Protocols for the Performance Verification of In Situ Nutrient Analyzers Submitted to the Nutrient Sensor Challenge

April 2016

ACT PV16-01

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Protocols for the ACT Nutrient Sensor Challenge

1.0 Background and Objectives

The Alliance for Coastal Technologies (ACT) has partnered with the Challenging Nutrients Coalition on the Nutrient Sensor Challenge to help address the environmental and ecological problems associated with nutrient pollution. A critical step in this process is facilitating the development and adoption of the next-generation of *in-situ* nutrient sensors and analyzers. The Nutrient Sensor Challenge is a market stimulation and innovation effort to accelerate the development, adoption, and use of affordable, reliable, and accurate sensors measuring nitrate and orthophosphate in water. The goal is to accelerate these new technologies to commercial availability by 2017.

ACT is a NOAA- and EPA-funded partnership of research institutions, state and regional resource managers, and private sector companies that are interested in developing, improving, and applying sensor technologies for studying and monitoring coastal environments. ACT was established on the premise that instrument validation of existing and emerging technologies is essential to support both coastal science and resource management. 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 Nutrient Sensor Challenge is similar to all past ACT Technology Verifications in that instrument performance will be evaluated in laboratory and field tests against reference water samples analyzed using EPA-approved standard methods. Unlike previous ACT technology verifications, however, results from these verification tests will be used by an independent Challenge judging panel in order to address all of the requirements of the competition according to the criteria and weighting factors in Table 1. Points will be assigned using weights to assess both exceedance and partial attainment of the targets. In the event that no sensors meet all target sensor features, awards will still be given to first, second, and third place performers in both the nitrate and phosphate categories.

Table 1: Target Nutrient Sensor Features

Measurement Criterion	Nitrate (± nitrite)	Orthophosphate	Weights
Accuracy	± 5 % or 0.01 mg/L - N (at upper range) from reference value	± 5 % or 0.005 mg/L - P (at upper range) from reference value	**20%**
Precision	± 5 % or 0.01 mg/L - N (at upper range) from reference value	± 5 % or 0.005 mg/L - P (at upper range) from reference value	**15%**
Range	0.005 - 60 mg/L – N	0.005 - 2 mg/L – P	**15%**
Deployment Length	3 months (at 15 minute sampling interval)		**25%**
Cost	Bill of materials for 1) sensor and 2) package		**25%**

These Test Protocols describe how ACT will evaluate the performance characteristics of nutrient sensors through the collection and analysis of quality-assured environmental data.

2.0 Introduction to Technology

Most measurements of nutrients are still made by taking water samples for later analysis in the lab. However, a variety of instruments have become available that automatically measure nutrient concentrations *in situ*. These instruments allow a much higher temporal resolution of measurements than what can be achieved by taking discrete field samples. Many of the initial field-based nutrient analyzers were based on proven wet-chemical laboratory analysis methods. Recently, however, nitrate analyzers, based on the absorbance of ultraviolet light by nitrate in water, have also been introduced.

A variety of wet chemical nutrient analyzers exist on the market. The wet chemical nutrient analyzers require the addition of chemical reagents to determine the target nutrient concentration. The resulting solution develops a particular property (e.g. color) depending on the concentration of the target nutrient, which then can be measured. Parameters limiting the deployment time of wet-chemical analyzers are typically reagent consumption, reagent degradation, power consumption, sample filtration capacity, waste collection and biofouling. Reagent based sensors have the ability to produce highly accurate measurements of a wide range of parameters, but can be large, expensive, and require significant training for expert operation and calibration.

Optical nitrate analyzers do not require reagents or waste storage, but typically measure nitrate concentrations based on the absorption characteristics of nitrate within the ultraviolet spectrum (200-400 nm). The deployment time of the optical instruments is most likely limited by power consumption, power source reliability and biofouling. However, these systems also are large and expensive and reliability and calibration problems have occurred.

Comprehensive nutrient monitoring networks have not been widely established due in large part to the costs and complexities associated with operating the current suite of *in situ* instruments. Hence, there is a critical need for low cost, reliable nutrient sensors which can be deployed in sufficient numbers to ensure that nutrient monitoring data are available at the appropriate geographic and temporal densities to allow stakeholders to make well-informed decisions on resource management.

3.0 Definition of Test Parameters

For the purposes of this Nutrient Sensor Challenge, "**nutrients**" are defined in terms of the dissolved nitrate (NO_3^-), dissolved nitrite (NO_2^-) and soluble reactive orthophosphate (PO_4^{3-}) concentration in water. Nitrate concentrations may be inclusive of nitrite (NO_2^-), if disclosed. Instruments will be tested for accuracy, precision, range, and completeness of data return under varying deployment lengths.

- **Accuracy:** Closeness of agreement between the result of a measurement and reference values, as measured using EPA approved methods, defined below. Accuracy is estimated by repeated comparisons between instrument measurements and reference water samples, and is reported as percent difference (or absolute difference, for high limits of quantification) between reference and measured values.

- **Precision:** Closeness of agreement between independent test results obtained under stipulated controlled conditions. Determined by repeated measures (N=3-5) during laboratory tests with instruments placed in, or exposed to, known stable conditions. Reported as percent difference (or absolute difference, for high limits of quantification) between repeated samples as compared to one another.

- **Range:** Upper and lower limits of detection and quantification. Determined by an analysis of the variance within repeated instrument readings on a known (prepared, sampled, and analyzed) dilution series of the measurement parameter.

- **Deployment Length:** Amount of time the instrument can operate in a submerged deployment setting at a depth of one meter below the surface without needed maintenance or recalibration. Successful deployment requires the sensor to perform within the targeted ranges of accuracy defined in Table 1 throughout the deployment duration. Also, 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.

4.0 Challenge Test Plan

These Test Protocols are based on consensus recommendations of the ACT Technical Advisory Committee, ACT staff, and participating Manufacturers. In summary, the test will:

- utilize standard, approved laboratory analytical methods to provide best possible measure of the 'true' nutrient concentration from field samples, which will serve as performance standards against which instrument estimations can be compared internally by the individual developer;

- all reference sample analysis will be conducted at the state certified Nutrient Analytical Services Laboratory (NASL) of the Chesapeake Biological Laboratory (CBL), Solomons, MD to determine true nutrient concentrations using USEPA approved methodologies;

- employ USEPA Method No. 365.5 for phosphate as applied in Standard Operating Procedures of the NASL (see details below);

- employ USEPA Method No. 353.4 for nitrate, as modified in the Standard Operating Procedures of NASL to utilize an enzymatic reduction step instead of cadmium reduction (see details below);

- include a laboratory evaluation of instrument performance;

- include moored/dock-based field trials under a wide range of environmental conditions including freshwater, estuarine and marine ecosystems and to the extent possible variable nutrient concentrations and water quality characteristics (e.g. turbidity).

All ACT personnel involved in the Nutrient Sensor Verification will be properly trained on standardized water sample collection, storage and shipping methods. ACT staff will be available to assist in the physical deployment and recovery of all submitted test instruments and will be responsible for the data management of test instrument results. Challenge participants will be responsible for initial set-up and calibration of their instrument. If requested, ACT can provide the chemicals and nutrient standards needed for all calibrations and instrument set-up. All laboratory nutrient analyses of our independent reference samples will be conducted at the CBL NASL using standardized automated wet chemistry. All numerical data will be recorded to two significant decimals where appropriate and nutrient concentrations will be reported in either elemental mass units (mgN/L and µgP/L) or as micromoles per liter (µM) for NO_3^- or PO_4^{3-}.

4.1 Laboratory Tests

Laboratory tests of accuracy, precision, and range will be conducted at the University of Maryland's Chesapeake Biological Laboratory (CBL) in Solomons, MD. Laboratory tests will be conducted on a second set of instruments supplied by Vendors. Immediately following the week long laboratory tests, these instruments will be used for the long-term field deployment test at CBL (see below). A series of five tests will be conducted to evaluate performance under controlled challenge conditions including: concentration range, temperature, salinity, turbidity, and dissolved organic carbon (details below). All Laboratory tests will be conducted in a 'bench-top' fashion within a temperature controlled room. All instruments will be sampling from a common, well-mixed, test bath of approximately 20L volume that is maintained at a documented level of known challenge condition. (Note: One of the test instruments has a sample through-put rate that will require recirculating the sample into the test bath. No physical or chemical modifications of the sample will be produced from the sampling process.) Instruments will be set-up and kept running at a fixed sampling interval during each of individual laboratory tests. Instruments will be exposed to each test condition for a period of two hours and programmed to sample at a minimum frequency of 30 minutes. Reference samples will be collected every 15 minutes during each test. Comparisons to instrument measurements will be done for the final 90 minutes of exposure, providing an initial 30 minute equilibration period and a minimum of three matched timepoints. A copy of the resultant test data will be downloaded at the conclusion of each Lab test. Whenever possible the original data will be kept within the instrument as well.

Laboratory tests will include the following 'controlled' challenge conditions and objectives:

Test 1: Accuracy and Precision over a broad concentration range

- Establish 6 fixed concentrations whose range covers stated ranges of detection for natural waters and meets judging criteria.
    - Range for NO_3 (mgN/L): 0.005, 0.1, 1.0, 5, 10, and 60
    - Range for PO_4 (mgP/L): 0.002, 0.01, 0.05, 0.1, 0.5, and 2.0
- Conduct a minimum of 3 comparative readings at each stable, fixed concentration to assess accuracy and precision against reference values.
- Use Type 1 deionized water as the test matrix to which we add known amounts of nutrient salts (KNO_3 and K_2HPO_4).
- All tests will be conducted at 20 ^oC in a temperature controlled room with samples drawn from a common well-mixed 20L test bath.
- Provide a two 2 hour sampling window at each of the six concentrations. Reference samples will be collected every 15 minutes during the final 90 minute test cycle, which will provide a match-up to instrument measurements at a minimum of 3 times, given a measurement frequency of 30 minutes.
- Collect 1 tank duplicate during the 6 reference sample timepoints to assess variability generated through sample collection, processing and analysis.
- All instrument results within the 90 min sampling time will be used to estimate precision according to the standard error of the mean and coefficient of variation.
- Vendors have to pick a single set-up or calibration for the entire test. Discussions on proper 'range fitting' for ideal environmental use can be discussed in the company response letter to the report.

Test 2: Temperature Response

- Test instrument response for three concentrations, corresponding to levels C2, C3, and C4 from the range test, at temperatures of 5 versus 20 ^oC.

- Responses at 20 °C are obtained in the initial range test on day 1 and responses at 5 °C will be testing on day 2.
- Temperature will be regulated and maintained within a temperature controlled room. Temperature of the testing tank will be independently verified with RBR temperature loggers with an accuracy of better than 0.01 °C.
- Instruments will be equilibrated to the new 5 °C test temperature overnight.
- Instruments will exposed for two hours at each of the 3 concentrations with a 90 minute comparative sampling period as defined above.

Test 3: Salinity Response

- Test accuracy and precision over three additional salinities (10-20-30) at the C3 concentration level of the range test at 20°C.
- Salinity levels will be developed using Instant Ocean additions to a DI water matrix.
- Instruments will be exposed for two hours at each of the 3 concentrations with a 90 minute comparative sampling period as defined above.

Test 4: Turbidity Response

- Test accuracy and precision over two additional turbidity levels (10 and 100 NTU) at the C3 concentration level of the range test at 20 °C.
- Turbidity concentrations will be established using the Elliot Silt Loam reference material (cat # 1B102M) available from the International Humic Substances Society (http://www.humicsubstances.org/sources.html). Turbidity concentrations will be established using a Type 1 DI water matrix.
- Instruments will exposed for two hours at each of the 3 concentrations with a 90 minute comparative sampling period as defined above.

Test 5: DOC Response

- Test accuracy and precision against 2 DOC levels (1 and 10 mg/L) at the C3 concentration level of the range test at 20 °C.
- DOC concentrations will be established using the Upper Mississippi River Natural Organic Matter standard (cat# 1R110N) available from the International Humic Substances Society (http://www.humicsubstances.org/sources.html). DOC concentrations will be established using a Type 1 DI water matrix.
- Instruments will exposed for two hours at each of the 3 concentrations with a 90 minute comparative sampling period as defined above.

4.2. Field Tests

In situ field performance evaluations of the test instruments will be conducted under extended mooring deployments at three ACT Partner Institution sites covering freshwater, estuarine, and marine conditions. Field site descriptions are provided in Section 11. The freshwater deployment will take place on the Maumee River in Waterville, OH and is intended to provide a high nutrient, high turbidity test environment. The duration of the Maumee River test will be one month. The estuarine deployment will take place at the research pier of the Chesapeake Biological Laboratory in Solomons, MD and is intended to provide for variable salinity and nutrient levels within a highly productive and biofouling environment. The duration of the CBL estuarine test will be three months. The marine deployment will take place in Kaneohe Bay at the Hawaii Institute of Marine Biology field lab and is intended to provide a full salinity, low nutrient test condition. The duration of the HIMB marine test will be one month. One nutrient analyzer from each developer will be deployed at each site. Retrieval of instruments will only occur at

the end of the test, unless an obvious problem or environmental condition develops that could jeopardize the safety of the instruments (e.g. flooding, major storms, or structural damage).

Instrument Setup - Prior to deployment, all instruments will be set up and calibrated as required at the field sites by a manufacturer representative, with assistance provided by ACT staff as necessary. Participants must supply all instrument specific materials and hardware (chemicals, power cords, cables, weights, etc.) to deploy their instrument(s) according to requirements defined for each field site (see below). If vendors want the local ACT Partner to purchase any chemicals directly then complete ordering information needs to be provided at least one month in advance. No servicing of the instruments will occur during the test deployment period unless observed physical damage has occurred from natural events and a repair or replacement is deemed necessary. Instruments will be set up as self-recording, either internally or to an external data logger, and be programmed to record data based on a time interval that will allow instruments to function for the specified number of days for the respective deployment. While specific sampling intervals may vary among test instruments, the Nutrient Sensor Challenge targets were set at15 minute sampling intervals (Table 1). A sampling schedule will be established so that all instruments being tested at the same time will have a common sampling time point at a minimum frequency of 2 hours. Internal clocks will be set to local time and synchronized against the time standard provided by www.time.gov. A photograph of each individual instrument and the entire instrument rack will be taken just prior to deployment and just after recovery to provide a qualitative estimate of biofouling during the field tests. All instruments will be delivered a low (0) and high (100 µgP/L; 10 mgN/L) reference standard (certified by analysis at CBL-NASL) before deployment to ensure good working order. For instruments that incorporate an on-board standard as part of their measurement system we will collect a sub-sample of the standard immediately before and after the deployment period for independent analysis by CBL-NASL to help account for any possible accuracy offset that may arise from the degradation of the standard.

Instrument Deployment - The individual start times for the various test instruments may be staggered slightly to allow individual attention for any given participant, but all instruments will be deployed within approximately three days of each other. Discharge of any waste stream into the environment if prohibited. All waste streams need to be collected in appropriate waste bags within the instrument or in attached containment bags. All wastes will be disposed of by the ACT representative from the test site according to their institutional requirements after consultation with the manufacturer regarding content and concentrations.

Deployment Moorings – ACT staff will work with the instrument developers to design an appropriate sensor deployment configuration at each site and will arrange instruments in a manner so that a single representative field sample can be collected without the potential of interference between instruments. For the two coastal deployments at CBL and Hawaii, the deployment frames will be arranged so that all of the sample inlets for the instruments remain at a fixed depth of 1 m below the water surface using a floating dock in tidal systems or a fixed dock in environments not affected by tidal changes. We will also try to pick deployment sites so that instruments can remain at least 1m off the bottom to reduce the risk of entraining bottom sediments. For the riverine test site, instruments will be deployed in a 180 gallon flow-through tank with a water depth of approximately 0.8m and exchange time of approximately 10 minutes. The sensor rack design will be standardized as much as possible from site to site; however, physical conditions at particular sites may require specialized modifications. Finally, mooring frames and attachment points will be designed in such a manner that no instrument will be disturbed during the deployment or retrieval of other instruments.

Reference Water Sampling Schedule – The reference sampling schedule will generate approximately 50 comparative reference samples and be structured to examine changes in nutrient concentrations over daily to monthly time scales. Specifically, once each week ACT staff will conduct an

intensive sampling event that consists of 4 consecutive samples spaced at two-hour intervals. For the remaining 4 days of the week, ACT staff will sample once or twice per day, spaced out to cover early morning and late-afternoon timepoints or anticipated flow or tidal events. The initial intensive sampling event will occur within the first two days of the deployment after all instruments have been deployed, and the final intensive sampling event will occur during the last two days of the deployment. This schedule will provide a higher density of comparative data at the beginning when instruments should be functioning at optimum performance and again after the challenge of a long-term deployment. To the extent possible, the middle two intensive sampling events will be selected to correspond to specific meteorological or hydrological events that are likely to correspond to significant changes in ambient nutrient concentrations. This sampling schedule should be sufficient to capture both diurnal and event based scales of variation and provide a 'continuous' check on instrument performance throughout the deployment. The specific timing of when water samples will be collected will be left up to the individual sites, but with the goal of capturing maximum variations in nutrient concentrations. In the event of weather limitations or un-avoidable schedule conflicts it will be permissible to miss a sampling day and collect additional samples on the following day to keep a similar number of reference points for each test site. All sampling times will be recorded on log sheets and entered into a database for final instrument measurement comparisons.

Reference Water Sample Collection - A standard 2L Van Dorn bottle will be used at the CBL and HI field sites to collect water samples for laboratory nutrient concentration analysis. For the riverine test site a 1L acid-cleaned, polypropylene bottle will be filled directly from the tank. These samples will be used as the reference samples for examining instrument performance and stability over time. For the tank sampling, the sampling bottle will be rinsed three times before filling. For the mooring sites, the sampling bottle will be lowered to the same depth and as close as physically possible to the sampling inlets of all instruments and less than 1 m from any individual sampling inlet. The sampling bottle will be allowed to soak at sampling depth for 1 minute prior to sampling. If water is not flowing the bottle will be moved to ensure that it is being flushed with the ambient water. The bottle will be triggered to close at the same time that the test instrument are initiating sample uptake, to ensure that the same water mass is being compared with regards to nutrient concentrations. All environmental reference samples will be processed within 10 minutes of collection while wearing clean laboratory gloves to minimize potential sources of contamination. The entire water sample will be transferred to an acid washed 1L polypropylene bottle. The bottle will be rinsed 3x with small amounts of the sample before filling. The sample will be filtered through a 47mm Whatman GFF filter into an acid cleaned vacuum flask. The first 50 ml of filtrate will be discarded as a rinse. The remaining filtrate will be distributed into 8 individual acid-cleaned, 30 ml polypropylene bottles to provide three analytical replicates each for NO_3 and PO_4 plus two replicates to hold as back-ups. All final sample bottles will be rinsed once before filling and filled no more than ¾ full to allow adequate headspace for freezing. The final reference samples will be immediately frozen and vials will remain upright during the freezing processes, and caps will be re-tightened after the water has frozen as they may loosen during freezing.

Cleaning Sampling Apparatus – Between each consecutive sample taken on the same day, sample bottles and filtration equipment will be rinsed with the new sample water. Filtration apparatus and sample storage vessels will be cleaned daily by acid washing with 10% HCl and copious rinses (5x) with Type 1 deionized water back at the laboratory.

Sample Handling and Chain of Custody - All collected reference samples at each test site will be handled in the same manner. Each reference sample will be dated and coded according to site and sample sequence. Each sample container will be labeled with a number for identification. The reference sample number will be used in all laboratory records and Chain-of-Custody (COC) forms to identify the sample. Samples stored for any period of time will be routinely inspected to assure proper preservation and label

integrity. The storage containers and storage devices (i.e. freezers and refrigerators) must be visually inspected on a routine basis to assure proper operation and integrity. Results of all inspections will be included in the sample records. All logs will be duplicated weekly with the original retained at the ACT Partner site and an electronic copy sent to the ACT Chief Scientist.

Sample Shipping - Samples will be shipped on dry ice to CBL-NASL for nutrient analysis every two weeks. NASL may composite the samples until the end of each test deployment prior to analysis if the storage time falls within their required SOPs. Each test site will conduct a preliminary 'shipping test' to ensure that samples will remain frozen under the established packing conditions. Shipping containers will be sent for next morning delivery, or the soonest possible delivery time possible from a given shipping location. All samples, including the condition shipped and received, will be recorded onto Chain of Custody (COC) forms and a copy will be sent with the samples. The COC specifies time, date, sample location, unique sample number, requested analyses, sampler name, and required turnaround time, time and date of transaction between field and laboratory staff, and name of receiving party at the laboratory. NASL will confirm receipt and condition of samples within 24 hours of their arrival by signing and faxing a copy of the form to the test site. Original copies of these forms will be maintained on site. The additional two replicates will be stored at the field test site until all analysis are completed and QA/QC'd to provide a back-up should there be a problem in the shipment or analysis at the NASL.

4.3 Reference Sample Analysis

Phosphate concentrations for all reference and quality control samples will be determined by the NASL at CBL following their Standard Operating Procedures Manual (CEES, UMD, Publication Series No. SS-80-04-CBL). The methodology is based on U.S. EPA Method 365.1, in Methods for chemical analysis of water and wastes. (United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600-4-79-020 March 1979). In brief, ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphate to form an antimony-phospho-molybdate complex. The complex is reduced to an intensely blue-colored complex by ascorbic acid. The color produced is proportional to the phosphate concentration present in the sample.

Nitrate and nitrite concentrations for all reference and quality control samples will be determined by the NASL at CBL following their Standard Operating Procedures Manual (CEES, UMD, Publication Series No. SS-80-04-CBL). The methodology is based on U.S. EPA Method 353.2, in Methods for chemical analysis of water and wastes. (United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600-4-79-020 March 1979). In brief, nitrate is reduced to nitrite using the cadmium reduction method. The nitrite is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine di hydrochloride to form a color azo dye. The absorbance measured at 540 nm is linearly proportional to the concentration of nitrate + nitrite in the sample. Nitrate concentrations are obtained by subtracting nitrite values, which have been separately determined without the cadmium reduction procedure.

All laboratory nutrient analyses will be conducted on an Aquakem 250 auto-analyzer. For phosphates, a statistically-determined method of detection limit for this instrument has been established at 0.0007 mgP/L by prior laboratory studies for a wide range of salinities. An expected working concentration range for this Verification and SOP is between 0.002 and 1.48 mgP/L. The detection limits for nitrate and nitrite are at 0.0007 mgN/L and 0.0006 mgN/L respectively. The typical working concentration range for the nitrate method and SOP is between 0.0049 – 5.6 mgN /L. The typical working concentration range for the nitrite method and SOP is between 0.0042 – 0.28 mgN /L. The

system contains an auto-dilutor to bring any higher concentrations down to the established linear calibration range. A sample reagent blank is analyzed in conjunction with every sample as part of the routine operation of the Aqaukem 250. Approximately 40 samples per hour can be analyzed. All internal standards will be verified and calibrated using certified external nutrient standards (such as Spex Certi-Prep or NIST). In addition, Field Trip Blanks and Field Sample Spike Additions (defined below) will be conducted once per week as part of established quality assurance/quality control (QA/QC) protocols.

4.4 Ancillary Environmental Data

Basic water quality ancillary data will also be collected during field deployments to both fully characterize the different field conditions during testing and to provide qualitative comparisons as to whether particular environmental parameters correlate with instrument nutrient measurements. At each of the mooring test sites, a calibrated CTD package and a multi-parameter water quality sonde will be positioned at the same depth as the test instruments to provide an independent record of temperature, conductivity, turbidity, pH, fDOM, and chlorophyll at 15 minute intervals. In addition we will deploy 2 additional RBR temperature loggers on the mooring rack approximately 0.5m above the sensor depth to characterize any micro-stratification that may exist at the site due to surface heating/cooling or salinity differences from advective flows. All CTD and sonde data, collected from each site, will be provided to all participants and included in the final reports. Where available, access to real-time data output of ancillary measurements will be provided.

5. Test Schedule

A three-day period of time will be provided at the start of testing at each site for manufacturers to perform initial set-up and calibration of their instruments and work with ACT staff to prepare them for deployment. Vendors will also be given a half-day to train ACT staff on required protocols for instrument use, retrieval and data down-loading to be performed by ACT staff at the completion of each test.

Field Test 1: Maumee River, Waterville, OH.
Set-up and Training: May 23-25
Field Deployment: May 26
Retrieval: June 28
Instruments Shipped to Vendors: July 7

Laboratory Test: Chesapeake Bay, Solomons, MD.
Set-up and Training: July 8-10
Lab Test: July 11-15
Instruments to be immediately transitioned to the CBL Field Test

Field Test 2: Chesapeake Bay, Solomons, MD.
Prep for Field Deployment: July 16-17
Field Deployment: July 18
Retrieval: October 11
Instruments Shipped to Vendors: October 18

Field Test 3: Kaneohe Bay, HI.
Set-up and Training: October 3-5
Field Deployment: October 6

Retrieval: November 3
Instruments Shipped to Vendors: November 10

To meet our schedule for completing the challenge requires that companies supply two sets of instruments during the Verification. The first unit supplied for the Maumee Riverine test will be returned to the Vendor immediately upon completion and may be factory serviced for use at the HIMB test site. A second unit will be required for the Laboratory and field tests conducted at CBL.

6. Data Collection, Review and Distribution

A variety of data will be acquired and recorded electronically and manually by ACT staff during each deployment. Results from the reference method and ancillary measurement will generally be documented in a field/laboratory record book and on the data sheet/chain-of-custody forms (Table 1). An electronic copy of these raw data will be transferred to the ACT Chief Scientist, who will organize and store the study data.

Table 1. Summary of ancillary and reference method information to be recorded by ACT staff during each field deployment as part of the Nutrient Sensor Challenge.

Data to be Recorded	**Responsible Party**	**Where Recorded**	**How Often Recorded**	**Purpose of Data**
Dates, times of sampling events	Each ACT Site	Field 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)	Each ACT Site	Field record books/data sheets	Each reference sample collection	Used to define site characteristics; manually incorporate data into spreadsheets - stored in study binder
Reference analytical results	CBL Nutrient Analytical Services Lab	Laboratory record Book/data sheets	At the conclusion of each analytical sample batch.	Used to check test results; manually incorporate data into spreadsheets - stored in study binder
Reference calibration data	CBL Nutrient Analytical Services Laboratory	Laboratory record books/data sheets	Whenever zero and calibration checks are done	Document correct performance of reference method

All data will be recorded directly in the field/laboratory record book as soon as they are available. Records will be written in water-proof ink, written legibly, and have any corrections initialed by the person performing the correction. Any corrections will be crossed out with a line (not blackened or white-out), and the correction made, with initials and date of correction. These data will include electronic data, entries in field/laboratory record books, operating data from the test sites, and equipment calibration

records. Records will be spot-checked within one week of the measurement to ensure that the data are recorded correctly. The reviewer will not be the individual who originally entered the data. Data entries will be checked in general for obvious errors and a minimum of 10 percent of all records will be checked in detail. Errors detected in this manner will be corrected immediately. The person performing the review will add their initials and the date to a hard copy of the record being reviewed. ACT staff will place this hard copy in the files at each ACT test site and a copy will be provided to the ACT Chief Scientist.

7. Quality Management

All technical activities conducted by ACT comply with ACT's Quality Management System (QMS), which includes the policies, objectives, procedures, authority, and accountability needed to ensure quality in ACT's work processes, products, and services. 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. 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. ACT's QMS meets the requirements of ISO/IEC 17025:2005(E), *General requirements for the competence of testing and calibration laboratories*; the American National Standards Institute (ANSI)/American Society for Quality (ASQ) E4-2004 *Quality Systems for Environmental Data and Technology Programs*; and U.S. Environmental Protection Agency, quality standards for environmental data collection, production, and use.

Preventive actions will be taken throughout the tests to anticipate and resolve any problems before the quality of performance is compromised. QA/QC procedures for this Nutrient Sensor Challenge will follow the requirements described in these Protocols, any participant specified requirements, and the general principles and specific QA/QC from technical documents for measuring nutrients 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.

7.1. Quality Control for Field Samples and Laboratory Analyses

Field quality control represents the total integrated program for assuring the reliability of measurement data. It consists of the daily field logs and sample handling and custody procedures described above. QC samples will include:

- Field Trip Blank: Sample containers filled with reagent water (Type 1 reagent grade deionized water) are taken to the field and processed identically to field reference samples to evaluate contamination introduced during sampling, storage and transport. Four field trip blanks will be collected for each field test spaced evenly throughout the deployment period.

- Field Sample Spike-Additions: An aliquot of a reference sample to which a known quantity of the analyte of interest is added. The field sample spike is analyzed exactly as the initial reference sample and is designed to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the sample matrix must be determined independently

and subtracted from the field spike. Four field sample spikes will conducted at each field test spaced evenly throughout the deployment period. Spike samples will be made into 100ml of filtered sample in a volumetric flask to produce N and P concentrations approximately 2x natural concentration.

- Field Duplicates – we will collect two reference sample water bottles simultaneously at approximately 10% of the sampling points to examine fine scale spatial heterogeneity within the mooring arrangement.

As part of laboratory QC, all laboratory instrumentation at NASL used to measure nutrient concentrations of the reference samples will be calibrated by a highly trained technician using established SOPs that have met both State of Maryland and ACT audit checks. NASL will maintain a log of all calibration and reference QC samples analyzed during the Challenge. The logs will include at least the following information: name and identification number of instrument, date of calibration, and calibration results. These logs will be provided to the ACT Chief Scientist and maintained in a master calibration file as part of the QA/QC records. QC samples will include:

- Internal Nutrient Calibration Standards - Solutions prepared from stock standard solutions to calibrate the laboratory instruments with respect to analyte concentrations. Five standards will be measured in duplicate during each set of analyses. Consistency in absorbance values for each standard will be compared to long-term daily records.

- External Certified Nutrient Standards - An external certified nutrient standard will be prepared and analyzed in duplicate during each set of analyses. External standards are used to verify the accuracy and consistency of the internal calibration standards.

- Laboratory Reagent Blanks - an aliquot of reagent water that is treated exactly as the laboratory calibration standards including exposure to glassware, equipment, and reagents.

7.2. Quality Assurance Technical Assessments

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.

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 Nutrient Sensor Challenge will include: (1) technical system audits (TSAs) and audits of data quality (ADQs).

A 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.

An 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 ACT QA Manager conducts the ADQ after data have been 100% verified by the ACT Chief Scientist. 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.

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 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 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 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.

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 test data is addressed in the test report.

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.

8. Roles and Responsibilities

The ACT Chief Scientist has the overall responsibility for ensuring that the technical goals and

schedule established for the beta test are met. The ACT Chief Scientist will:

- Prepare the Test Protocols in consultation with ACT TAC and staff.
- Coordinate testing, measurement parameters, and schedules at each ACT Partner institution testing site.
- Ensure that all quality procedures specified in the Test Protocols are followed.
- Respond to any issues that may arise during the tests.
- Serve as the primary point of contact for participants and ACT staff.
- Ensure that confidentiality of proprietary participant technology and information is maintained.

The ACT QA Manager will:

- Review the Challenge Test Protocols.
- Conduct technical audit and data quality assessments.
- 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
- Verify implementation of any necessary corrective action.
- Prepare audit reports.

ACT Technical Coordinators at each ACT Partner institution will:

- Assist in developing the Test Protocols.
- Select a secure location for the tests.
- Support participants in the deployment and recovery of instruments as needed.
- Perform sample collections as detailed in the Test Protocols.
- Provide all test data to the ACT Chief Scientist electronically, in a mutually agreed upon format.

The Nutrient Analytical Services Laboratory at CBL will:

- Perform reference sample analyses.
- Perform all QA/QC analysis as detailed in the Test Protocols.
- Provide the ACT Chief Scientist and QA Manager access to and/or copies of appropriate QA documentation of test equipment and procedures (e.g., SOPs, calibration data).

Challenge participants will:

- Commit to a specific set of locations and dates for testing according to the Test Protocols.
- Setup, calibrate, deploy, and recover test instruments at the locations and dates agreed to.
- Provide all materials, supplies and equipment needed to setup, calibrate, deploy, operate, maintain and recover test instruments.

Nutrient Analyzer Technical Advisory Committee will:

- Review and comment on Test Protocols.
- Provide specific advice during testing, as needed.

9. Nutrient Challenge Technical Advisory Committee

- Suzanne Bricker, National Oceanic and Atmospheric Administration
- Brian Pellerin, U.S. Geological Survey
- Dwane Young, U.S. Environmental Protection Agency
- Matt Cohen, University of Florida
- R. David Holbrook, National Institute for Standards and Technology
- Chris Gross, U.S. Department of Agriculture NRCS
- Joe Rudek, Environmental Defense Fund

10. Field Test Site Descriptions

University of Maryland, Chesapeake Biological Laboratory

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 can range from 0° to 35°C (likely 28° to 32°C in August) and salinities range from 5 to 20 psu depending on season, rainfall, wind, and other external factors.

University of Michigan, Cooperative Institute for Limnology and Ecosystems Research

The ACT Partner at the University of Michigan will establish a flow-through system on the Maumee River near Waterville Ohio (83 44.32 N, 41 28.65 W), located within the pump house of the City of Bowling Green Municipal Water Treatment Plant. The Maumee River main stem flows 137 km before flowing into the Maumee Bay of Lake Erie at the city of Toledo, Ohio. The Maumee watershed is the largest watershed of any Great Lakes river with 8,316 square miles. The majority of the watershed is cultivated crop land, mostly corn and soybeans, though concentrated areas of pasture are located in the northwestern and southeastern areas of the watershed. Developed land is approximately 11.5 percent of the land use. Currently the Maumee River watershed contributes over 50% of sediment that flow into Lake Erie. In addition the Maumee watershed provides over 40% of the phosphorous load to Lake Erie, predominately from agriculture.

Hawaii Institute of Marine Biology

The ACT Partner at the Hawaii Institute of Marine Biology (HIMB) is part of the University of Hawaii with a 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. Water temperatures at this site vary between 21 and 29°C and salinities are between 34 and 36 psu.

11. Ease of Use Survey

An Ease of Use Survey regarding requirements for instrument set-up and operation will be developed with input from the participating vendors. The survey will be completed by each of the ACT Technical staff operating the equipment. Survey results will be part of judging for Nutrient Challenge, but specific weighting factors for these results were not determined. It is acknowledged that responses are not quantitative. Results of the surveys will be provided to the participating vendors but will not be included in published Verification reports.