP_SOP_Hydrolab_v1_0.pdf

6.6 Collecting samples during continuous dissolved oxygen probe deployment

6.6.1 Refer to SOP for Adjusting Continuous Dissolved Oxygen Data based on Winkler Check Standards.

7.0 Records Management

7.1 Each sample collection will be fully described in the field notebook with waterproof ink, e.g. date, time, location identification, sample laboratory identification number, sample type, analyses to be performed, and ancillary data. Entries will be kept neat and concise. Measures will be taken to avoid losing the field notebook.

7.2 Sample locations will be described in enough detail to find on a USGS 7.5 minute map or an Environmental Information Management (EIM) System map cover. Otherwise, a global positioning system (GPS) unit will be used to record an accurate location. Coordinates will be recorded as per EIM requirements.

8.0 Quality Control and Quality Assurance

8.1 QA/QC procedures will be addressed thoroughly on a project-by-project basis in the QAPP for the project. Typically, one duplicate Winkler sample is collected for every ten Winkler samples. The variation between duplicate Winklers is expected to be at or below 2.5% relative standard deviation (RSD), where % RSD equals the standard deviation divided by the mean and multiplied by 100.

9.0 Safety

9.1 Preferably, gloves should be worn to avoid exposure to corrosive chemicals and potentially contaminated water. If gloves are not worn, hands should be cleaned using anti-bacterial soap or hand sanitizer after completing work at each sampling station or, at a minimum, after completing work at sampling stations with known high bacteria counts and before ingesting food or drink.

9.2 Refer to attached MSDS sheets for proper first aid and cleanup procedures should a chemical spill or exposure occur.

9.3 After sampling assume your hands and anything they touch are contaminated with bacteria and use care accordingly.

9.4 For further field health and safety measures refer to the:
Environmental Assessment Program (EAP) Safety Manual

10.0 References

10.1 APHA, 1998. Standard Methods for the Examination of Waste and Wastewater. 20th Edition.

10.2 MEL, 2005. Manchester Environmental Laboratory Lab Users Manual Eighth Edition. Environmental Assessment Program. Washington State Department of Ecology. Manchester, WA.