Protocols for the Performance Verification of *In situ* Dissolved Oxygen Sensors

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ACT PV14-01

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1.0 Background on ACT Technology Evaluations

The Alliance for Coastal Technologies (ACT) has initiated this Performance Verification of commercially available in situ dissolved oxygen (DO) sensors as a follow up to the first ACT Technology Evaluation conducted on DO sensors in 2003/2004. Over the past 10 years, there have been significant advancements in this class of instrumentation, while the need for accurate and reliable spatially and temporally intensive measurements of DO remains a high priority in fresh, coastal and ocean waters around the world. ACT selected in situ DO sensors for its next technology evaluation based on 1) DO sensors being an NOS priority for habitat quality monitoring and ecological forecasting; and 2) a consensus from stakeholders of the need for performance information on the "next generation" DO sensors that have been developed over the last decade since ACT's previous evaluation. The DO sensor verification will follow ACT's standard evaluation process.

These test protocols delineate how ACT will evaluate the performance characteristics of *in situ* DO sensors through the collection and analysis of quality-assured environmental data. The overall goals of ACT's verification program are to provide industry with an opportunity to have a third-party (ACT) test their instruments in both controlled laboratory settings and in diverse field applications within a range of coastal environments, and to provide users of this technology with an independent and credible assessment of instrument performance. The Verification program also provides an opportunity to promote emerging technologies to the scientific and management communities. The instrument performance characteristics examined in the verification reflect the needs of the broader research and management communities.

The fundamental objectives of this Performance Verification are to: (1) highlight the capabilities of particular in situ DO sensors by demonstrating their utility in a range of coastal environments; (2) verify the claims of manufacturers on the performance characteristics of commercially available DO sensors when tested in a controlled laboratory setting, and (3) verify performance characteristics of commercially available DO sensors when applied in real world applications in a diverse range of coastal environments.

ACT does not certify technologies, nor guarantee that technologies will always operate at the verified standards, especially under conditions other than those used in testing; ACT does not seek to determine regulatory compliance; does not rank technologies, nor directly compare performance between specific instruments; ACT does not label, nor list technologies as "acceptable" or "unacceptable;" and does not seek to determine "best available technology" in any way. ACT will avoid any statements that imply "winners or losers". Thus, although the following protocols will be used to test all instruments evaluated in this program, there will be no direct comparisons of instruments. After the tests are complete, Instrument Performance Verification Statements for each instrument will be released to the public.

2.0 Technical Advisory Committee

These *Protocols for the Performance Verification of in situ Dissolved Oxygen Sensors* were developed under the guidance of our external Technical Advisory Committee (TAC) and in collaboration with accepted applicants and ACT staff. The members of the Technical Advisory

Committee are as follows:

Dr. Erik Smith, Baruch Institute for Marine and Coastal Sciences
Ms. Janice Fulford, U.S. Geological Survey
Dr. Henry Bittig, Helmholtz Centre for Ocean Research Kiel
Mr. Steve Ruberg, NOAA Great Lakes Environmental Research Laboratory

3.0 Definition of Test Parameters

Initial laboratory mesocosm tests will focus on verifying accuracy, precision, and response linearity in a controlled environment, over a range of salinity, temperature and DO conditions. The field tests will focus on reliability and the ability of the instrument to consistently track natural changes in DO.

- Accuracy – a measure of the closeness of a measured value to the true value. For this verification, the accuracies of the test instruments will be determined in a controlled laboratory test by making repeated comparisons between instrument measurements and reference water sample DO determinations measured by Winkler titration following procedures of Carignan et. al. 1998 as defined below.

- Precision – Precision is a measure of the repeatability of a measurement. Instrument precision will be determined in the laboratory test by calculating the standard deviation of a minimum of 30 consecutive measurements of a reference condition under stable temperature and salinity conditions

- Stability - Calibration stability will be assessed based on the difference between paired instrument and reference DO measurements throughout the lab test and field deployments. A minimum of 300 comparative measurements will be conducted for the laboratory test and a minimum of 100 paired comparisons will be conducted for each moored field deployment.

- Reliability –Reliability is the ability to maintain integrity or stability of the instrument and data collections over time. Reliability of instruments will be determined in two ways. In field tests, comparisons will be made of the percent of data recovered as a proportion of the data that an instrument was designed to collect during its deployment period. Comments on the physical condition of the instruments (e.g., physical damage, flooding, corrosion, battery failure, etc.) will also be recorded.

4.0 Introduction to Technology

There are two primary types of dissolved oxygen sensing technologies available: the optical based sensing method which is commonly referred to as luminescent and the Clark electrochemical or membrane-covered electrode. The optical-based DO sensors can be designed to measure either the lifetime or the intensity of luminescence as it is affected by the presence of oxygen. Typically the sensor uses a blue LED light to excite a lumiphore which then emits red photons. This emission is quenched in the presence of dissolved oxygen and the lifetime of the emission has an inverse relationship with the partial pressure of oxygen. A major advantage of the luminescent approach is that oxygen molecules are not consumed during the measurement

process. Oxygen concentration, in mg/L is derived from the observed phase in the emission signal and from concurrent measurement of temperature made by the sensor. In this current verification, ACT will be testing instruments from eight different manufacturers each of which is utilizing the basic luminescent technology.

5.0 Verification Test Plan

During this evaluation ACT has (a) established a DO Technical Advisory Committee, (b) released a Request for Technologies for companies to participate in the verification, (c) developed Test Plans for laboratory and field testing, and (d) will perform a 2 month extended laboratory evaluation of instrument performance, (e) will perform a 4 month winter freshwater field testing of instruments under ice in the Keweenaw Waterway of Lake Superior, (f) will perform a 3 month brackish water field testing of instruments at the mouth of the Patuxent River, (g) will test the instruments in thermally stratified water with both normoxic and hypoxic hypolimnion in Lake Michigan and Muskegon Lake, and (h) will perform a 4 month deployment in Kaneohe Bay, HI. Laboratory tests are intended to evaluate instrument precision, bias, and stability over a range of temperature and dissolved oxygen conditions; and field tests involve long, unattended deployments to address instrument stability and reliability. All instruments tested, both in laboratory and in situ, will be incorporated in a stand-alone package, which includes data logging, data transformation/conversion equations, and independent power, provided by manufacturers. One individual sensor from each manufacturer will be selected for the laboratory exercise. A second sensor will be required to accommodate the schedule of the field testing.

5.1 Laboratory Tests

Laboratory tests of accuracy, precision, response time, and stability will be conducted at Moss Landing Marine Lab. All tests will be run under ambient pressure (logged hourly from a barometer at the laboratory) and involve the comparison of dissolved oxygen concentration reported by the instrument versus Winkler titration values of water samples taken from the test baths. All tests will be run in thermally controlled tanks at specific temperature, salinity, and DO concentrations. Tanks will be well mixed with two submersible Aquatic Ecosystem Model 5 pumps with flow rates of 25 L/min. Temperatures will be controlled to within approximately 0.2^{o}C of set point using Thermo Nestlab RTE 17 or equivalent circulating thermostats flowing to closed coils distributed within the tank. Four RBR temperature loggers will deployed within the tank to verify actual temperature to better than 0.02^{o}C. Salinity will be varied by addition of commercial salts (Instant Ocean) to Type 1 deionized water. Salinity will be verified at the beginning and end of each test condition by analysis on a Guildline Portasal salinometer or calibrated CTD. Dissolved oxygen concentrations will be controlled by use of compressed gases of known oxygen concentration sparging through diffusers within the tank. Tanks will be covered with a layer of floating closed-cell plastic insulation that will continuously seal the water surface and minimize atmospheric exchange. If required by the manufacturer, instruments will only be calibrated prior to the start of the first lab test, and then again prior to the stability test which would begin approximately one month later. The following series of test will be

conducted in the laboratory trials:

5.1.1 Accuracy at various T/S and DO conditions

We will conduct a series of measurements under 36 discrete conditions involving 3 temperature ranges, 3 salinity ranges, and 4 DO ranges as follows:

- Temperature Conditions: 5 – 15 - 30
- 3 Salinity Conditions: 0 – 10 - 34
- Gas Concentrations (%O_2 in source Tank): 0 – 10 – 20 – 30 Pure N_2 will be used for the 0% O_2 concentration.

The test will be run such that all 4 DO concentrations will be tested for a fixed temperature and salinity on the same day. We will start at the lowest DO concentrations and increase stepwise to the highest concentration. Instruments will be allowed to equilibrate at the fixed temperature and salinity overnight. Sparging with each DO gas concentration will be conducted for a minimum of 90 minutes prior to the start of data collection and reference sampling. For each test condition, the test instruments will be programmed to sample on 1 minute intervals and we will collect reference samples at 6 timepoints spaced 5 minute apart for each of the fixed conditions. For three of the timepoints we will collect duplicate samples from two different sampling ports mounted at opposite ends of the tank to access heterogeneity within the tank. All reference samples will be collected while the gas sparging is off and is estimated to take approximately 1 minute. Reference samples will be processed and analyzed as defined below.

We anticipate the order of the test conditions to be 15 then 5 then 30 ^oC, going from 0 then 10 then 34 salinity at each temperature.

5.1.2 Precision Test at various DO concentrations

A high sampling rate test will be conducted at one fixed temperature and salinity condition (T= 15^oC and S=10) for three different gas concentrations (0%, 10%, and 30% O_2) to examine the precision of test instruments. The sampling frequency for the test instruments and corresponding reference samples will be 1 minute with matched-up timepoints. We will collect 30 samples over a 30 minute time-period. Instruments will be equilibrated to each test condition for a minimum of three hours prior to testing. Reference samples will be processed and analyzed as defined below.

5.1.3 Response Time Test

A response time test will be conducted by examining measurements during a rapid exchange across a large gradient in dissolved oxygen for a fixed temperature (20^oC) in deionized water, following the approach described in Bittig et al 2014. The reservoirs of the thermostat baths will be constantly bubbled with either N_2 gas or air to maintain discrete DO levels. A submersible pump will be added to each bath to ensure uniform flow and oxygen conditions and instruments will be mounted at a fixed position within the baths to minimize variance due to instrument manipulation. Instruments will be programmed to measure at their highest possible frequency and will measure continuously for minutes following the exchange. For instruments with the

capability, real-time monitoring of instrument output will be monitored to verify a steady state reading has been obtained. Instruments will be moved from the high DO concentration to the low DO concentration and subsequently reversed to check for response hysteresis. Reference samples from each reservoir will be taken at the beginning and end of the exposure. The test instrument will be equilibrated in the high DO reservoir for at least 30 min prior to the exchange to ensure temperature equilibration.

5.1.4 Lab-based Stability Test

A laboratory run stability test will be conducted to examine potential instrument drift in a non-biofouling environment. These results will be contrasted to the stability of measurement accuracy observed in the long-term field mooring deployments. The test will occur over 60 days with daily temperature fluctuations of approximately 10^{o}C. Reference samples will be collected at minimum and maximum temperatures at least 3 times per week.
The test will be conducted in deionized water at saturated air conditions. Tanks will be well circulated and open to the atmosphere. Water in the test tank will be exchanged as needed if there are any indications of biological growth. Instruments will stay continuously submerged and not be exposed to air during any water exchange. The goal of comparisons of accuracy over time between the field and a sensor deployed similarly in the laboratory is intended to provide insight into drift and reliability intrinsic to the instrument relative to changes that may result from biofouling.

5.2. Field Tests

In situ field performance evaluations of the test instruments will be conducted under extended (3-4 months) mooring deployments and with water-column profiling on a rosette.

5.2.1 Moored Deployment Test Applications

Deployment Over Winter and Under Ice

A four month moored deployment will be conducted at Michigan Technological University's Great Lakes Research Center dock in Houghton, MI. Instruments will be deployed in January and kept under ice cover until April. Instruments will be programmed to sample at a minimum frequency of 1 hour. Manufacturer may choose to sample more frequently if they want to demonstrate that capability. ACT will collect reference samples twice per day for 4 days per week during the entire deployment. Instruments will be moored at approximately 4m depth and surface access through the ice will be maintained by gentle circulation with a propeller to allow deployment of the Van Dorn sampling bottle. The goal of this test application is to demonstrate instrument performance (reliability, accuracy, and stability) in winter-time environmental conditions and to demonstrate the ability to operate continuous observations under ice.

Deployment in a High Biofouling Estuarine Environment

A three month moored deployment will be conducted at Chesapeake Biological Lab Pier, Solomons, MD. Instrument will be deployed between May and August during a period of

warming temperatures and high biological production. Instruments will be moored at fixed depth of 1m on a floating dock. Instruments will be programmed to sample at a minimum frequency of 1 hour. Manufacturers may choose to sample more frequently if they want to demonstrate that capability. ACT will collect reference samples twice per day for 3 days per week and collect six samples on one day per week during the entire deployment. The intensive sampling will be spaced to capture the maximum range of expected diurnal variation in dissolved oxygen concentrations. The goal of this test application is to demonstrate instrument performance (reliability, accuracy, and stability) under high biofouling conditions and over a range of salinity and temperature conditions in a coastal estuarine environment.

Deployment in a Warm Tropical, Coastal Ocean Environment

A four month moored deployment will be conducted in a shore patch reef at the Hawaii Institute of Marine Biology (HIMB), Coconut Island, Kaneohe, HI. Instruments will be moored at approximately 1m depth on a bottom mounted PVC rack. Instruments will be programmed to sample at a minimum frequency of 1 hour. Manufacturers may choose to sample more frequently if they want to demonstrate that capability. ACT will collect reference samples twice per day for 3 days per week and collect six samples on one day per week during the entire deployment. The intensive sampling will be spaced to capture the maximum range of expected diurnal variation in dissolved oxygen concentrations. The goal of this test application is to demonstrate instrument performance (reliability, accuracy, and stability) under high biofouling conditions in warm, full salinity coastal ocean conditions.

Procedures:

The moored deployments will be run sequentially, but due to the overlap with the Lab tests and turn-around time between sites each manufacturer will be required to provide two separate instruments for testing. Instrument packages will be returned to manufacturers for a maximum of 4 weeks for reconditioning and calibration in between each successive field test.
Prior to deployment, all instruments will be handled and calibrated, if required, at the field sites as directed by the manufacturer and demonstrated at the training workshop. Sensors will then be programmed to record dissolved oxygen data at a minimum of once per hour at the top of the hour for the duration of the planned deployment. All instrument internal clocks will be set to local time and updated before programming using www.time.gov as the time standard. A photograph of each individual sensor and the entire sensor rack will be taken just prior to deployment and just after recovery to provide a qualitative estimate of biofouling during the field tests. In the final step before deployment, all instruments will be placed in a well aerated fresh water bath, with a known temperature, for 45 minutes and allowed to record three data points as a baseline reference. Reference samples will be drawn at the corresponding sampling times and analyzed for dissolved oxygen using Winkler titration method described below.

All instrument packages will be deployed on a single box shaped rack that allows all sensor heads to be at the same depth, with instruments side by side and all sensor heads deployed at the closest proximity that their designs will allow. The rack will be deployed so that all of the sensor heads remain at a fixed depth of 1 m below the water surface, except as noted above. A standard and calibrated CTD package will be deployed at each test site and programmed to provide an

independent record of conductivity and temperature at the sensor rack during each instrument sampling event. Four additional RBR temperature loggers will be placed on the rack to capture any spatial variation in the temperature across the rack.

A standard 4 L Van Dorn bottle will be used at each test site to collect water samples for Winkler titrations. The bottles will be lowered into the center of the sensor rack, at the same depth and as close as physically and safely possible to the sensor heads. The bottle will be triggered to close at the same time as the instruments are measuring to ensure that the same water mass is being compared for DO content. Three replicate 125 ml BOD bottles will be filled from each reference sample and immediately fixed in the field for subsequent Winkler titration analysis as described above. The order of each sub-sample will be recorded and tracked to examine any variation that arises from sample handling. Approximately 10 – 12 independent sampling events will be conducted each week. At least once per week we will conduct an intensive sampling event to capture the maximum diurnal range of dissolved oxygen concentrations. Once per week we will collect field duplicates to examine fine-scale variability around the mooring site. Approximately 120 comparative reference samples will be collected over the 3 – 4 month-long deployments.

In conjunction with each water sample collection, each deployment site will also record site-specific conditions. The following information, logged on standardized datasheets will be transmitted electronically on a weekly basis to the ACT Chief Scientist, for data archiving and site performance review:

- Date, time (local and GMT) of water sample collection.
- Barometric pressure from nearest weather station at time of water sample collection.
- Weather conditions (e.g., haze, % cloud cover, rain, wind speed/direction) and air temperature at time of water sample collection.
- Recent large weather event or other potential natural or anthropogenic disturbances.
- Tidal state and distance from bottom of sensor rack at time of water sample collection.
- Any obvious problems or failures with instruments.

ACT will be responsible for accurately characterizing temperature and salinity surrounding the mooring with the goal of characterizing micro-stratification or heterogeneity surrounding the mooring. We will deploy four RBR Solo temperature loggers and two SeaBird CTDs at each mooring site. Sensors will be mounted both at the instrument sampling depth and approximately 0.5 m above the sampling depth. Companies may add additional sensors to their instruments for internal use, but the results would not be included in the final Verification reports. Each ACT test site will identify the nearest meteorological station to provide continuous time series of air temp, barometric sensors, wind speed and direction, relative humidity, (PAR if available). We will also identify the closest tide gauge to monitor tidal fluctuation for the HI and CBL deployments.

At the end of each mooring deployment we will conduct a pre- and post-cleaned comparison of sensor response to a 100 % saturated water bath. Upon retrieval the sensor will be wrapped in

a damp towel and returned to the lab as quickly as possible. Prior to any cleaning, the sensor will be submerged in a 100 % DO water bath (via bubbling with air) and DO recorded for a minimum of three readings after an initial 30 minute equilibration period. Then the sensor will be removed from the bath and cleaned of any visible biofouling according to recommended manufacturer procedures. Following cleaning the sensor will be submerged in a second 100% DO water bath to avoid any biofouling debris carryover and DO recorded for a minimum of three readings after an initial 30 minute equilibration period. Temperature of the both water baths will be monitored continuously and maintained at a constant condition within 0.5^{o}C. DO concentration will be maintained at a constant saturated level with bubbling and confirmed by Winkler titration at the beginning and final instrument reading timepoints.

5.2.2 Water-Column Profiling

Instruments will be tested in a profiling application on a CTD rosette aboard the R/V Laurentian in the Great Lakes. Profiling tests will be conducted during strong thermal stratification (late August, expected thermal gradient of >15 °C) and in two different regions including a normoxic and hypoxic hypolimnion. The normoxic hypolimnion site will be in Lake Michigan within a 100m deep water column approximately 15 km offshore of Muskegon, MI. The hypoxic site profiling will be conducted in Muskegon Lake, a drowned river mouth lake adjacent to Lake Michigan.

At each site we will run two full water-column CTD casts and two surface (0-10m) casts. Separate casts for surface and hypolimnion sample are being done to minimize holding and processing time of the reference samples. For each profile, the rosette will be equilibrated for a minimum of two minutes just below the surface before initiating the continuous downward cast. As soon as we reach the desired sampling depth (10m for the surface sample and 2m off the bottom for the hypolimnion sample) five samples will be collected at 2 minute intervals. During the last sampling timepoint we will collect a duplicate Niskin bottle to examine sampling heterogeneity. The CTD will then be immediately returned to the ship for sample processing. Triplicate BOD bottles will be filled from each Niskin and immediately fixed for Winkler titrations.

6.0 Reference Sample Analysis

The Winkler titration for quantifying dissolved oxygen will be used as the standard for comparison. The specific method is described in detail below and is based on the procedures described in, *Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method* (Carignan et. al. 1998). All Winkler titrations will be done at the individual laboratory and field sites by trained ACT staff using standardized techniques and equipment. ACT will conduct a sample storage test at each site to examine any impacts on processing time between sample fixation and sample titration. We will fill multiple bottles from a common, homogenous 20L source with a floating barrier to minimize air exchange during filling. All samples will be fixed immediately upon filling and then we will titrate 4 replicates each over timepoints of 0, 8, 16, 36 hours. The current default plan is to analyze fixed samples

between 12 – 24 hours after they are fixed. In the event that we have equipment failure of the autotitrator we will send a duplicate system from another ACT partner site. This response should allow for any test to proceed with no more than 3 days delay.

Initial Preparation:

The volumes of each BOD bottles (≈ 125 mL) must first be determined with a precision better than 0.005%. We will measure the volume of each bottle gravimetrically (± 0.01 mL) near 20°C, after filling with degassed (boiled 10 min and cooled) distilled water. Since the procedure's precision approaches 1 µg $O_2 . L^{-1}$, particular care will be taken to avoid contamination of the glassware and working space from any trace amounts of thiosulfate, iodate, I_2, and manganese. We will use the reagents recommended by Carritt and Carpenter (1966) and titrate whole bottles to minimize the loss of volatile I_2 and the oxidation of iodide to I_2 at low pH.

Reagents:

(1) Manganous chloride solution (3M Mn^{2+}): dissolve 300 g of $MnCl_2 \cdot 4H_2O$ in 300 mL of distilled water. Bring to 500 mL.
(2) Alkaline iodide solution (8M OH^-, 4M I^-): separately dissolve 160 g of NaOH and 300 g of NaI in ca 160 mL of distilled water. Mix with stirring and bring to 500 mL.
(3) 23N Sulfuric acid solution: slowly add 313 mL of concentrated H_2SO_4 to 175 mL of distilled water. Carefully mix and cool and bring to 500 mL.
(4) Thiosulfate titrant 0.03N: add 300 mL 0.1N $Na_2S_2O_3 \cdot 5H_2O$ (Fisher SS368-1) to 700 mL DI. The thiosulfate is standardized daily with KIO_3 according to the procedure described below. Note: The normality of thiosulfate will be adjusted to ensure that a complete sample can be titrated within one burette volume (less than 10 mLs), but kept as low as possible to maximize precision.
(5) Potassium iodate standard, 0.1000N +/- 0.005N commercially available stock (Fisher SP232-1).

Sample Fixing Procedures:

(1) Samples are fixed immediately after collection into the BOD bottles. Filling order will be noted on log sheets along with bottle and sample IDs. Gently dispense 1.0 ± 0.05 mL of $MnCl_2$ just below the water surface, followed by 1.0 ± 0.05 mL of alkaline iodide using positive displacement pipettors. The pipettors should be washed with distilled water every day to prevent valve and plunger malfunction due to salt crystallization.
(2) Immediately close the bottle and shake vigorously. Allow the precipitate to settle for about two thirds of the bottle and shake again to resuspend the precipitate a second time. Immediately add a water seal to the neck of the bottle to prevent air suction by the contained water sample.
(3) Samples will be stored in the dark and room temperature (ca. 20°C) and temperature variations will be minimized. Samples will be titrated within 18 - 24 hours of being fixed. We will try to have a consistent processing time for all samples.
(4) Samples will be acidified just prior to titration. With the precipitate settled to the lower third of the bottle, add 1.0 ± 0.05 mL of 23N H_2SO_4. The H_2SO_4 must be allowed to flow gently along the neck of the bottle. Close and shake vigorously, until precipitate is dissolved

(5) If titration must be delayed beyond the 24 hour window, the fixed sample will remain stored in darkness and at a temperature equal to or slightly lower than the temperature of the samples, with a water seal maintained at all times. The sample will be acidified only immediately before titration. Note: Storage at temperatures above the sample temperature will cause the loss of I_2 due to the thermal expansion of the solution (0.06 mL·°C^{-1}).

Sample Titration Procedures:

Whole bottles are titrated using a Metrohm automated (model 916 or equivalent) titrator equipped with a 10-mL burette and a Metrohm Pt ITrode. The Pt ring of the electrode should be polished weekly. The titrator is used in the dynamic equivalence point titration (DET) mode, with a measuring point density of 4, a 1.0-µL minimum increment, and a 2 mV·min^{-1} signal drift condition. In this method, the solution's potential (controlled by the I_2/I^- and $S_2O_3^{2-}/S_4O_6^{2-}$ – redox couples) is monitored after successive additions of titrant, where optimal increment volumes are calculated by the titrator's software. During titration, the size and rotation speed of the magnetic stirring bar will be controlled in such a way that complete mixing of the I_2 generated during standardization occurs in 3–4 s, without vortex formation. To reduce the titration time (3–4 min) and I_2 volatilization, an initial volume of titrant equivalent to 85–90% of the expected O_2 concentration is added at the beginning of the titration. Because the molar volume of water and the normality of the titrant vary appreciably with temperature, care must be taken to standardize the titrant and conduct all titrations of a given batch of samples at constant temperature (± 1°C).

(1) Remove the stopper of the BOD bottle and, using a wash bottle fitted with a 200-µL pipette tip, rinse the I_2 present on the side and conical part of the stopper into the BOD bottle with 1–2 mL of distilled water.

(2) BOD bottles (Corning No. 5400-125) have been selected that can accommodate the displacement of the electrode without having to remove any volume of the fixed sample.

(3) Using plastic or stainless steel forceps, insert the stirring bar into the bottle.

(4) Immerse the delivery tip and the electrode, turn the stirrer on, begin the titration. The electrode must not touch the neck of the bottle.

(5) Start titration and then note the equivalence point volume (*V*T) once titration has finished.

Thiosulfate Standardization:

The Thiosulfate is standardized at room temperature as the first and last step in daily analysis. Either triplicate assays of a fixed volume of iodate standard will be run, or a range of volumes (≥ 3) bracketing the normal sample titration range (eg. 0.500, 1.000, 1.500, 2.000 mL for well oxygenated waters.) A clean BOD bottle and clean glassware will be dedicated to this purpose.

(1) Insert a stirring bar into a 200 mL beaker.

(2) With mixing add 1.0 mL of the H_2SO_4 reagent followed by 1.0 mL of the alkaline iodide and then 1.0 mL Mn^{2+}reagent .

(3) Using a gravimetrically calibrated pipet add a suitable volume of the KIO_3 standard to the stirring solution

(4) Insert the electrode and delivery tube and immediately begin titration

(5) The normality of the thiosulfate is calculated from the equivalence point volume as Vol_{KIO3} / Vol_{Thio})* N KIO_3 using replicates of single KIO_3 volume additions or from the slope of a range of KIO_3 addition volumes.

Blank determination:

Reagent blanks will be determined as follows:

1.) A volume of 1-2 L of site water will be brought to a boil in a clean glass reagent bottle.
2.) While cooling it will be sparged with N_2 for no less than 30 minutes.
3.) While maintaining the N_2 bubble, samples of the deoxygenated liquid will be collected into three 125 mL sample flasks, taking care not to introduce any oxygen to the fluid.
4.) The sample will then be rapidly fixed as a normal sample, and run in sets of three on the auto titrator.
5.) Blanks will be run for each new batch of reagents and at the start and finish of field sampling efforts.

7.0 Verification Schedule

The Verification will consist of three main components: (1) and initial training session by a manufacturer representative, (2) Laboratory-based testing, and (3) Field test applications. The dates, location, and duration of each of these activities are as follows:

Manufacturer Training

Training of ACT staff on the use of each test instrument will be conducted November 10 -12, 2014 in Moss Landing, CA. Each company representative will be given a half-day to work with all ACT personnel that will be conducting the Verification. During training, approximately two hours should be dedicated to demonstrating set-up, operations, software, and data handling followed by two hours of ACT staff demonstrating independent usage of the instrument and verifying functionality of instrument and software that are being provided for the initial Laboratory testing component. Standard manuals should also be provided.

If a company rep is not available to lead training on site, then written protocols and/or video documentation on all programming, calibrating, and data management procedures must be provided to the Chief Scientist prior to November 10th. Alternatively, a company representative may work with one of the ACT Partners at their specific institution prior to the Training workshop and those instructions will be shared to all other staff during the workshop by the ACT Partner. In such a situation, written protocols and video should also be provided.

Each of the two instruments provided by each manufacturer will be deployed on the following schedule:

Laboratory Testing

Moss Landing: LAB mid-Nov until mid-January (sensor1)

Field Testing

1. Houghton, MI: Moored Deployment, Jan – April 2015, 4 months (sensor2)

2. CBL Long-term Mooring: May – early August, 3 months (sensor1)
3. Great Lakes: Profiling, late Aug, 2015, 2 week (sensor2)
4. Hawaii: mid-September – December, 2015 - 4 months (sensor1)

Companies may service or replace instruments in the minimum 1-month interval between deployments. Alternatively they may provide written protocols on how to clean and prepare the instruments for the next text. Companies may supply replaceable parts if needed.

8.0 Data Recording, Processing and Storage

This section describes methods to be employed during data recording, processing, and storage to minimize errors and assure high quality analyses in the Performance Verification Statements.

8.1 Documentation and Records

A variety of data will be acquired and recorded either electronically or manually by ACT staff in the laboratory and field components of this Verification. Operational information and results from the reference method will generally be documented in a field/laboratory record book and on the data sheet/chain-of-custody forms (see below). An electronic copy of these raw data will be transferred each week to the ACT Chief Scientist, who will store it permanently along with the rest of the study data.

The results from the test instruments will also be recorded electronically. Test data will only be downloaded and analyzed upon completion of the individual laboratory tests or field deployments. As feasible, all original data will be left on the instrument and returned to the manufacturer. Once collected, one copy of these data will reside at the corresponding ACT test facility and a second copy will be archived at ACT Headquarters or with the Chief Scientist until the entire Verification is finished. The types of data to be recorded and the process for recording that data are summarized below.

- Dates, times, and site conditions of sampling events are recorded on log sheets/field record books for each reference sample. These are scanned and stored at the site and with the Chief Scientist.
- Test instrument calibration data will be stored on the instrument when possible as well as downloaded and stored electronically at the site. After completion of the lab or field deployment, the data will be stored electronically with the Chief Scientist.
- Test instrument data will be stored on the instrument when possible as well as downloaded and stored electronically at the site. Upon completion of the deployment all instrument data will be stored electronically with the Chief Scientist.
- Reference calibration data will be stored in laboratory record books or data sheets and will be located with the reference instrument.
- Performance evaluation audit results will be stored in laboratory record books or data sheets held by the independent auditor

8.2 Data Review

Records generated by any ACT staff during the verification test will be reviewed by the ACT Chief Scientist before the records are used to calculate, evaluate, or report verification results. The ACT QA Manager will conduct an audit of data quality on at least 10% of the test data (see 10.2.1).

9.0 Verification Reports

Individual company Verification reports will be produced approximately six months after the end of the field test produced and a single report will be prepared that includes both the Laboratory and Field testing components. The reports will include:

- Time series of derived instrument dissolved oxygen values as directly downloaded from the test instruments.
- Means, standard deviations, and number of replicates of laboratory-determined dissolved oxygen values for corresponding reference samples at the same time of the instrument measurements.
- Time series and statistical summaries of differences between instrument and reference sample dissolved oxygen measurements for matched sample pairs.
- Time series and statistical summaries of independently determined temperature and salinity record collected at several locations surrounding the mooring using two SeaBird CTDs and four RBR Solo temperature loggers.
- Summaries of any initial calibration reading, and initial and final readings of reference solutions made pre- and post-deployment.
- Any post-corrections of data based on improper calibration or drift are the responsibility of the companies. Post-corrected data can be included in the companies' response page that is included within each report.

All reports will undergo scientific review by the Technical Advisory Committee before they are submitted to the companies. Companies will be given a minimum of two weeks to prepare a 2-3 page response which will be included as an Appendix within the report before reports are posted live on ACT's website for public access. All reports are specific to the submitted instrument only. The reports will not provide any comparisons among different companies' instruments. Upon completion of the Verification, ACT can provide all reference data to the companies but we request that it be used only as a basis for comparing their own results. ACT will seek direct agreements from the company before any data can be used outside of the Verification reports (i.e., scientific publications or presentations). We encourage collaboration for this deeper analysis and use of data where appropriate to maximize the value and outcomes of the testing program.

10.0 Quality Management

The ACT quality management system (QMS) is a comprehensive set of policies, processes, and procedures that ensure that the quality of data, products, and services provided by ACT consistently meet or exceed meeting the clients stated quality requirements and comply with all applicable quality standards. The QMS also ensures that all ACT data collection and processing activities are carried out in a consistent manner, to produce data of known and documented quality that can be used with a high degree of certainty by the intended user to support specific decisions or actions regarding technology performance. The QMS provides the framework for quality assurance (QA) functions, which cover planning, implementation, and review of data collection activities and the use of data in decision making, and quality control (QC), which is a technical function that includes all the scientific precautions that are needed to acquire data of known and adequate quality.

Preventive actions will be taken throughout the DO Verification to anticipate and resolve any problems before the quality of performance is compromised. QA/QC procedures for this DO Sensor Verification will follow the requirements described in these Protocols, any vendor specified requirements, and the general principles and specific QA/QC from technical documents for measuring DO in aquatic systems. ACT technical staff has the responsibility to identify problems that could affect data quality or the ability to use the data. Any problems that are identified will be reported to the ACT Chief Scientist, who will work with the ACT Quality Assurance (QA) Manager and Technical Advisory Committee to resolve any issues. Action will be taken to control the problem, identify a solution to the problem, and minimize losses and correct data, where possible.

10.1 Quality Control Requirements

Quality control measures are implemented by ACT technical staff and monitored by the ACT Chief Scientist. These provide information on data quality on a day-to-day basis to ensure the integrity, correctness, and completeness of the collected data and include:

- Duplicate sampling to ensure sample representativeness with respect to sampling and handling procedures. The acceptable range of relative percent difference between a sample and its duplicate is 2 % (or an expected standard deviation of less than 0.4 µmol/L for natural surface water).
- Replicate analysis to ensure sample representativeness with respect to sample processing and analysis. Triplicate DO readings will be done on every field sample. The acceptable range of relative standard deviation among replicate analyzes is 0.05 % (or an expected standard deviation of less than 0.1 µmol/L for natural surface water).
- Calibration and maintenance procedures, schedules, and standards (certified reference materials) for all equipment used in the test.

The responsibility for interpreting the results of QC checks and resolving any potential problems resides with the Chief Scientist.

10.2 Quality Assessment

ACT assessments include technical audits and data quality assessments. Fundamental principles of the ACT assessment process include:

- Assessments are performed by the ACT QA Manager, who is independent of direct responsibility for performance of the Verification.
- Each assessment is fully documented.
- Each assessment must be responded to by the appropriate level of the ACT team. ACT quality assessment reports require a written response by the person performing the inspected activity, and acknowledgment of the assessment by the ACT Director.
- Corrective action must be documented and approved on the original assessment report, with detailed narrative in response to the assessor's finding. Initials and date are required for each corrective action response. Acknowledgment of the response will be provided by the ACT Director.

10.2.1 Technical Audits

Technical audits are systematic and objective examinations of the verification test implementation to determine whether data collection activities and related results comply with the Test Protocols, are implemented effectively, and are suitable to achieve its data quality goals. Audits for the DO Sensor Verification will include: (1) technical system audits (TSAs) and audits of data quality (ADQs). The ACT Chief Scientist is responsible for ensuring that audits are conducted as part of this verification.

Technical System Audit

A Technical System Audit (TSA) is a thorough, systematic, and qualitative evaluation of the sampling and measurement systems associated with a Verification test. The objective of the TSA is to assess and document the conformance of on-site testing procedures with the requirements of the Test Protocols, published reference methods, and associated SOPs. The TSA assesses test facilities, equipment maintenance and calibration procedures, reporting requirements, sample collection, analytical activities, and QC procedures. Both laboratory and field TSAs are performed.

The QA Manager will conduct a TSA of the laboratory component and at least one field test during the verification. The TSA is performed following the EPA document *Guidance on Technical Audits and Related Assessments for Environmental Data Operations*, EPA QA/G-7, January; 2000.A TSA checklist based on the Test Protocol is prepared by the QA Manager prior to the TSA and reviewed by the ACT Chief Scientist. At the close of the TSA, an immediate informal debriefing will be conducted. Non-conformances are addressed through corrective action. The QA Manager will document the results of TSAs and any corrective actions in a formal audit report.

Audit of Data Quality

An Audit of Data Quality (ADQ) is a quantitative evaluation of the verification test data. The objective of the ADQ is to determine if the test data were collected according to the requirements

of the Test Protocols and associated SOPs and whether the data were accumulated, transferred, reduced, calculated, summarized, and reported correctly. The ADQ assesses data accuracy, completeness, quality, and traceability.

The ADQ is conducted after data have been 100% verified by the ACT Chief Scientist. The ACT QA Manager conducts the ADQ. The ADQ entails tracing data through their processing steps and duplicating intermediate calculations. A representative set of the data (10%) is traced in detail from raw data and instrument readouts through data transcription or transference through data manipulation through data reduction to summary data, data calculations, and final reported data. The focus is on identifying a clear, logical connection between the steps. Particular attention is paid to the use of QC data in evaluating and reporting the data set.

Problems that could impact data quality are immediately communicated to the ACT Chief Scientist. The results of the ADQ are documented in a formal audit report with conclusions about the quality of the data from the verification and their fitness for their intended use.

Audit Reporting

The ACT QA Manager is responsible for all audit reports. These written reports:

- identify and document problems that affect quality and the achievement of objectives required by the Test Protocols and any associated SOPs;
- propose recommendations (if requested) for resolving problems that affect quality;
- independently confirm implementation and effectiveness of solutions;
- identify and cite noteworthy practices that may be shared with others to improve the quality of their operations and products;
- provide documented assurance that when problems are identified, further work performed is monitored carefully until the problems are suitably resolved.

10.2.2 Data Quality Assessment

ACT reviews technology testing data to ensure that only sound data that are of known and documented quality and meet ACT technology testing quality objectives are used in making decisions about technology performance. Data assessment is conducted in two phases. The first phase consists of reviewing and determining the validity of the analytical data – data verification and validation. The second phase consists of interpreting the data to determine its applicability for its intended use – usability assessment.

Data Verification

Data verification is the process of evaluating the completeness, correctness, and consistency of the test data sets against the requirements specified in the Test Protocols. Data verification is conducted by the ACT QA Manager. The process includes verifying that:

- the raw data records are complete, understandable, well-labeled, and traceable;
- all data identified in the Test Protocols has been collected;
- instrument calibration and QC criteria were achieved;
- data calculations are accurate.

Corrective action procedures are implemented if data verification identifies any non-compliance issues.

Data Validation

Data validation evaluates data quality in terms of accomplishment of measurement quality objectives, such as precision, bias, representativeness, completeness, comparability, and sensitivity. Data validation:

- establishes that required sampling methods were used and that any deviations were noted;
- ensures that the sampling procedures and field measurements met performance criteria and that any deviations were noted;
- establishes that required analytical methods were used and that any deviations were noted;
- verifies that QC measures were obtained and criteria were achieved; and that any deviations were noted.

Data validation is performed by the ACT QA Manager. Any limitations on the data and recommendations for limitations on data usability are documented.

Data Usability

Data usability assessments determine the adequacy of the verified and validated data as related to the data quality objectives defined in the Test Protocols. All types of data and associated information (e.g., sampling design, sampling technique, analytical methodologies) are evaluated to determine if the data appear to be appropriate and sufficient to support decisions on technology performance.

A data usability assessment has an analytical and a field component. An analytical data usability assessment is used to evaluate whether analytical data points are scientifically valid and of a sufficient level of precision, accuracy, and sensitivity. The field data usability assessment evaluates whether the sampling procedure (e.g., sampling method, sample preservation and hold times) ensures that the sample that is collected for analysis is representative.

10.3 Corrective Action

Corrective action is implemented in response to any situation that compromises the quality of testing or data generated by ACT. The need for corrective action can be identified by any ACT personnel and implemented with the prior approval of the ACT Chief Scientist, in consultation with the QA Manager. The Chief Scientist is responsible for determining appropriate corrective action to address an issue. Any findings that have a direct impact on the conduct of the verification test will be corrected immediately following notification of the finding. Implementation of corrective actions must be verified by the ACT QA Manager to ensure that corrective actions are adequate and have been completed. This will be done in real-time if corrective actions can be immediately performed. All corrective actions are documented. Any impact that an adverse finding had on the quality of the verification test data is addressed in the test report.

11.0 Field Test Site Descriptions

11.1 Moored Deployment: MTU Great Lakes Research Center Pier, Houghton Cut, Lake Superior

Figure 1. Arial view of MTU Site

Figure 2. MTU deployment Site

The Great Lakes Research Center deployment site is located at 47° 7.233'N, 88° 32.736'W, at the end of the pier at the Great Lakes Research Center docks. This site is located on the south side of the Keweenaw Waterway, and is connected to Lake Superior in both the NW and SE directions. The instrumentation rack will be lowered off of the end of the takeout pier with a ½ ton crane (Fig. 2), and will rest on the bottom, under the ice, in 4.5m of water. A small shelter will be constructed at the end of the pier to provide shelter during winter sampling efforts.

11.2 Moored Deployment: Chesapeake Biological Laboratory Pier, Solomons, MD.

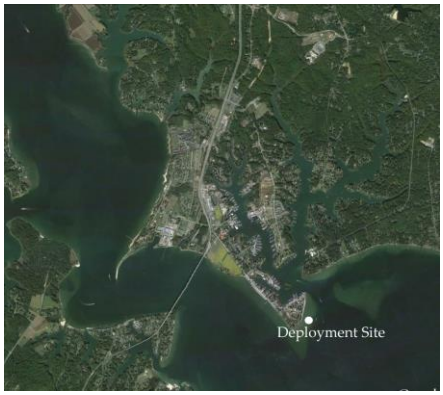

Figure 3. Arial view of CBL Site

Figure 4. CBL deployment Site

The moored deployment site in Chesapeake Bay is located at 38.317°N, 76.356°W attached to the end of a pier at the mouth of the Patuxent River, a tributary of the Chesapeake Bay (Figure 4.) The average water depth of the test site is 2.2 m The site is brackish with salinity ranging from 5 PSU to 20 PSU and temperature ranging from 0°C to 35°C depending on the season, rainfall, wind, and other external factors.

11.3 Moored Deployment: Hawaii Institute of Marin Biology, Kaneohe Bay

Figure 5. Arial view of HIMB Site

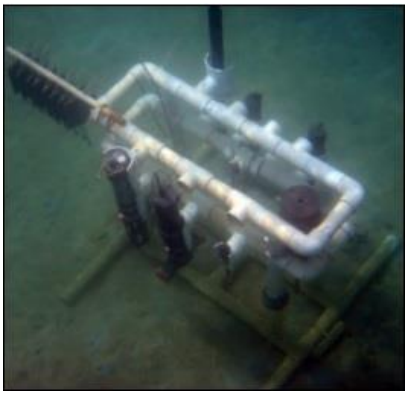

Figure 6. HIMB deployment Site

The mooring test site in Kaneohe Bay is located on the fringing reef flat surrounding Coconut Island. The instruments will be placed on a standing rack (Fig. 6) in a water depth of 3 meters with tidal variations typically less than .5 m at this site. Salinity values range from 33 to 35.5 and water temperatures range from 26.1 to 29.6°C.

11.4 Profiling: Muskegon Lake and Lake Michigan Profiling Sites

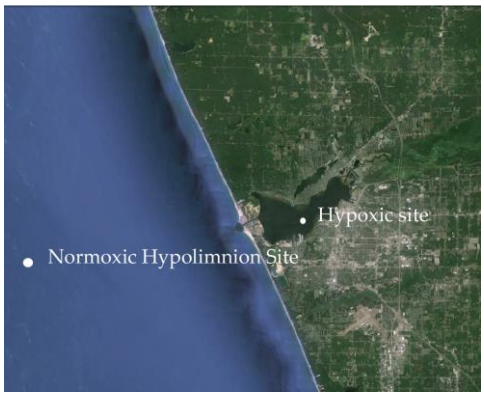

Figure 7. Arial view of Profiling Sites

Figure 8. R/V Laurentian

Great Lakes profiling tests will be performed aboard the R/V Laurentian (Fig. 8) at two separate locations (Fig. 7) in order to experience both normoxic and hypoxic hypolimnion. The normoxic site will in in Lake Michigan within a 100m deep water column, while the hypoxic site will be in Muskegon Lake.

12.0 References

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