Protocols for Verifying the Performance of Algal Toxin Detection Field Sensors and Kits

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1. Background and Objectives

The Alliance for Coastal Technologies (ACT) is a NOAA- and EPA-funded component of the US Integrated Ocean Observing System (IOOS) involving a partnership of research institutions, state and regional resource managers, and private sector companies that are interested in developing, improving, and applying sensor technologies for monitoring coastal and freshwater 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 goal of ACT's verification program is 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. The ACT verification program aims to provide users of this technology with an independent and credible assessment of instrument performance.

The following protocols describe how ACT will verify the environmental performance characteristics of commercial-ready, or prototype, algal toxin detection field kits through the evaluation of objective and quality assured data. Specifically, the evaluation will demonstrate capacity (performance, ease of use, reliability) of new field portable technologies for measuring harmful algal bloom (HAB) toxins and HAB toxin-producing species. ACT will verify range of detection, accuracy, and precision against accepted reference methods, and will help quantify matrix effects or challenges that may affect sensor performance.

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 pertinent information that users need. ACT will not simply verify vendor claims, but instead looks to the broader community to define the data and operational parameters that are valuable in guiding instrument purchase and user 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. Also, instrument-specific Verification Statements will be released to the public for each instrument type as a final report.

2. Introduction to Technology

Rapid detection of toxin presence and concentration are vital for ensuring public safety and environmental health. Accurately and efficiently detecting and measuring harmful algal bloom (HAB) toxins in freshwater and marine systems requires specific, accurate, and time/cost-efficient technologies. Standard methods for detecting and quantifying toxins (e.g. LC-MS/MS, HPLC, mouse bioassay, receptor-binding assay) are highly accurate but tend to be time-, cost-, and labor-intensive. The time, cost and effort required to generate data from samples often means that few samples are analyzed and that there are significant time lags in generating those data.

There are several field-portable/-deployable assays, test kits, and sensor-based approaches that detect HAB toxins via immunological (i.e. antibody) and/or molecular methods. As these approaches and instruments are incorporated into harmful algal bloom monitoring and management efforts, it is important to understand their performance. This ACT Performance Demonstration will focus on the suite of instruments and/or assays with the specific application of detecting HAB toxins in marine and freshwater systems, particularly those sensors that are field-portable or field-deployable.

3. Definition of Test Parameters

The evaluation will quantify instrument/assay accuracy, precision, range/detection limits, and reliability against standard methods. Laboratory tests will focus on quantifying accuracy, precision, dynamic range, and detection limit under controlled environments and taking into account matrix effects. Limited field tests will follow that focus on instrument reliability and ability to detect and quantify toxins against a complex natural background.

- **Accuracy –** Closeness of agreement between the result of a measurement and reference, as measured using EPA approved methods, defined in the following paragraphs. Accuracy is estimated by repeated comparisons between instrument measurements and reference water samples, and is reported as the absolute and relative difference between reference and measured values.
- **Precision –** Repeatability of a measurement obtained under stipulated controlled conditions. Precision is determined by repeated measures during laboratory tests with instruments placed in, or exposed to, known stable conditions, and is reported as the absolute and relative difference between repeated samples as compared to one another.
- **Range –** Upper and lower limits of detection and quantification. Range is determined by an analysis of the variance within repeated instrument readings on a known (prepared, sampled, analyzed) dilution series of the measurement parameter.
- **Reliability –** Ability to maintain integrity or stability of the instrument and data collections over time. Reliability of instruments will be determined from both laboratory and field tests through comparisons of the percent of data recovered versus expected. Comments on the physical condition of the instruments (e.g., physical damage, flooding, corrosion, battery failure, etc.) will also be recorded.

4. Test Applications

ACT will conduct two laboratory tests and four field tests as part of the toxin/HAB species instrument evaluation. One of the lab tests will focus on freshwater species and associated toxins, and the second will evaluate marine species and associated toxins. The field tests are chosen to represent a broad range of environmental conditions and will incorporate both freshwater and marine environments. Prior to laboratory testing, ACT will work individually with each company for 4-5 hours to be trained on the general operations and handling of their specific instrumentation. Training will also provide an opportunity to check operational status of instruments immediately prior to the first laboratory test. The training activity will occur from July 9-10, 2018 at Bowling Green State University (BGSU). ACT Partners will provide equipment and materials needed to collect reference samples and conduct field and laboratory

tests. This includes vessel support, dockside power supply, laboratory space, phytoplankton culture lines, test tanks, and ancillary sensors. Manufacturers will be responsible for providing their test instruments and all ancillary components specific to the targeted analysis to the appropriate testing site one week before scheduled use. ACT will work with the companies to ensure that all required labware and consumable reagents are on hand at the test site. ACT will help with the purchasing of required consumables to the extent possible based on available resources. Details of the test sites and sampling plan are given below.

4.1 Lab Tests

Laboratory tests to evaluate range, accuracy, and precision of detecting freshwater HAB species and their toxins will be conducted at BGSU within the laboratory of Dr. Timothy Davis. Laboratory tests to evaluate range, accuracy, and precision of detecting marine HAB species and their toxins will be conducted at Moss Landing Marine Laboratory (MLML) within the laboratory of Dr. G. Jason Smith. Each laboratory test will last approximately one week in duration and will assess analytical accuracy of the test instruments compared to reference sample analysis which will include independent analysis of toxin concentrations using U.S. EPA approved ELISA methods and LCMS measurements conducted by Dr. Raphe Kudela using state of California certified protocols. In addition, we will conduct independent qPCR and microscopic counts of targeted HAB species.

Laboratory testing will involve three stages:

Stage 1. Fortified Media Blank

Test instruments will be exposed to a fortified (spiked) freshwater media blank. Five replicate measurements will be conducted and used for a precision estimate. The spiked blank will also be used to evaluate the matrix effect, or extraction efficiency, of the various culture lines. The spiked blank will be performed at one toxin concentration. This analysis will be conducted first to also make sure the test instruments are working as expected before going into lysate and extraction challenges.

Stage 2. Analysis of a commonly produced lysate from known HAB cultures at various cell densities.

Each submitted test instrument will be exposed to a common lysate from two different monocultures of a known toxin producing HAB species produced using the EPA freeze/thaw method (EPA Method 546). Each instrument will record a single measurement at three levels of cell abundance, targeted at: 10,000, 50,000, and 100,000 cells/ml for freshwater species and one-hundredth that for seawater species. The lower abundance levels will be created through dilutions of the 100,000 cells/ml concentration using the same sterile media. Analysis will be conducted on whole-water sample fractions of the lysate. In addition we will conduct one analysis on a dissolved fraction of the lysate produced for the middle concentration. The dissolved fraction will be generated by an initial centrifugation (5 min at 4000 rpm), followed by filtration through a 0.2 μM nylon syringe filter. This test is intended to reduce potential variability in instrument response related primarily to the extraction/cell lysis protocols.

Stage 2. Analysis of a selected toxin/species HAB culture at various densities using manufacturer specific sample processing to create the lysate.

Each instrument will record a single measurement at six levels of cell/toxin concentrations of a known toxin producing HAB monoculture. The range of concentrations tested will be established to cover the expected full linear range of detection and down to the limit of detection. Analysis will be conducted on whole water samples of the lysate. In addition, we will conduct analysis on a dissolved fraction of the lysate produced for one of the six test concentrations. All cultures used will have known strains/congeners.

Freshwater Lab Testing: Bowling Green State University, OH

Dates: July 11-15, 2018

Targeted Toxins and Species: Freshwater lab tests will focus on the microcystin producer, LE3, and the cylindrospermopsin producer, CSRO 505.

Estimated Number of Samples: 38

Participants: All companies who have applied to the ACT evaluation are expected to participate in the freshwater lab testing.

Saltwater Lab Testing: Moss Landing Marine Lab, CA

Dates: September 11-14, 2018

Targeted Toxins and Species: Saltwater lab tests will use MLML cultures for the domoic acid producing *Pseudo-nitzschia* spp. and UCSC cultures for the saxitoxin producing *Alexandrium* spp.

Estimated Number of Samples: 38

Participants: Participation will be dependent upon the readiness of new analytical procedures for these specific species and toxins. At the time of the protocol development workshop companies were not certain of the availability of these methods. This phase of lab testing may be used to support further development of methodologies by the companies and not integrated into final performance evaluations.

4.2 Field Tests

A rigorous field testing program has been designed to provide a wide variety of algal toxins and toxin-producing species within various freshwater and marine ecosystems. The tests will provide a range of test conditions, including ranges of cell densities, toxin concentrations, and water quality parameters such as salinity, temperature, turbidity, CDOM, and alkalinity. Each test site will include sampling over multiple days and at multiple sites to provide the greatest variation in test conditions. Each site will provide 10-20 reference field samples which will be collected dock-side or by small boat with analysis performed at field site dock. Each instrument will be tested at various field locations depending on which toxins and/or algal species they are designed to detect. For each unique environment tested, we will conduct a standard addition of a

known amount of certified toxin to evaluate variability in matrix effects of the various water quality conditions and phytoplankton populations. Freshwater test sites will include two locations within the Great Lakes, western Lake Erie and Sandusky Bay, as well as Pinto Lake and additional inland hot spots neat Santa Cruz, CA. Saltwater test sites will be conducted in both the northern Atlantic and the mid Pacific. Test sites on the east coast will be selected based on current monitoring sites within Long Island Sound established by Dr. Chris Gobler. Test sites on the west coast will be selected based on current monitoring sites within Monterey Bay and Santa Cruz wharf established by Dr. Smith and Dr. Kudela, respectively.

In the situation where Phytoxigene will be the only manufacturer participating in testing, we will modify our reference sampling to include only particulate toxin analysis for both LCMS and ELISA since they are only detecting gene presence of targeted cells and there is no direct comparison of dissolved phases of toxins (for example, in Long Island Sound, NY). This approach will improve toxin detection ability without having to pre-concentrate a whole-water sample. In addition, we will **not** include a field matrix spike involving the addition of a certified toxin standard.

Long Island Sound, NY

Field testing within Long Island Sound will be conducted over a five day period, with a goal of sampling at four different sites as determined from the active monitoring program of Dr. Chris Gobler. Monitoring data provided by Dr. Gobler will provide weekly estimates of cell abundances for up to 10 different sites for a month leading up to the testing and we will select the top four that are located within distances that can be accessed within a given sampling day. Daily sampling trips to three sites will take place from Monday to Wednesday and laboratory analysis of the reference samples by ELISA and qPCR will be conducted on Thursday and Friday. One Field Reference Duplicate and one field trip blank will be collected during sampling events as part of our QA/QC analysis. All reference samples should be immediately processed at the collection site using a common, well-mixed composited sample in a 10-20 L carboy. Given the expected low cell densities of Alexandrium (ca. 100 per liter), we anticipate filtering up to 2-4 liters of water on a 10 μM screen for both particulate toxin and qPCR analyses. The carboy should be rolled and inverted three times before each sample aliquot is distributed. Phytoxigene will conduct their field-based analysis at the last site of the day after all collections are completed. All reference samples to be processed by ACT will be stored on ice until return to the laboratory and then stored at -80 ºC until analysis at the end of the week.

Dates: May 5-12, 2018

Targeted Toxins and Species: This marine test site is being used to evaluate the ability of submitted test instruments to detect the saxitoxin producing species, *Alexandrium* spp. Reference sampling will also conduct analysis specific to the quantification of the saxitoxin compound.

Estimated Number of Samples: 11 reference samples, including 1 field duplicate and 1 field trip blank. No matrix spike will be included for this test since the test instrument will not be conducting direct toxin analysis. The dissolved toxin measurements are not applicable to the instrument being tested at this location, and therefore, will be excluded from this field site.

Participants: Only Phytoxigene will be submitting an instrument for testing at this field site.

Western Lake Erie, OH.

Two sampling efforts will be conducted within western basin of Lake Erie, spaced approximately six weeks apart to capture a range of HAB conditions. Each sampling effort will cover four stations to capture some variation in expected cell densities and toxin concentrations. Sampling and testing time may limit the effort to only two stations per day, for which we would collect the other two on the next available day.

Dates: The first sampling trip will occur between July 24-26, 2018 and the second between September 5-7, 2018.

Targeted Toxins and Species: This freshwater test site is being used to evaluate the ability of submitted test instruments to detect the toxins microcystin and cylindrospermopsin, produced by *Microcystis* and *Planktothrix* spp. However, the toxin cylindrospermopsin is unlikely to be present in detectable levels.

Estimated Number of Samples: 13 reference samples including two field duplicates, two matrix spikes (one generated each sampling event) and one field trip blank.

Participants: ALL submitted test instruments

Sandusky Bay, Lake Erie, OH.

Two sampling trips will be conducted within Sandusky Bay, Lake Erie spaced one week apart. Each trip will access two stations to capture some variation in expected cell densities and toxin concentrations.

Dates: The first sampling trip will occur between August 14-16, 2018 and the second between August 21-23, 2018.

Targeted Toxins and Species: This freshwater test site is being used to evaluate the ability of submitted test instruments to detect the toxins microcystin and cylindrospermopsin, produced by *Microcystis* and *Planktothrix* spp. However, the toxin cylindrospermopsin is unlikely to be present in detectable levels.

Estimated Number of Samples: 13 reference samples including two field duplicates, two matrix spikes (one generated each sampling event) and one field trip blank.

Participants: ALL submitted test instruments

Monterey Bay/Santa Cruz Wharf, CA.

Field testing on the west coast will involve sampling within the marine coastal waters of both Monterey Bay and Santa Cruz Wharf. Within each region, sampling effort will target two – three stations per day, with sampling on three different occasions at each region to capture some variation in expected cell densities and toxin concentrations. Specific locations for sampling will depend upon the observed presence of target HAB toxins as determined by ongoing monitoring efforts.

Dates: West coast marine test sampling will occur between the dates of September 17- October 5, 2018.

Targeted Toxins and Species: This marine test site is being used to evaluate the ability of submitted test instruments to detect the saxitoxin producing species, *Alexandrium* spp., and saxitoxins. In addition, test instruments may be submitted to test for the detection of *Pseudo-nitzschia* spp. and the toxin domoic acid.

Estimated Number of Samples: 26 reference samples including four field duplicates, two matrix spikes, and one field trip blank.

Participants: Participation will be dependent upon the readiness of new analytical procedures for these specific species and toxins. Phytoxigene will be submitting a qPCR based instrument for full testing. At the time of the protocol development workshop the other companies were not certain of the availability of these methods. This phase of Lab testing may be used to support further development of methodologies by the companies and not integrated into final performance evaluations.

Pinto Lake/Freshwater Systems, CA

Field testing within freshwater systems located near Santa Cruz, CA region will be conducted over two – three sampling events with a goal of covering approximately 10 total sample collections. Sampling will be opportunistic and based on observed conditions of HAB presence and density. We will try to select sites that provide a wide range of cell densities and toxin concentrations.

Dates: West coast freshwater test sampling will occur between the dates of September 17 - October 5, 2018. The specific locations sampled will depend upon the presence of target HAB toxins as determined by ongoing monitoring efforts.

Targeted Toxins and Species: This freshwater test site is being used to evaluate the ability of submitted test instruments to detect the toxins microcystin and cylindrospermopsin, produced by *Microcystis* and *Planktothrix* spp.

Estimated Number of Samples: 14 reference samples including 2 field duplicates and 2 matrix spikes.

Participants: ALL submitted test instruments.

5. Reference Sample Collection and Analytical Methods

Reference samples will be collected during all field and laboratory tests for direct comparison between test instruments and independently analyzed laboratory results. All samples will be processed to analyze toxin concentrations, toxin-producing genes, and phytoplankton abundance. Toxin concentrations will be determined using liquid chromatography/mass spectrometry (LCMS) and enzyme-linked immunosorbent assay (ELISA). All LCMS analysis will be performed by Dr. Raphael Kudela at University of California – Santa Cruz, while ELISA and quantitative polymerase chain reaction (qPCR) analysis will be conducted by ACT staff at each field or laboratory site. The qPCR analysis will be conducted using a common dedicated instrument across all sites. ACT staff will also perform microscopic cell counts of target HAB species on ambient samples preserved in acidified Lugol's. Reference samples will be collected from the environment using a 4 L horizontal Van Dorn sampler to collect water 1 m below the surface and will avoid inclusion of any surface scums. When larger volumes are needed for

processing, water will be composited into a common carboy for homogenization before aliquoted to each analysis.

All reference sample collections and processing will be documented on standard log sheets, completed at the time of collection. At the end of each week scanned electronic copies of the sampling logs will be sent to the Chief Scientist. Any reference sample shipped to an outside lab for analysis or left behind at a Partner testing site will be logged with a standardized Chain of Custody Form. In the case of shipped samples, the recipient will confirm the content and state of condition of the samples immediately upon receipt and acknowledge the required handling has occurred. Signed copies of the COC forms will be sent electronically to the sender and copied to the Chief Scientist.

ACT will examine matrix effects on extraction efficiency and analytical accuracy through spiked additions of certified toxin standards. Analysis will be run on the original ambient sample and the spiked sample to examine potential challenges or variation in quantification based on phytoplankton composition and ambient water quality characteristics. We will conduct a matrix spike for each unique environmental region of field testing, and for a limited number of culture lines during the lab tests. Every test site will also conduct one blank spike in clean distilled water, or saltwater if appropriate, for a given test technology. This test will be done as early as possible to confirm instruments and analysis are working properly.

For lab or field testing involving only Phytoxigene, LCMS and ELISA reference sample analysis will only be performed on filtered samples (particulate or intracellular) instead of whole water and dissolved fractions since there is no direct measurement of toxins with this qPCR technology.

5.1 Liquid Chromatography/Mass Spectrometry

Sample Collection

Samples for toxin analysis by LCMS will be collected for both whole water and the dissolved fraction. The dissolved fraction will be analyzed from a 10 ml sample filtered through 0.2 µM nylon filters into amber glass vials and stored at -80ºC. The whole water fraction will be analyzed from a 50 ml sample poured into an amber glass bottles stored at -80ºC. All vials will be rinsed two times with sample before filling. All reference samples will be collected with a duplicate holdback, and samples will be shipped in batches on dry ice to UCSC for analysis with the holdback remaining frozen at the local test site until results are QA'd and finalized.

For situations when we are analyzing for particulate (intracellular) toxins, two replicates will be produced by filtering a required volume of sample water through a 25mm, 10µm Millipore/Merck Isopore filter (TCTP02500). The volume filtered will be determined from previous monitoring results (2-4L per filter) to try to ensure reaching detection levels. Filters will be store in 2 ml polypropylene CryoVial and kept on ice until placed in a -80 °C freezer. At end of the field site test, one of the replicates from each sample will be shipped to Dr. Kudela at UCSC and one kept as hold-back at the local site in a -80 °C freezer.

Sample Analysis

In the Kudela lab, samples will be processed according to methods described in Mekebri et al. 2009, Kudela 2011, for microcystins, nodularin-R with the following modifications (Miller et al.

2010, Kudela et al 2011). Anatoxin-a and Cylindrospermopsin will be analyzed using a modified (to account for instrument differences) version of
EPA545 as described in Agilent Application Note 5991-4725EN.

Samples will be received and kept frozen until extracted and extracted samples were kept frozen until analysis LC/MS using an Agilent 6130 instrument. The established MDL based on 7x replicate analysis is 1 ug/L (on column), adjusted for sample size. Blanks will be included for every 10 samples, and a standard curves run at the beginning/end of each set of samples. A Matrix Spike recovery will be completed with each sample matrix type.

The LCMS will use the 5-6 main microcystin congeners to analyze for both dissolved and whole water fraction of toxins. Every analytical batch will include matrix additions, blanks, and standard runs. The analysis will be run in full scan mode but with lower sensitivity. It should be noted that the direct comparison of ELISA and LC/MS/MS toxin concentrations is not recommended without a conversion of the LC/MS/MS data based on cross-reactivity of the detected congeners before summing concentrations from all congeners. This effect could result in LCMS reported values being up to 40% less than ELISA results. Therefore, individual LC/MS/MS microcystin and nodularin congener concentrations will be converted from µg/L of the given congener to µg/L of microcystin-LR equivalents following the method of Loftin et al. 2008. However, we recognize that the extent to which cross-reactivity corrected LC/MS values improve the comparison to respective ELISA, will depend on the degree of potential matrix effect's with ELISA, and whether the measured LCMS toxins represent the majority of the microcystins measured by ELISA.

5.2 Enzyme-Linked Immunosorbent Assays (ELISA)

Sample Collection

Samples for toxin analysis by ELISA will be collected for both whole water and the dissolved fraction. The dissolved fraction will be analyzed from a 10 ml sample filtered through 0.2 µM nylon filters into amber glass vials and stored at -80ºC. The whole water fraction will be analyzed from 50 ml sample poured into an amber glass bottles stored at -80ºC. All vials will be rinsed two times with the sample before filling. All reference samples will be collected with a duplicate holdback which will remain frozen at the local test site until results are QA'd and finalized. ELISA analysis will be performed by ACT staff at the local test-site laboratory.

For situations when we are analyzing for particulate (intracellular) toxins, three replicates will be produced by filtering a required volume of sample water through a 25mm, 10µm Millipore/Merck Isopore filter (TCTP02500). The volume filtered will be determined from previous monitoring results (up to 2L per filter) to try to ensure reaching detection levels. Two replicates will be analyzed for each reference samples and one saved as a back-up.

Sample Analysis

ELISA analysis for microcystins will be performed according to EPA Method 546 and the Abraxis kit (catalog #520011). ELISA for particulate saxitoxin will be analyzed using the BIOO Scientific Saxitoxin (PSP) ELISA Test Kit (catalog #1034). This procedure includes a 96-well microtiter plate and competitive binding of microcystins and microcystin-protein analogues within the wells. The ELISA method employs reagent blanks, calibration standards, fortified

blanks, and fortified sample matrix and duplicates. Each extract will be sub-sampled into two wells on the plate for analytical dups. So each reference samples will use 4 wells.

To extract the filters, remove from freezer and allow warming to room temperature. Add 3 ml of 50% MeOH and sonicate with a probe at 10W power for about 30 seconds. Filter should be ground into a milkshake like consistency. If longer sonication is needed take care not to warm the sample by using an ice bath. Pour sonicated sample into a 5ml Luer lock syringe with a 0.2 um nylon syringe unit and filter extracted into a new clean glass vial. Per the Bioo kit manual, transfer 100 µL of clear filtrate into a new tube, then add 1.9 mls of 1x sample extraction buffer/methanol and mix well. Use 50 µL of final extract for the assay.

5.3 Quantitative Polymerase Chain Reaction (qPCR)

Sample Collection

Filter three replicates using a 25mm, 10µm Millipore/Merck Isopore filter (TCTP02500) for the saltwater collections. For freshwater test sites for cyanobacteria use a 25mm, 2 µM pore size filter to collect the triplicates. Volume filtered will be determined from previous monitoring results (2-4 L per filter). Store filter in 2ml polypropylene CryoVial; keep on ice until placed in a -80 °C freezer. At the end of sample collection we will extract and analyze **two** of the filters and keep one as a hold-back to reanalyze if needed. Each extract will be sub-sampled into two wells on the plate for analytical dups.

Sample Analysis

Quantitative polymerase chain reaction (qPCR) analysis will be performed on all samples according to established standard methods using a Qiagen DNeasy Blood and Tissue kit. During qPCR, DNA is extracted from cells, and genes of interest are detected, amplified, and quantified. ACT's qPCR analysis will focus on genes coding phytoplankton toxin production. Triplicate samples will be collected for each reference point, with two replicates being immediately analyzed and one saved as back-ups. Two analytical replicates will be conducted per filter extract, so each reference samples will use 4 wells..

5.4 Cell Counts

Phytoplankton cell abundances will be quantified for each reference sample to determine relative abundance of target species. Saltwater sample counts will focus on dinoflagellates and diatoms, while freshwater sample counts will focus on cyanobacteria. These data will build upon the 16S rRNA analysis performed by qPCR.

For the cell counts, whole water samples (480 mls) will be fixed with20 mls of acidified Lugol's for a final preservative concentration of 4% (v/v). Cell abundance of target species will be enumerated microscopically after concentrating as necessary by settling or gentle centrifugation (3000 rpm, 10 min).

Acid-Lugol recipe: In a fume hood, dissolve, 100 g of KI and 50 g of I_2 in approximately 800 mL of reagent water in a 1-L volumetric flask. Mix until the chemicals are completely dissolved. Add 100 mL of glacial acetic acid and bring volume up to 1 L with reagent water.

5.5 Ancillary Measurements

In addition to reference sample analysis, site-specific conditions will be recorded with a multi-parameter YSI EXO 2 sonde during each field and laboratory test. The EXO2 sonde will be calibrated prior to use at each site and collect water quality characterization for temperature, conductivity/salinity, dissolved oxygen, pH, turbidity, fDOM, and pigment fluorescence during reference sample collection. For Long Island, we will use Dr. Gobler's calibrated hand-held YSI sondes.

6. 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 Performance Verification 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.

6.1 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. Field trip blanks will be collected at approximately 10% of the sampling points 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 b conducted at each field test spaced evenly throughout the deployment.
- Field Duplicates: we will collect two field reference samples simultaneously for approximately 20% of the sampling points to examine relative precision of the test instruments and to establish an expected level of analytical variability for each of the reference sample analysis. Variability will also include fine-scale spatial heterogeneity within the environment as well as sampling handling processes.

6.2 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 Algal Toxin Detection Verification 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.

7. 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.

Verification 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 upon.
- Provide all materials, supplies and equipment needed to setup, calibrate, deploy, operate, maintain and recover test instruments.

The Technical Advisory Committee will:

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

8. Summary of Overall Schedule

Dates	Event
May 2, 2018	Final Verification protocols and ACT Verification Contract sent to manufacturers
May 7, 2018	Signed contracts due back to ACT headquarters
May 7-25, 2018	Field Test 1 at Long Island
July 9-10, 2018	ACT personnel and manufacturer representatives meet at Bowling Green State University for training
July 11-15, 2018	Freshwater Laboratory Test at Bowling Green State University
July 25-26, 2018	Field Tests 2 at Western Lake Erie
Aug 14-16, 2018	Field Tests 3 at Sandusky Bay

Aug 21-23, 2018	Field Tests 4 at Sandusky Bay
Sept 5-7, 2018	Field Tests 5 at Western Lake Erie
Sept 1-7, 2018	Saltwater Laboratory Test at Moss Landing Marine Laboratory
Sept 8-30, 2018	Field Test 6 Marine testing at Monterey Bay and Santa Cruz Wharf…
Sept 8-30, 2018	Field Test 6 Freshwater testing at Santa Cruz
December 2018	Data Review
March 2019	Draft reports to TAC
April 2019	Report to companies
May 2019	Final Reports published on ACT website

9. Technical Advisory Committee

- Dr. Joel Allen, EPA
- Dr. Dianne Greenfield, USC
- Dr. Meredith Howard, SCCWRP
- Dr. Keith Loftin, USGS

10. References

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