

Protocols for the ACT Verification of In Situ Dissolved Oxygen Sensors

1. Background on ACT Evaluations

Instrument validation is necessary to enable effective existing technologies to be recognized and so that promising new technologies can be made available to support coastal science, resource management and the long-term development of an Integrated Ocean Observing System. The Alliance for Coastal Technologies (ACT) has therefore been established to provide an unbiased, third party testbed for evaluating new and developing coastal sensors and sensor platforms for use in coastal environments.

The following protocols describe how ACT will verify the environmental performance characteristics of commercial-ready, in situ dissolved oxygen sensors through the evaluation of objective and quality assured data. The goal of this evaluation program is to provide technology users with an independent and credible assessment of instrument performance in a variety of environments. Therefore, the data and information on performance characteristics will cover legitimate information that users need. ACT will not simply verify all claims of vendors, but instead looks to the broader community to define the data and operational parameters that are valuable in guiding instrument purchase and deployment decisions.

It is important to note that ACT does not certify technologies or guarantee that a technology will always, or under circumstances other than those used in testing, operate at the levels verified. ACT does not seek to determine regulatory compliance; does not rank technologies or compare their performance; does not label or list technologies as acceptable or unacceptable; and does not seek to determine “best available technology” in any form. ACT will avoid all potential pathways to picking “winners and losers”. Therefore, although the following protocols will apply to all instruments evaluated, no direct comparisons will be made between instruments from different manufacturers and instrument-specific Verification Statements will be released to the public for each instrument type as a final report.

2. Introduction to Technology

As part of our service to the coastal community, ACT Partner Institutions and Stakeholder Council has chosen the performance verification of commercially available in situ dissolved oxygen sensors as the first ACT Technology Evaluation. There are a variety of dissolved oxygen sensors/electrodes currently available, many of which use polarographic membrane measuring techniques. Recently, however, new optical luminescence dissolved oxygen sensors have been developed and are now on the market. Precise and reliable measurements of dissolved oxygen concentration (DO) with effective and reliable in situ sensors are critical for understanding many physiological and ecological processes and are required for a variety of coastal science and management activities. For example, many coastal resource managers and scientists are interested in regions of very low levels of dissolved oxygen (hypoxia) in bottom-water or "dead zones". These areas of seasonally-depleted oxygen levels are natural phenomena, but can be intensified spatially and temporally as a result of human activities (i.e., nutrient over-enrichment).

ACT has performed a customer needs and use assessment for in situ dissolved oxygen sensors. Scientists, resource managers, and other users of these technologies were asked to

respond to a questionnaire regarding their current use or application of these instruments, limitations or problems with their current dissolved oxygen sensors, and the important parameters they use when selecting a dissolved oxygen sensor. The results of this assessment were used to identify the main applications and key parameters that ACT will evaluate in this Technology Verification.

3. Objectives and Focus of DO Sensor Performance Verification

The majority of respondents to our needs and use assessment indicated that in situ dissolved oxygen sensors were deployed on remote platforms in estuarine and near shore environments, and in relatively shallow water (< 10 meters depth). Therefore, the present performance verification of manufacturers' claims will focus on these applications. It was also clear from the user survey that accuracy, reliability, precision, instrument drift/calibration life, and operating life are the most important parameters guiding instrument selection decisions. Protocols were therefore developed with the aid of manufacturers to evaluate these specific areas excluding operating life. Although any malfunctions and problems with instruments will be noted during verification testing, evaluating the operational life of dissolved oxygen sensors would take several years and is therefore beyond the scope of the ACT Technology Verification Program.

The parameters to be verified:

- **Accuracy** – combination of bias and precision of an analytical procedure, which reflects the closeness of the measured value to the true value. Accuracy will be determined in the laboratory and field by a comparison of differences in mean values reported by the instrument package versus values determined by Winkler titration (details below) on water sample in proximity to the sensor.
- **Bias** – consistent deviation of instrument measured values from the true value, here designated as the DO concentration determined by Winkler titration, caused by systematic errors in a procedure (e.g. calibration error, sensor design, data processing).
- **Precision** – measure of the degree of agreement among replicate measurements of a sample, usually expressed as a standard deviation. Precision will be determined by a comparison of differences in mean values produce by the instruments versus values determined by Winkler titration of corresponding water samples at a fixed dissolved oxygen concentrations with 30 replicate samples/measures for each during a laboratory test.
- **Instrument drift** – changes in the deviation of instrument measurements of DO concentration relative to the Winkler titration reference values over the duration of the instrument deployment in laboratory or field conditions or from time of last calibration.
- **Reliability** – measure of the ability to maintain integrity of the instrument and data collections over time. Reliability will be determined in the laboratory and field by comparing percent of data recovered versus percent of data expected. Comments on potential causes lost data, malfunctions, downtime, etc., will also be recorded. Records will be kept on any problems associated physical damage, flooding, corrosion, battery failure, etc.

4. Verification Approach

The protocols described in detail below were based on an amalgamation of protocols for sensor calibration and testing provided by the manufacturers participating in this ACT Technology Verification. Initial generic protocols were further refined through direct discussions during an ACT DO Sensor Performance Verification Workshop held on 25-26 March, 2004. Participants of this workshop included ACT Headquarters Staff, ACT Partner Institution Technical Coordinators, ACT Quality Assurance Manager, a Dissolved Oxygen Technical Advisory Committee, and representatives from each of the participating manufacturers.

It was decided that the protocols will follow a format that A) employs the Winkler titration as the standard of reference for determining instrument performance characteristics, B) include controlled laboratory tests, and C) include field tests to evaluate performance under a variety of environmental conditions. Each of these components are described in detail below.

Qualified personnel affiliated with ACT will conduct all tests. All personnel involved in this verification exercise will be properly trained on use of instruments by manufacturer representatives and on a standardized water sampling method and Winkler titration methods by ACT staff. For more details see the Quality Assurance Plan below.

All numerical data will be recorded to four significant digits and dissolved oxygen values will be listed as mg/l. Results will be presented as: A) means, standard deviations, and number of replicates (n) of instrument measurements, B) means, standard deviations, and number of replicates of instrument measurements of corresponding Winkler titrations, and when appropriate, C) measured barometric pressure and calculated pO₂ and solubility based on known temperatures and salinities. Field data will be presented as the two sets of measured values (sensor and Winkler) versus time. Inter-instrument comparisons will only be made when multiple sensor packages from the same manufacturer are tested in parallel in order to evaluate between sensor variability in performance under different field conditions or defined laboratory conditions.

4.1. Reference/Standard for Comparison

The Winkler titration for quantifying dissolved oxygen will be used as the standard for comparison. The specific method to be used is described in detail below and is based on the well accepted WOCE protocols (Culberson, C. H., G. Knapp, M. C. Stalcup, R. T. Williams and F. Zemlyak. 1991. A comparison of methods for the determination of dissolved oxygen in seawater. *WOCE Report 73/91*, 77 pp.). All Winkler titrations will be done at the individual laboratory and field sites where tests are conducted by trained staff with standardized techniques.

Winkler Reagents/Equipment

- *Manganous Chloride* – Dissolve 60g MnCl₂·H₂O in 100 ml DI water. This produces a 3M Mn²⁺ solution.
- *Alkaline Iodide* – Dissolve 32g NaOH and 60 g NaI separately in a minimum amount of DI water. It may help to dissolve NaOH under mild heating and stirring. After preparing both solutions, mix the two together and bring the volume of the mixed solution up to 100 ml. This produces an 8 M OH⁻ and 4 M I⁻ solution.
- *Dilute sulphuric acid* – Slowly add 30 ml concentrated acid to 60 ml DI water under a fume hood. Make up total volume to 100 ml. This produces a 5 M H⁺ solution.

- *Thiosulfate* – Dilute purchased Thiosulfate solution (0.1 N $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, Aldrich cat#: 31,954-6) by a factor of 2.5 by pouring 100 ml of concentrated solution in a flask and adding DI H_2O until the final volume of the solution is 250 ml. This produces a 0.04 M solution.
- *Potassium Iodate Standard* – 0.1 N KIO_3 solution
- *Starch* – Add 5 g of laboratory grade starch to a small volume of DI water and mix until a milky consistency is obtained. Next, slowly add the concentrated starch solution to ~500 ml of boiling water in a 1-L Erlenmyer flask and allow the solution to continue to boil for another 5 minutes. Allow solution to settle for 24 hours, keeping the top of the container covered (stopper/parafilm) to prevent contamination. After which time, remove the clear top portion from the solution and place in a 250-500 ml amber glass bottle and store under refrigeration. Add 1 ml chloroform per liter of solution to help preserve solution.
- *Auto Pipetter* – calibrated prior to use.
- *Bottles* – use 300 ml glass flasks with ground glass stoppers. Make sure all flasks and stoppers have the same number, are kept in pairs, and the exact volume for each flask is known.
- *Redox Probe* – Electrode for measurement of reduction/oxidation potential and redox titrations.
- *Dosimat* – for automated precise titration.

Winkler Procedures

Fixing the sample – Add 1.5 ml of the Manganous solution and 1.5 ml Alkaline Iodide solution to the sample. Mix by multiple inversions (which has greater mixing than simply shaking) for 20 seconds. Allow precipitate to settle for 10-20 minutes.

Analyzing the sample –

1. Add 1.5 ml of dilute sulphuric acid solution to fixed samples under stirring until all precipitate has been dissolved and solution is a uniform color. To allow for insertion of Redox probe and dosimat output tube, remove ~20 mL of water from the top of the flask before adding acid.
2. Titrate acidified sample with Thiosulphate solution using the Dosimat until pale yellow color is barely visible. Next, add 1 ml of starch solution.
3. Continue to slowly titrate sample using the Dosimat (no more than 1 μl /seconds) until a dramatic change in redox is noted (levels below 300 millivolts) and the solution turns completely clear. Be careful not to over-titrate. Record the volume of Thiosulphate solution needed to titrate the standard.
4. Add the finished volume to a solution of 15g NaHCO_3 in 200ml to bring solution pH up to ~8.

Standardizing the Thiosulphate solution – The oxidation potential of the Thiosulphate solution will decay over time and is light sensitive, so the solution must be standardized before each set of Winkler titrations are performed (no more than once per day). This is the most critical step in controlling the accuracy of the titration.

1. Add ~75 ml of DI water to a 125 ml Erlenmyer flask. Next, add 1 ml Dilute sulphuric acid solution, followed by 1 ml Alkaline iodide solution and 1 ml Manganous solution in that order. Flask should be kept under stirring to properly mix all reagents.
2. Pipette exactly 200 μl KIO_3 solution to the flask. Calibrate the pipette before or after use to get exact volume. Be sure solution is room temp prior to addition is stock is stored in refrigerator.

3. Titrate standard with Thiosulphate solution following the same procedure as for samples. Record the volume of Thiosulphate solution needed to titrate the standard.

Getting a reagent blank – When you add the fixing reagents to the sample, they bring in a limited amount of oxygen with them that you will ultimately titrate with the sample. To find this blank:

1. Sparge a body of seawater with N₂ for ~30 minutes.
2. Collect a sample of the sparged seawater taking care not to introduce any oxygen to the fluid.
3. Fix and analyze as any normal sample. Calculate the mass of O₂ by the reagents added from the formula below.

Winkler Calculations:

- Concentration of Thiosulphate:
$$[Thio] = \frac{6V_{IO_3^-}}{V_{Thio}} [IO_3^-]$$
- Concentration of dissolved oxygen:
$$[O_2] = \frac{1}{V_{flask}^*} \left(\frac{V_{Thio} [Thio]}{4} - O_2^b \right)$$
- Actual Volume of flask:
$$V_{flask}^* = V_{flask} - \Sigma V_{reagents}$$
- Oxygen blank:
$$O_2^b = \frac{V_{Thio}^b [Thio]^b}{4}$$

4.2. Laboratory Tests

Laboratory tests of accuracy, precision, instrument drift, and reliability will be conducted at the University of Michigan Cooperative Institute of Limnology and Ecosystem Research, the ACT Great Lakes Partner Institution. 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 for the test baths.

All instruments tested, both in laboratory and in situ, will be incorporated a stand-alone package, which includes data logging, data transformation/conversion equations, and independent power, provided by manufacturers'. A total of eight sensors will be evaluated during this verification, four with the manufacturers biofouling prevention system and four without. Two individual sensors from each manufacturer (one with a biofouling prevention and one without) will be randomly selected for the laboratory exercise. This paired testing will allow the evaluation of performance by independent sensors from each manufacturer (2 of the 8 submitted by each manufacturer).

The accuracy of individual sensor will be determined by comparisons of differences in mean values recorded by the instrument versus corresponding Winkler titration values of dissolved oxygen from corresponding water samples. Three replicate measures of dissolved

oxygen will be recorded by the instruments and by Winkler titrations under a set matrix of environmental conditions, varying temperatures, salinities, and dissolved oxygen levels. The various conditions below will be produced in three thermally controlled, well-mixed (using submersible pumps) baths by adding marine salts and bubbling with various gasses (laboratory grade mixed or pure gasses).

Instruments will be tested under each combination of the following conditions:

- **Temperature** – 2°C, 17°C and 40°C
- **Salinity** – 0 ppt, 17 ppt, and 34 ppt
- **Gas** – sparging with 100% nitrogen, bubbling with 10 % oxygen + 90% N₂ (v/v), bubbling with 20.9 % oxygen + 79.1% N₂ (v/v), and bubbling with 40 % oxygen + 60% N₂ (v/v).

3 temperatures x 3 salinities x 4 gasses = 36 independent testing conditions

Each instrument will be set up and calibrated according to the manufacturers' standard protocols prior to initiation of the laboratory testing. The three thermally controlled baths will be set and maintained at the three test temperatures listed above (temperature will be monitored continuously at two locations within baths) and each sensor will first be tested in fresh water while purging with nitrogen to lower levels of dissolved oxygen close to anoxia. Then the various mixed gasses will be bubbled into the water in order of increasing oxygen concentrations. After the entire series of gasses is complete, the marine salts will be added to bring the salinity to 17 ppt in all three temperature-controlled baths. The gas series will then be repeated. Finally, additional salts will be added to bring the salinity to 34 ppt for the final series of measurements at each temperature and oxygen level taken.

At each test point in the matrix of temperatures, salinities, and gasses, the bath will be allowed 1 hour to stabilize. Once the bath has equilibrated, a continuous/integrated 500 ml sample will be taken from a spigot in each of the baths over 60 seconds. The sample will be split into three replicates, and all three will be analyzed by Winkler titrations. As the water samples are being taken the time will be noted so the three closest instrument recordings can be used for comparison to Winkler values.

The goal is to test all instruments (two replicate sensors from each manufacturer) at the same time, in the same bath, under the same conditions using a single water sample for comparison. This will be possible for instruments that have relatively rapid response times (< 2 minutes), which can all be set to record dissolved oxygen levels every 5 minutes during the laboratory exercise. However, instruments with longer response times (> 20 minutes) may have to be tested in the laboratory independently because of the time required between recording of data and sample collections.

The precision of instruments will be evaluated using the same methods described above but at only one point on the matrix. A total of 30 instrument measurements will be taken when the bath is set at 20 °C, freshwater, and bubbled with 20.9 % oxygen. An integrated 10 l water sample from the bath will be taken over 10 minutes (while the instruments are taking their corresponding measurements) and split into 30 replicates for evaluation by Winkler titration.

The mean and standard deviation of the instrument values will be compared to the mean and standard deviation of the Winkler values.

Although a main focus of the field tests is to determine instrument drift (or the length of time an instrument provided measurements that are accurate), the drift over time in instrument-recorded data will also be examined in the laboratory. One sensor from each manufacturer will be “deployed” in a bath for 26 days held at 20 °C, freshwater, and bubbled with an aquarium pump. Data and samples will be recorded, collected, and analyzed using the same methods and the field deployments described below. This will provide insight to drift over time as a function of the instrument itself versus environmental factors acting on the sensor or sensor package. Finally, the individual sensor from each manufacturer deployed in the laboratory bath will also be monitored for reliability. The percent of data recovered after 28 days will be determined and any malfunctions, failures, etc., will also be recorded.

4.3. Field Tests

In situ evaluations of instrument performance will be conducted at each of the seven ACT Partner Institution sites (see descriptions below). One sensor with biofouling prevention and one without from each manufacturer will be deployed for 26 days at each site. Although it may not be possible to identify a clear causal relationship, comparisons of accuracy over time between the paired field instruments, and a third sensor deployed similarly in the laboratory, may provide insight into drift and reliability as a result of biofouling versus the instrument itself. Because each manufacturer will provide only eight total instruments, two sets of consecutive field tests will be run (four sites then three sites). Instrument packages will, however, be returned to manufacturers for a maximum of 3 weeks for reconditioning and calibration in between the two sets of field tests.

Prior to deployment, all instruments will be calibrated at the field sites as suggested in individual manufacturer manuals. Sensors will then be programmed to record dissolved oxygen data every 15 minutes on the quarter hour during the entire field deployment and their internal clocks set to local time using www.time.gov as the time standard. A photograph of each individual sensor and the entire sensor rack (see below) 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. One liter of water will be removed from the bath, divided into three samples, and all three analyzed for dissolved oxygen using Winkler titration method described above. This same baseline reference procedure will be repeated immediately (within 30 minutes) after the instruments are recovered following the 26-day deployment.

All instrument packages will be deployed on a single box shaped rack that allows all sensor heads to be at the same depth, with each manufacturer’s instrument side by side and all instrument 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 (using a float system or on a floating dock). A standard and calibrated CTD package will be deployed at each test locale and programmed to provide an independent record of conductivity and temperature at the sensor rack during each instrument sampling event. The

sensor rack design will also be standardized as much as possible from site to site. However, physical conditions at particular sites may require specialized modifications.

A standard 2-l Van Dorn bottle will be used at each test site to collect water samples for Winkler titrations on Mondays through Fridays during the 4-week field test. 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 instrument sampling, to ensure that the same water mass is being compared for DO content. Water samples will then be split into 3 replicates, and fixed in the field for subsequent Winkler titration analysis of all three replicates as described above.

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

- 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.
- Dissolved oxygen at the surface, at 1 m depth, and near bottom at time of water sample collection, determined using an independent portable hand deployed DO sensor (Orion Model 830A).

The goal of this last measurement is to estimate the stratification of the water column when and where water samples and instrument readings are taken and as a third, independent measure of dissolved oxygen. Although no direct comparisons will ever be made between the instruments being evaluated and this third sensor, it may in some cases help identify if a problem exists with a particular water sample or Winkler titration that warrants further consideration. Each site will use an Orion 830A handheld dissolved oxygen meter that will be calibrated daily before use for this activity.

During the first 24 and last 24 hours of the 26-day deployment, each site will collect Van Dorn water samples for Winkler titrations every 2 hours for a 12-hour period (6 sample each split into 3 replicates during the first 24 hours on day 1 and 6 samples each split into 3 replicates the last 24 hours on day 26). During the remainder of the field test deployments, each test site will take 2 field water samples every weekday (M-F), each timed to correspond to the instrument sampling time (2 samples split into 3 replicates each). The timing of water sampling on days 2 through 25 of the field tests will be left up to the individual site with the goal of capturing natural daily variations in dissolved oxygen and will therefore be separated by a minimum of 2 hour.

5. Verification Schedule (planned dates but may vary).

- The Final Verification Protocols and ACT Verification Contract will be sent to Manufacturers on **30 April, 2004**.
- Signed contracts are due back to ACT Headquarters by **24 May, 2004**.
- All instruments to be test will be delivered to CILER/University of Michigan by **31 May, 2004**.
- ACT Chief Scientist, Technical Coordinators, Quality Manager, and Manufacturer Representatives will meet at the University of Michigan for instrument use/operation/deployment, sample collection, and Winkler titration training on **7 – 9 June, 2004**.
- Selected ACT staff will conduct the laboratory verification tests on **10 – 22 June, 2004**.
- All instruments will be delivered to the first four ACT test sites by **25 June, 2004**.
- The first four 26-day field deployment verification tests will begin on **28 June, 2004**.
- All instruments will be sent back to individual Manufacturers for reconditioning and calibration on **26 July, 2004**.
- Instruments will be sent back from Manufacturers to the second set of three ACT test sites and received by **20 August, 2004**.
- The second set of three 26-day field deployment verification tests will begin on **23 August, 2004**.
- All instruments will be sent back to individual Manufacturers on **21 September, 2004**.
- ACT Chief Scientist, Technical Coordinators, Technical Advisory Committee, and Quality Manager, will meet for 3 days to analyze results and evaluated the Verification processes during the last week of September or the first week of October, 2004.
- ACT Verification Statements for each individual instrument will be drafted and sent out for review by Manufacturers, Technical Advisory Committee, Technical Coordinators, Quality Manager, Partners, and Stakeholders in **November, 2004**.
- Final Verification Statements will be released to the public in **December, 2004**.

6. Data Recording, Processing and Storage

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

6.1. Documentation and Records

A variety of data will be acquired and recorded electronically and manually by ACT staff in this verification test. 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 to the ACT Chief Scientist weekly, who will store it permanently along with the rest of the study data.

The results from the test dissolved oxygen probes will also be recorded electronically. Test instrument data will be logged by individual sensor packages and will only be download and analyzed upon completion of the 26-day field deployments. Once collected, one copy of these data will reside at the corresponding ACT test facility and a second copy at ACT

Headquarters and until the entire verification is finished. The table below summarizes the types of data to be recorded and the process for recording data.

Data to be Recorded	Responsible Party	Where Recorded	How Often Recorded	Purpose of Data
Dates, times of sampling events	ACT Partner	Field/laboratory record books/data sheets	Each reference sample collection and laboratory analysis	Used to organize/check test results; manually incorporate data into spreadsheets - stored in study binder
Test parameters (site conditions)	ACT Partner	Field/laboratory record books/data sheets	Each reference sample collection	Used to define site characteristics; manually incorporate data into spreadsheets - stored in study binder
Test instrument calibration data	ACT Partner	Laboratory record book/data sheets	Start/end of test	Document correct performance of test instrument
Test instrument data - digital display - electronic output	ACT Partner	- Data sheets - Instrument data acquisition system (data logger)	After completion of the 26-day field deployments	Used as part of test results; incorporate data into electronic spreadsheets - stored in study binder
Reference analytical results	ACT Partner	Laboratory record book/data sheets	After sample collection; data recorded after reference method performed	Used to check test results; manually incorporate data into spreadsheets - stored in study binder
Reference calibration data	ACT Partner	Laboratory record books/data sheets	Whenever zero and calibration checks are done	Document correct performance of reference method
Performance evaluation audit results	ACT HQ	Laboratory record books/data sheets	At times of performance evaluation audits	Test reference method with independent standards/measurements

6.2. Data Review

All data are to be recorded directly in the field/laboratory record book as soon as they are available. Records are to be written in ink, written legibly, and have any corrections initialed by the person performing the correction. These data will include electronic data, entries in field/laboratory record books, operating data from the ACT Partner test facility, and equipment calibration records. Records will be spot-checked within two weeks of the measurement to ensure that the data are recorded correctly. The checker shall not be the individual who originally entered the data. Data entries shall be checked in general for obvious errors and a minimum of 10 percent of all records shall be checked in detail. Errors detected in this manner shall be corrected immediately. The person performing the review will add his/her initials and the date to a hard copy of the record being reviewed. The ACT Technical Coordinator (TC) will place this hard copy in the files for this verification test. In addition, data generated by each ACT Partner test site will be provided to the ACT Chief Scientist and reviewed before they are used to calculate, evaluate, or report verification results.

7. Quality Assurance/Quality Control

Technology performance verifications are implemented according to the test/QA plans and technical documents (e, g. Standard Operating Procedures) prepared during planning of the verification test. Prescribed procedures and a sequence for the work are defined during the planning stages, and work performed shall follow those procedures and sequence. Technical procedures shall include methods to assure proper handling and care of test instruments. All implementation activities are documented and are traceable to the test/QA plan and SOPs and to test personnel.

7.1 Laboratory Quality Control

Both the test and laboratory reference instrumentation to be used in this verification test will be calibrated by the ACT TC at the University of Michigan according to the SOPs for the instrumentation prior to field deployment. Each TC for each instrument will maintain a calibration log. The logs shall include at least the following information: name of instrument, serial number and/or identification number of instrument, date of calibration, and calibration results. These logs shall be provided to the ACT Chief Scientist and maintained in a master calibration file as part of the QA/QC records.

7.2. Field Quality Control

Field quality control represents the total integrated program for assuring the reliability of measurement data. It consists of the daily field logs, quality control samples, and sample custody procedures.

Field Logs – Standard, uniform field logs should be maintained for all fieldwork. These logs should report name of staff conducting fieldwork, date (month, day, and year), operating status of all equipment, and manual readings of environmental conditions.

Field Quality Control Samples – To ensure that the reference sample collection and analysis procedures are properly controlled, field blanks and laboratory replicate samples will be taken once a week during the test period. These will be analyzed in the same manner as the collected reference samples. Field blanks are sample containers filled with distilled or deionized water that have been saturated with O₂, taken to the field, fixed, and returned to the laboratory. These samples assess contamination during transport and storage. Replicate samples determine the variability associated with sample collection.

Sample Custody – All collected reference samples at each test site will be handled in the same manner. All reference samples will be accompanied by the sample collection sheet and chain-of-custody form (see below). Proper labeling of sample bottles is critical. Each reference sample should be dated and coded according to site and sample sequence. The actual sample container should be labeled with a number for identification. The reference sample number should be used in all laboratory records to identify the sample. Transfer of reference samples from field personnel to lab personnel is also recorded and records are maintained in the lab with the names and signature of persons leaving and receiving the custody. Samples stored for any period of time shall be routinely inspected by the TC to assure proper preservation and label integrity. Results of these inspections shall be included in the sample records. All logs shall be duplicated

weekly. The original shall be retained at the ACT Partner site and a copy shall be sent to the ACT Chief Scientist.

7.3. Audits

Independent of each Partner test facility QA activities, the ACT Chief Scientist will be responsible for ensuring that the following audits are conducted as part of this verification test at a minimum of three ACT Partner test sites. Audits shall be performed by Quality Assurance personnel, who shall be independent of direct responsibility for performance of the verification test.

Performance Evaluation Audits – A performance evaluation audit will be conducted to assess the quality of the reference measurements made in this verification test. Each type of reference measurement will be compared with a NIST-traceable standard that is independent of those used during the testing. This audit will be performed once during the verification test.

Technical Systems Audits – ACT’s Quality Assurance personnel will perform a TSA at least once during this verification test. The purpose of this audit is to ensure that the verification test is being performed in accordance with the test/QA plan, published reference methods, and any SOPs used by the Partner test facility. In this audit, the ACT Quality Assurance personnel may review the reference methods used, compare actual test procedures to those specified or referenced in the test/QA plan, and review data acquisition and handling procedures. A TSA report will be prepared, including a statement of findings and the actions taken to address any adverse findings.

Data Quality Audits – ACT’s Quality Assurance personnel will audit at least 10% of the verification data acquired in the verification test to determine if data have been collected in accordance to the test/QA plan with respect to compliance, correctness, consistency, and completeness the ACT Quality Assurance personnel will trace the data from initial acquisition to final reporting.

Assessment Reports – Each assessment and audit will be documented, and assessment reports will include the following:

- Identification of any adverse findings or potential problems,
- Response to adverse findings or potential problems,
- Possible recommendations for resolving problems,
- Citation of any noteworthy practices that may be of use to others, and
- Confirmation that solutions have been implemented and are effective.

7.4. Corrective Action

The ACT Chief Scientist, during the course of any assessment or audit, will identify to the ACT Technical Coordinators performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the ACT Chief Scientist is authorized to stop work. Once the assessment report has been prepared, the ACT Chief Scientist will ensure that a response is provided for each adverse finding or potential problem and will implement any necessary follow-up corrective action. The ACT Quality Assurance Manager will ensure that follow-up corrective action has been taken.

7.5. QA/QC Document Control

It is the responsibility of the ACT Chief Scientist to maintain QA/QC records, which shall include the following:

- records of the disposition of samples and data.
- records of calibration of instruments.
- records of QA/QC activities, including audits and corrective actions.

8. Roles and Responsibilities

The verification test is coordinated and supervised by the ACT Chief Scientist and ACT Partner institution personnel. Staffs from the Partner institutions participate in this test by installing, maintaining, and operating the respective technologies throughout the test; operating the reference equipment, collecting the water samples, downloading the data from the dissolved oxygen sensor systems, and informing the ACT Chief Scientist staff of any problems encountered. Manufacturer representatives shall train ACT Partner staffs in the use of their respective technologies and, at their discretion, observe the calibration, installation, maintenance, and operation of their respective technologies throughout the test. QA oversight is provided by the ACT Quality Managers. In addition to aiding the development of these protocols, the ACT Dissolved Oxygen Technical Advisory Committee will be consulted during the evaluation in the event problems occur, will assist in the analyses of results, and will review the final Verification Statement prior to release. Specific responsibilities are detailed below.

The ACT Chief Scientist has the overall responsibility for ensuring that the technical goals and schedule established for the verification test are met. The ACT Chief Scientist shall:

- Prepare the draft test/QA plan and verification statements.
- Revise the draft test/QA plan and verification statements in response to reviewers' comments.
- Coordinate distribution of the final test/QA plan and verification statements.
- Coordinate testing, measurement parameters, and schedules at each ACT Partner institution testing site.
- Ensure that all quality procedures specified in the test/QA plan are followed.
- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary.
- Serve as the primary point of contact for manufacturers and ACT Partner Technical Coordinators.
- Ensure that confidentiality of proprietary manufacturer technology and information is maintained.

ACT Quality Managers for the verification test shall:

- Review the draft test/QA plan.
- Conduct a technical systems audit (TSA) once during the verification test.
- Audit at least 10% of the verification data.
- Prepare and distribute an assessment report for each audit.
- Verify implementation of any necessary corrective action.
- Notify the ACT Chief Scientist if a stop work order should be issued if audits indicate that data quality is being compromised or if proper safety practices are not followed.

- Provide a summary of the audit activities and results for the verification reports.
- Review the draft verification reports and statements.
- Have overall responsibility for ensuring that the test/QA plan and ACT QMP are followed.
- Ensure that confidentiality of proprietary manufacturer technology and information is maintained.

ACT Technical Coordinators at each ACT Partner institution shall:

- Assist in developing the test/QA plan for the verification test.
- Allow facility access to the manufacturers and ACT Headquarters representatives during the field test periods.
- Select a secure location for the tests.
- Install, maintain, and operate the DO sensor systems at the test location at their respective institution.
- Perform sample collections and analyses as detailed in the test procedures section of the test/QA plan.
- Perform reference measurements.
- Provide all test data to the ACT Chief Scientist electronically, in mutually agreed upon format.
- Remove sensor systems and other related equipment from the test facility upon completing the verification test.
- Provide the ACT Chief Scientist and Quality Managers access to and /or copies of appropriate QA documentation of test equipment and procedures (e.g., SOPs, calibration data).
- Provide information regarding education and experience of each staff member involved in the verification.
- Assist in ACT's reporting of their respective test facility's QA/quality control results.
- Review portions of the draft verification statements to assure accurate descriptions of their respective test facility operations and to provide technical insight on verification results.

Manufacturers shall:

- Review the draft test/QA plan and provide comments and recommendations.
- Approve the revised test/QA plan.
- Work with ACT to commit to a specific schedule for the verification test.
- Provide duplicate commercial-ready sensor systems for testing.
- Provide an on-site operator(s) to train ACT staff in the installation, operation, and maintenance of the sensor systems.
- Review and comment upon their respective draft verification statements.

9. Field Test Site Descriptions

Chesapeake Biological Laboratory Field Test Site –

The ACT Partner at Chesapeake Biological Laboratory (CBL), University of Maryland Center for Environmental Science, has established a Technology Verification Field Test Site on a fixed pier (Lat: 38°19.039 N, Lon: 76°27.065 W, with an average depth of 7 ft) at the mouth of the Patuxent River, a tributary of the Chesapeake Bay. The Chesapeake is a nutrient rich estuary with a watershed that encompasses portions of six states and the District of Columbia. Water temperatures at the testing location range from 0° to 35°C and salinities range from 5ppt to 20ppt depending on season, rainfall, wind, and other external factors.



Cooperative Institute of Limnology and Ecosystem Research Field Test Site –

The ACT Partner at the Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, has established a Technology Verification Field Test Site on the French Landing Dam (Lat: 42°12.915 N, Lon: 83°26.372) at the outflow of Belleville Lake, a freshwater impoundment on the Huron River in southeastern Michigan. Belleville Lake has a surface area of 1270 acres and has an average depth of 14.0 feet. Lake temperatures range from 0 to 20°C. The Huron River enters the western end of Lake Erie soon after this impoundment.



Gulf of Maine Ocean Observing System Test Site –

The ACT Partner at the Gulf of Maine Ocean Observing System (GoMOOS) has established a Technology Verification Field Test Site at the University of Maine's, Darling Marine Center in Walpole, Maine. The Center occupies 170 acres of largely wooded property bordering 2 km of pristine water frontage on the Damariscotta River Estuary. The Darling Marine Center offers a secure and easy access to the estuary and maintains a pier and boating facility on site. There is a laboratory facility near the pier, where water sample analysis can be conducted during sensor evaluations. The Damariscotta River estuary is a tide dominated embayment approximately 5 km from the open waters of the Gulf of Maine. The site experiences a predominantly semi-diurnal tide with an approximate amplitude of 3m. Local marine environments include rocky shores, sandy beaches, mud flats, sea grass beds, and expansive sponge communities. High organic production in the Gulf of Maine supports a diversity of benthic and pelagic species. The Damariscotta River is one of several local estuaries that provide a gradient of environments varying in fresh water input, with resultant changes in types and quantities of organic production and consequent changes in biogeographic zonation. The complexity of the Maine coastline allows for a wide range of exposure to waves and ice, further adding to the diversity of habitats. Sea temperatures range from 2 to 15 C° in the open ocean and from -2 to 20 C° in the upper reaches of the estuary. Salinity at the Center's dock ranges from 28 to 32 ppt.



Moss Landing Marine Laboratories Field Test Site –

The ACT Partner at Moss Landing Marine Laboratories (MLML) has established its Technology Verification Field Test Site at the MLML Small Boat Facilities (36.8068N, 121.7878W). This secure deployment site is located in Moss Landing Harbor on the junction of northern tributary of the Salinas River and Elkhorn Slough National Estuarine Reserve on the central coast of California. Instrumentation is deployed off a secure floating dock in waters with a tidal range of 2 meters and a maximum depth below the floating dock of 4 meters. It is an estuarine environment with a mean temperature of 12.858 °C (range: 11.287 to 15.767°C) and a mean conductivity/salinity of 3.615 S m⁻¹ / 30.577 PSU (range: 1.358 to 4.036 S m⁻¹ and 10.851 to 32.942 PSU) at 1 meter depth.



Skidaway Institute of Oceanography Field Test Site –

The ACT Partner at the Skidaway Institute of Oceanography (SkIO) has established a Technology Verification Field Test Site on a floating dock adjacent to the Priest Landing Dock located on the eastern shore of Skidaway Island (Lat: 31° 57.768' N, Lon: 81° 00.705' W).

Skidaway Island is sheltered from the Atlantic Ocean by a chain of barrier islands. The site experiences a semi-diurnal tide with a 2 m amplitude. The SkIO site is located within a typical subtropical estuary dominated by *Spartina alterniflora*. The Priest Landing dock is a large “T” shaped concrete structure that juts easterly into the north/south running Wilmington River. A minimum depth at test site is 14.28 ft. or 4.35m at MLW, water temperature range is 10 - 32°C and salinity range is 10 – 35 ppt.



University of Hawaii Field Test Site –

The University of Hawaii field site will be on the Kaneohe Bay Barrier Reef flat (157°48'W, 21°28.5') in waters ~2 m deep. Kaneohe Bay sits on the northeast, or windward, side of Oahu. The barrier reef acts as a physical divider separating coastal waters from the Kaneohe Bay lagoon and coastal ocean, as well as impeding the passage of surface wave energy into the bay interior. Significant wave heights at the study site are typically < 1 m with mean cross-reef currents only on the order of a few cm s⁻¹. Both wave heights and cross-reef currents appear to be heavily modulated by the tides. Water temperatures at this site vary between 21 and 29°C with highest values in summer. Tidal variations are typically less than 0.5 m and salinities are between 34.5 and 35.5 psu. Diurnal variations in dissolved oxygen are expected to be on the order of tens of uM.



University of South Florida Field Test Site –

The ACT Partner at the University of South Florida (USF) has established its Technology Verification Field Site directly behind the College of Marine Science in Bayboro Harbor. Bayboro Harbor is located in the southwestern region of Tampa Bay, the largest Florida estuary and the second largest estuary in the eastern US. The deployment site ($27^{\circ} 45.612$ N and $82^{\circ} 38.003$ W) is located at the end of a fixed dock, extending westward into the Harbor. This harbor protects two marinas, allowing for consistent and heavy recreational boat traffic. These waters have a summer temperature range from 27.5°C to 31.5°C with a mean of 29.5°C . The salinity varies from 20 psu to about 31 psu and is strongly dependent on rainfall amount. The site has a mean depth of 3.4 m and a mixed tidal range of about 1m. The dock sits on a soft bottom consisting mostly of unconsolidated sediments.



10. Data Reporting Forms

ACT DO Sensor Verification Training Record Form

Name _____

Organization _____

Date _____

Page _____

The named has been trained for the following procedures in accordance with the ACT Dissolved Oxygen Sensor Verification Protocols.

Activity Training	Approved/comments	Date	Trainer (initials)	Trainee (initials)
Water Sample Collection w/Van Dorn Bottle				
Fixing DO in samples				
Storing DO Samples				
Conducting Winkler Titrations				
Preparing DO fixing Chemicals				
Cleaning Glassware Procedures				
Chain of Custody Procedures				
QA/QC Procedures				
Greenspan Inst. Manual, setup, operation				
In Situ Inst. Manual, setup, operation				
Aanderra Inst. Manual, setup, operation				
YSI Inst. Manual, setup, operation				
Hach/Hydrolab Manual, Operation				
Lab Calibration & predeployment procedures				

Instructions were read and discussed and a practical demonstration of required techniques was presented. The trainee was monitored for correct technique. I have received the above training, read the pertinent procedures and plans, and understand the instructions presented to me.

Trainee: _____

Date: _____

Instructor: _____

Date: _____

ACT DO Sensor Verification Laboratory Sample Log Sheet

Site/partner: Cooperative Institute for Limnology & Ecosystems Research,
University of Michigan

Date (dd mon yy): _____ Time: _____

Technician (print): _____

Technician Signature: _____ QA Checked: _____

Temperature: _____ **Salinity:** _____ **Gas:** _____

DO Bottle Numbers (1 sample draw, 3 replicates from each sample):										
Corresponding Instrument Reading (mg/l)										
	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 5	Sensor 6	Sensor 7	Sensor 8	Sensor 9	Sensor 10
T1										
T2										
T3										

(Cross out errors, do not erase. If sampling procedure fails to be complete and needs to be redone, keep this log, explain incident, and start with fresh field log and clean bottles)

Barometric Pressure: _____ (Identify station)

Comments:

Photocopy immediately following sample collection (and titration if applicable). Send original to Technical Coordinator, file copy in an offsite file box.

ACT DO Sensor Verification Field Sample Log Sheet

Site/partner: e.g., Darling Marine Science Lab, Walpole, Maine, GoMOOS

Date (dd mon yy): _____ Time: _____

Technician (print): _____

Technician Signature: _____ QA Checked: _____

Water column DO profile at mooring prior to sample collection, reported in mg/l

Depth (meters)	0.25 m	1 m	Near-bottom	
Probe DO value				

DO Bottle Numbers (1 sample draw, 3 replicates from each sample):

(Cross out errors, do not erase. If sampling procedure fails to be complete and needs to be redone, keep this log, explain incident, and start with fresh field log and clean bottles.)

Weather Observations (e.g., haze, % clouds, rain, wind speed/direction, air temp)

Barometric Pressure: _____ (Identify station for site)

Tide Stage: _____ (include reference)

Other Observations (e.g., flotsam, oil slick, blooms, storm activity prior to sample)

Photocopy immediately following sample collection (and titration if applicable). Send original to Technical Coordinator, file copy in an offsite file box.

ACT DO Sensor Verification Sample Chain of Custody Log

Site/partner: e.g., Darling Marine Science Lab, Walpole, Maine, GoMOOS

Date (dd mon yy)	Time	Bottle #	Bottle #	Bottle #	Technician Initials	Photocopy Sample Log*	Storage Check ++	Date of Analysis	Technician Initials	Photocopy Sample Custody Log (daily) *	QA Checked (Initials)

* Original log forms should be sent to or secured by the Technical Coordinator at each partner site. The photocopy should be held offsite in a secure file box. The Sample Custody Log should be copied daily, and follow same procedure.

++ Storage of DO Bottles for analysis should be in a closed and locked box, in a dark, cool room out of the way of heating units and direct sunlight.