INTRODUCTION

Domoic acid (DA) is a potent toxin produced by bloom-forming phytoplankton in the genus Pseudo-nitzschia (Fig. 1). It is a glutamate analog, which acts as a potent excitatory neurotransmitter and causes amnesic shellfish poisoning (ASP) in humans. ASP symptoms include vomiting, diarrhea, and in more severe cases confusion, loss of memory, disorientation, and even coma or death. This paper describes the development and validation of a rapid, sensitive enzyme linked immunosorbent assay test kit for detecting DA using a monoclonal antibody. The assay gives equivalent results to those obtained using standard high performance liquid chromatography, fluoronylhemoxycarbonyl high performance liquid chromatography, or liquid chromatography—mass spectrometry methods. It has a linear range from 0.1–3 ppb and was used successfully to measure DA in razor clams, mussels, scallops, and phytoplankton. The assay requires approximately 1.5 h to complete and has a standard 96-well format where each strip of eight wells is removable and can be stored at 4°C until needed. The first two wells of each strip serve as an internal control eliminating the need to run a standard curve. This allows as few as 3 or as many as 36 duplicate samples to be run at a time enabling real-time sample processing and limiting degradation of DA, which can occur during storage. There was minimal cross-reactivity in this assay with glutamine, glutamic acid, kainic acid, epi- or iso-DA. This accurate, rapid, cost-effective, assay offers environmental managers and public health officials an effective tool for monitoring DA concentrations in environment samples.

KEY WORDS: ASP, domoic acid poisoning, ELISA, mussels, scallops, razor clams, test kit

RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID

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ABSTRACT

Domoic acid (DA) is a potent toxin produced by bloom-forming phytoplankton in the genus Pseudo-nitzschia, which is responsible for causing amnesic shellfish poisoning (ASP) in humans. ASP symptoms include vomiting, diarrhea, and in more severe cases confusion, loss of memory, disorientation, and even coma or death. This paper describes the development and validation of a rapid, sensitive enzyme linked immunosorbent assay test kit for detecting DA using a monoclonal antibody. The assay gives equivalent results to those obtained using standard high performance liquid chromatography, fluoronylhemoxycarbonyl high performance liquid chromatography, or liquid chromatography—mass spectrometry methods. It has a linear range from 0.1–3 ppb and was used successfully to measure DA in razor clams, mussels, scallops, and phytoplankton. The assay requires approximately 1.5 h to complete and has a standard 96-well format where each strip of eight wells is removable and can be stored at 4°C until needed. The first two wells of each strip serve as an internal control eliminating the need to run a standard curve. This allows as few as 3 or as many as 36 duplicate samples to be run at a time enabling real-time sample processing and limiting degradation of DA, which can occur during storage. There was minimal cross-reactivity in this assay with glutamine, glutamic acid, kainic acid, epi- or iso-DA. This accurate, rapid, cost-effective, assay offers environmental managers and public health officials an effective tool for monitoring DA concentrations in environment samples.

ASP, domoic acid poisoning, ELISA, mussels, scallops, razor clams, test kit
by centralized state facilities with results typically not available for 3–14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, WA State Department of Health, pers. comm.). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples. This paper describes the development and optimization of a robust monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) test kit for DA that will meet management needs for rapid detection of DA in environmental samples.

MATERIALS AND METHODS

Assay Kit Overview

The DA assay kit was developed jointly by NOAA’s National Centers for Coastal Ocean Science, National Ocean Service, and the Northwest Fisheries Science Center, together with an industry partner Mercury Science, Inc., Durham, NC (NOAA/MSI). It was designed as a sequential competitive enzyme linked immunosorbent assay (ELISA) utilizing a high avidity monoclonal antibody (mAb) to DA to ensure assay specificity and consistency across production lots. In the current format, a fixed number of anti-DA mAb binding sites are incubated with dissolved DA in the sample followed by the addition of a DA—horseradish peroxidase (HRP) conjugate. As these binding events occur, the anti-DA mAb molecules are simultaneously captured by antimouse antibodies affixed to the surface of the microtiter plate wells. Subsequent HRP derived color development, readable on standard microplate readers, was inversely proportional to the concentration of DA in the sample matrix. The assay reagents were titrated so that the amount of mAb and the DA–HRP conjugate added produced a maximal absorbance signal of 3 absorbance units when no DA was present. The implementation of this ELISA system required the development and validation of two essential reagents, a high avidity monoclonal antibody to DA and a stable DA-HRP conjugate recognized by the same mAb.

Production of the Anti-Domoic Acid Antibody

Domoic acid (Sigma-Aldrich, St. Louis, MO), was conjugated with bovine serum albumin (BSA) using dicyclohexyl carbodiimide and N-hydroxysuccinimide by a two-step synthetic pathway (Adamczyk et al. 1994). Ten mice were immunized with the DA-BSA immunogen. Serum titers were determined five days after each boost. A fusion was performed on the three mice that showed the greatest response. Hybridoma cell lines and monoclonal antibody production was performed according to the method of Fenderson et al. (1984). The 10 clones with highest affinity mAbs were selected for further growth and their affinity to DA was compared. The most sensitive clone was ultimately selected as the primary mAb for use in the assay development.

DA-HRP Conjugate

Domoic acid (Sigma) was cross-linked to horseradish peroxidase (HRP) using the procedure of Yoon et al. (1993). The reagent was tested for stability and was used to screen for high affinity mAbs after the fusion and for assay development.
**Domoic Acid Standards**

The DA standards used to calibrate the assay were purchased from the Certified Reference Materials Program at the National Research Council of Canada Institute for Marine Biosciences (Halifax, Nova Scotia, Canada).

**Assay Calibration**

A series of dose response curves using varying amounts of antibody and DA-HRP were performed to optimize the assay sensitivity. The optimal assay conditions were found to have an effective linear range from approximately 0.1–3.0 ppb. These conditions were used in all the subsequent phases of assay development. The antibody was also tested for cross-reactivity with varying concentrations of kainic acid, glutamine and glutamic acid. These compounds are structurally similar to various portions of DA molecule and have the potential to cross-react with anti-DA mAbs. Glutamine and glutamic acid, in particular, are common in animal tissues, including shellfish.

**Calculation of the Parameters Needed to Construct an Internal Domoic Acid Standard for Each Well Strip**

Using the optimized DA assay, multiple dose response curves were made using the NRC standards diluted to between 0 and 10 ppb (1–10 ng mL$^{-1}$) in the assay reaction buffer. The average response derived from each of the individual response curves was calculated and a dose response curve was generated using a four parameter logit-log curve fitting analysis (Ritchie et al. 1981; Fig. 2). Four parameters were derived from this analysis. This first was $B_o$, the maximal signal, which occurred when no sample DA was present (Fig. 3A). The second was $B$, the signal produced by a known amount of sample DA. The third was the slope of the logistic transformed data [proportional to the linear portion of the sigmoidal curve describing the relationship between the ln sample DA concentration versus signal ($B_i$)]. And the fourth was $ED_{50}$, the DA concentration at the mid point of the slope curve where half the available anti-DA mAbs in the well are bound to DA-HRP (Fig. 3A). Because the concentration ratio of anti-DA antibody and DA–HRP conjugates are standardized within reagent lots, the kinetics of the reaction were fixed between assay runs (assuming constant temperature), such that the slope and $ED_{50}$ values remain constant. This made it possible to calculate DA concentrations using the four parameter model.

$$DA\ concentration = \frac{ED_{50}[(B_o/B) - 1]}{\text{slope}}$$

Because the slope and $ED_{50}$ are constants, all that was needed to calculate the DA concentrations was an accurate $B_o$ and the $B$ estimates from individual samples. In the assay, the mean value for $B_o$ for each strip of wells was determined by adding sample dilution buffer lacking DA to the first two wells in that strip. Duplicate aliquots from each of three extracted samples diluted with sample buffer were then added to the six remaining wells to obtain the $B$ values. Duplicates were run to ensure assay replicability. It should be noted that $B_o$ (the maximal value with no DA added) can have noticeable variation between assays depending on differences in temperature and development time as shown in Figure 2A. However, when the $B$ values for each strip are divided by $B_o$, the kinetics of the curve become normalized (i.e., replicable between strips and between runs) (see Fig. 2B). In this way the average $B_o$ values serves as an internal standard that can be used in place of a standard curve provided the variation in the $B_o$ is not above or below certain limits, which are specified in the calculation software described later.

**Domoic Acid ELISA Test Kit Procedure**

The 96-well assay tray used in the assay contained 12 strips. Each strip of 8 wells could be removed and stored until it was needed. The first two wells of each strip were used as a control (no DA added). The remaining six wells were used to analyze three samples in duplicate. This format provided the flexibility of running anywhere from 3–36 duplicate samples at a time. For unknown sample analysis, extracts were diluted to a final concentration ranging from 0.3–3 to ppb using the sample buffer (phosphate salt solution, pH 7.8, containing casein). For
clam tissues containing DA, sample dilutions of 1:50 and 1:1000 were typically used. Preliminary tests with razor clam extracts showed that a 25-fold dilution in sample dilution buffer eliminated matrix effects in ELISA analysis.

The assay was initiated by adding 50 μL of the anti-DA antibody to each well using a multi channel pipettor. Next, 50 μL of the control solution (sample buffer without DA) was added to the first two wells in each row. Duplicate 50 μL aliquots from the diluted DA extracts were then added to the remaining wells in each strip and the plate incubated at room temperature for 30 min on an orbital shaker to vigorously mix the solution in each well (PerkinElmer Waltham, MA 1296–004 DELFIA Plateshake set on high). Vigorous mixing is key to obtaining replicable results from one run to the next. In this step, the bulk of the native DA will bind to available mAbs in proportion to the DA concentration. At the end of the incubation, 50 μL of DA HRP conjugate was added to each well and the plate incubated a second time for 30 min at room temperature on an orbital shaker. The DA-HRP will then bind to remaining available mAb sites. After the incubation, the plate was washed three times with wash solution [Tris-HCl buffered salt solution (pH 7.8) containing Tween 20 and sodium azide as a preservative] using a commercial plate washer, making certain the fluid was completely aspirated from all the wells. Alternatively, these washes can be done manually by adding wash solution to wells using a multichannel pipettor and then flicking all fluid from the wells. The manual method may result in slightly higher variability. Next, 100 μL of K-Blue TMB substrate (5.5′-tetramethylbenzidine, Neogen Corporation, Lexington, KY) was added to each well. The plate was placed on an orbital shaker for no more than 5 min, or until adequate color development was observed. Color development was terminated by adding 100 μL stop solution (1N hydrochloric acid) to each well. The absorbance in each well was measured at 450 nm using a Thermo Ascent MultiSkan plate reader (Thermo Scientific, Waltham, MA). The DA concentrations were determined using the sample (B) and control (Bo) absorbances, the original tissue weights, and the volume of 20% or 50% methanol used to extract each sample. The actual calculations were made using a Microsoft Excel work sheet (Microsoft Corporation, Redmond, WA), which incorporates the constants for the four parameter model described above. This worksheet can be downloaded from Stewart (2008). Processing time for this assay was ~1.5 h.

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**Routine Tissue Extraction**

In the case of razor clams and scallops, pooled samples of 10–12 individual shellfish were cleaned, and ground to a smooth and uniform homogenate in a commercial blender (Waring model HGBSS56, Torrington, CT). Clams were pooled because previous studies of DA in razor clams from the Washington coast indicated that the coefficient of variation for DA between clams in a population exceeded 100% (Wekell et al. 2002). If the homogenate appeared to be forming a gel caused by unusually high lipid content, an equal weight of water was added and the dilution noted. Approximately 2 g of homogenized tissue were added to a tared 50 mL conical tube and the weight recorded to the nearest 0.01 g. Next, 18 mL of 50% methanol were added and the samples mixed at high speed on a vortex mixer for 2 min. Once the extraction was completed the tubes were spun in a table top centrifuge for 20 min at 10,000 × g or until a tight pellet and clear supernatant were obtained. If the samples did not clear despite the spinning at high speed, the supernatant was poured into a syringe, then passed through a 0.45 μm Millex HA syringe filter (Millipore, Billerica, MA) to remove proteins and other compounds that can form micelles, whereas soluble DA remained in the filtrate. At this point the homogenate was ready for analysis by ELISA and HPLC. If necessary, the sample was stored at 4°C for up to 24 h in an explosion proof refrigerator prior to analysis.
**Phytoplankton Extraction**

Approximately 0.1–1.0 L of cultured cells or sea water samples were filtered onto a GF/F filter, which was immediately frozen at -80°C until the filter could be processed. For processing, the filter was placed in a 5 mL conical BD Falcon Tube (Becton Dickinson, Franklin Lakes, NJ) and 3 mL of 20% methanol were added. The samples were then sonicated using a Thermo Fisher Scientific Model 100 Sonic Dismembrator with a 1/8 inch probe (model 15-338-80, Fisher Scientific, Waltham, MA) until the filter was completely homogenized. Care was taken to prevent the probe from rupturing the tube. The sonicator probe was cleaned very carefully with 20% methanol between samples to prevent cross-contamination. Next the homogenate was centrifuged at 3000 g for 10 min. The supernatant was then passed through a disposable Whatman GD/X 0.2 µm syringe filter (Florham Park, NJ) into a 5 mL tube. At this point the sample was split for analysis using both the ELISA and HPLC assays.

**HPLC Validation of DA concentration from Razor Clam Tissues**

HPLC is the accepted standard method for measuring DA and is the basis of the current official method for regulatory action in the U.S. (AOAC Official Method 991.26). The lower detection level for the standard assay is ~0.5 ppm. This technique was used to validate the DA concentration in the razor clams in this study. Briefly, 10–15 mL of the clarified supernatant prepared as described above was transferred into a 25 mL disposable plastic syringe and filtered through 0.45 micron HA Millipore filter (Bedford, MA) until the filter was completely homogenized. Care was taken to prevent the probe from rupturing the tube. The filter was placed in a 5 mL conical BD Falcon Tube (Becton Dickinson, Franklin Lakes, NJ) and 3 mL of 20% methanol were added. The samples were then sonicated using a Thermo Fisher Scientific Model 100 Sonic Dismembrator with a 1/8 inch probe (model 15-338-80, Fisher Scientific, Waltham, MA) until the filter was completely homogenized. Care was taken to prevent the probe from rupturing the tube. The sonicator probe was cleaned very carefully with 20% methanol between samples to prevent cross-contamination. Next the homogenate was centrifuged at 3000 g for 10 min. The supernatant was then passed through a disposable Whatman GD/X 0.2 µm syringe filter (Florham Park, NJ) into a 5 mL tube. At this point the sample was split for analysis using both the ELISA and HPLC assays.

**HPLC Detection of Domoic Acid in Phytoplankton Using Fluorenylmethoxycarbonyl (FMOC) Derivatization**

A more sensitive fluorescent fluorenylmethoxycarbonyl chloride (FMOC) derivatization method (Pocklington et al. 1990) was used to determine particulate DA concentrations in phytoplankton samples, which typically contained less DA than shellfish tissues. The samples were processed on a Hewlett-Packard 1090 HPLC using a Vydac 201TP, 5 µm, 25 cm column, HP 1046A fluorescence detector, and column heater set to 40°C with the following modification. In our analysis, solvents A (HPLC Water with 0.1% v/v TFA) and B (acetonitrile with 0.1% v/v TFA) were pumped at 0.2 mL/min and the linear gradient elution was changed allowing for increased separation and resolution of the domoic acid peak. The initial gradient went from 70% A and 30% B at time of injection to

<table>
<thead>
<tr>
<th>Organism</th>
<th>Combined epi and iso-DA by HPLC (ppb)</th>
<th>DA Concentration by NOAA ELISA (ppb)</th>
<th>% Total DA Detected by NOAA ELISA</th>
<th>DA Concentration by Biosense ELISA (ppb)</th>
<th>% Total DA Detected by Biosense ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chthamalus fissus/dalli</td>
<td>281.7</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Chthamalus fissus/dalli</td>
<td>1,137.1</td>
<td>15.41</td>
<td>1.36</td>
<td>1.53</td>
<td>0.13</td>
</tr>
<tr>
<td>Littorina scutulata</td>
<td>198.7</td>
<td>10.57</td>
<td>5.32</td>
<td>3.02</td>
<td>1.52</td>
</tr>
<tr>
<td>Littorina scutulata</td>
<td>682.0</td>
<td>15.98</td>
<td>2.34</td>
<td>1.02</td>
<td>0.15</td>
</tr>
<tr>
<td>Littorina scutulata</td>
<td>119.5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.17</td>
<td>0.14</td>
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<tr>
<td>Lottia digitalis</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.04</td>
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<tr>
<td>Lottia digitalis</td>
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<td>2.91</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Lottia digitalis</td>
<td>390.6</td>
<td>10.31</td>
<td>2.64</td>
<td>0.78</td>
<td>0.20</td>
</tr>
</tbody>
</table>
60% A and 40% B over 0–10 min, then held constant for 10 min; adjusted to 0% A and 100% B from 20–30 min, held isocratic for 2 min; adjusted from 0% A and 100% B to 70% A and 30% B over 2 min, and then held constant at these (initial) conditions until the end of the run at 45 min. Dihydrokainic acid was used as an internal standard, as described by Pocklington et al. (1990).

A subset of phytoplankton samples was validated to confirm the presence of DA (by mass) using liquid chromatography-mass spectrometry (LC-MS) on a ThermoFinnigan Quantum Discovery Max TSQ ESI Mass Spectrometer coupled to a HP 1100 series binary pump HPLC, following the general protocol of Quilliam et al. (1989a). Samples for LC-MS were prepared as for HPLC, but were then dried down under vacuum and redissolved in 100% methanol prior to injection. The HPLC conditions for the reverse phase were programmed for a linear gradient elution of 10:90% acetonitrile:deionized water (both containing 0.1% formic acid) up to 0:100% water:acetonitrile over 30 min.

Testing Cross-Reactivity of the ELISA Against Glutamine, Kainic Acid and Putative Isomers Epi-DA and Iso-DA

Domoic acid is structurally similar to glutamine, glutamic acid and kainic acid, all of which can potentially co-occur with DA in sample extracts (Fig. 1). To test for potential cross-reactivity with these compounds, the NOAA/MSI ELISA kit was run using concentrations of glutamine, glutamic acid and kainic acid ranging from 10 ppb to 5 ppm. The ED_{50} for each compound was calculated and then divided by ED_{50} for DA and multiplied by 100 to determine percent cross-reactivity (Table 2). A majority of DA in razor clams and phytoplankton is in the form shown at the top of Figure 1. However, samples sometimes contain a larger quantity of compounds closely eluting with DA on standard HPLC runs that have been identified as the DA conformers epi- and iso-DA (Wright et al. 1990, Kotaki et al. 2005). To determine if the mAb used in this assay could detect these DA isomers, and the extent of interference by such coeluting compounds present in crude extracts of intertidal barnacle, limpet, and snail samples, crude methanolic extracts of these tissues were assays using HPLC-UV and both the NOAA/MSI and Biosense (Biosense Laboratories, Bergen, Norway) ELISA methods. These intertidal invertebrate extracts exhibited high levels of the putative epi-DA and iso-DA isomers as called by comigration on HPLC chromatograms. These compounds are generally near detection limits in razor clams, crabs, and to a lesser extent in mussels, and therefore these extracts provided novel matrices for evaluating the accuracy of NOAA/MSI ELISA.

TABLE 2.

Cross-reactivity of the NOAA/MSI ELISA with kainic acid, glutamine, and glutamic acid.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Reactivity in the Domoic Acid Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domoic acid</td>
<td>100</td>
</tr>
<tr>
<td>Kainic acid</td>
<td>0.3</td>
</tr>
<tr>
<td>Glutamine</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Data Analyses

Analytical results for DA concentrations determined from razor clams, mussels, scallops and phytoplankton cells determined by HPLC, FMOC-HPLC, LC-MS and the NOAA/MSI ELISA were compared using linear regression analysis (Sokal & Rohlf 1995). The performance of the NOAA/MSI and Biosense ELISA kits was also compared using a subset of the phytoplankton samples. This comparison involved simultaneously analyzing phytoplankton extracts using the two kits and comparing the results with those obtained using FMOC-HPLC. All samples were run within a 24 h period to prevent differential degradation of DA, which may occur in some samples. Data were compared using linear regression analysis.

RESULTS AND DISCUSSION

The NOAA/MSI ELISA accurately measured NRC standard DA concentrations (Fig. 4) and gave equivalent results for razor clam (Fig. 5), mussel (Fig. 6), scallop (Fig. 7), and phytoplankton extracts (Fig. 8) as obtained when using HPLC, FMOC-HPLC, or LC-MS methods. When the variability in the NOAA/MSI ELISA and FMOC-HPLC method were compared using replicate phytoplankton extracts they were found to be comparable (Fig. 9). The primary advantage of the NOAA/MSI ELISA over HPLC methods, besides a significantly lower cost per sample was much higher throughput. As many as 36 samples can be completed in <1.5 h after tissue extraction.

The NOAA/MSI format was also flexible. An internal control was incorporated into each strip, which eliminated the necessity of running a standard curve each time the assay was performed. Any unused strips could be removed and stored in a desiccator pouch at 4°C for at least six months without compromising assay performance. This allowed as few as 3 samples to be run in real time thereby avoiding the degradation of DA that can occur during storage, particularly once the samples have been extracted (Smith et al. 2006). For example, when phytoplankton samples were run within 24 h using the Biosense ELISA kit, which has been validated by an international collaborative study, and is officially approved by the AOAC International for regulatory detection of DA in shellfish,
and the NOAA/MSI ELISA kit, equivalent results were obtained (Fig. 10, \(r^2 = 0.97\)). In contrast, when samples were run two weeks apart the correlation dropped to \(r^2 = 0.79\), indicating DA degradation.

The ability to efficiently run a small number of samples in real time was not incorporated into other DA ELISA formats. For example, the Biosense DA ELISA kit includes reagents for only two standard curves (product insert), therefore, only two batches of samples can be run per kit. This means that when small numbers of samples are being collected, they may have to be stored until a sufficient number of samples have been accumulated to maximize the number of samples per kit. This could lead to sample degradation and a critical delay in reporting when samples surpass the regulatory limit of 20 ppm.

Another advantage of the NOAA/MSI assay is that it could be run in either a quantitative or screening mode when assaying shellfish tissues. For quantitative analysis, several dilutions were assayed simultaneously to obtain an accurate DA concentration.
Alternatively, to rapidly screen for DA concentrations of concern, the sample extracts were diluted 1:1,000 before running the assay. Taking into account the 1:10 dilution that occurred during the extraction process, the 1:1,000 dilution reduced samples in the 20 ppm DA range to 2 ppb in the diluted sample. This concentration was within the linear range of the assay (0.1–3 ppb). Tissue samples with 5–10 fold less DA, and far below levels of concern, would show no detectible DA at this dilution. Tissues containing initial DA concentrations >30 ppm would be off scale and indicate a significant DA concentration requiring action. Any samples from this rapid screening that were of concern could then be diluted and run again to obtain an accurate concentration. The NOAA/MSI ELISA test kit also comes with a simple Excel spreadsheet, which allowed the toxin concentrations to be quickly and easily calculated in either a quantitative or rapid screening mode. All that had to be entered was the Bo (no DA added) and sample absorbance data from each strip, the weight of the extracted tissue samples, and the extraction volumes.

The NOAA/MSI and Biosense ELISA kits were tested against crude methanolic extracts of several intertidal invertebrates, which HPLC identified as containing >100 ppb levels of epi-DA and iso-DA. These compounds are reported to be less toxic DA congeners based on receptor binding assays (Sawant et al. 2007). Results from both ELISA kits revealed the presence of only trace amounts of DA equivalents in the extracts. The NOAA/MSI ELISA cross-reactivity with these compounds ranged from 0% to 5.3% and the Biosense ELISA cross-reactivity from 0.01% to 1.5% (Table 1) indicating that the ELISA assays are relatively insensitive to cogener interference. It should also be noted that the regulatory methods for assessing human safety are currently based on measuring DA alone, not the combination of DA, iso-DA and epi-DA. These results indicated that both the NOAA/MSI and Biosense
ELISA methods provide DA values comparable to the HPLC values currently used as a basis for regulatory decisions.

Measuring low concentrations of DA in real time is particularly important because the presence or absence of DA contamination is frequently patchy and associated with variable onshore transport of toxic phytoplankton blooms (e.g., Trainer et al. 2002). Depending on prevailing winds and currents, one harvest area can become highly contaminated over a short period whereas adjacent regions remain uncontaminated (Trainer et al. 2000). These differentially affected regions frequently include areas where significant commercial and recreational clam harvests occur. This variability complicates monitoring programs designed to protect human health. The current standard practice involves shipping shellfish samples to a centralized facility for HPLC analyses, introducing delays between 3–14 days from the date of sample collection to reporting results. This turnaround time is too slow to adequately protect subsistence shellfish harvesters who rely on clams consumed within a day or two of harvest. The cost of HPLC analysis is also relatively high per sample and requires a substantially higher capital investment compared with the NOAA/MSI ELISA method. Having an economical technique for better assessing the degree of contamination locally, and in real time, is of great value for local resource managers and public health officials.

The ability to detect DA in phytoplankton using the NOAA/MSI kits would further benefit environmental monitoring programs designed to detect the early onset of toxic *Pseudo-nitzschia* blooms. It is known that increases in the *Pseudo-nitzschia* capable of producing DA often precedes the contamination of shellfish and other filter feeders by a week or two (Trainer & Suddleson 2005). A combination of cell counts and direct toxicity measurements should provide timely predictions for marine resource managers and public health officials. The kit is now commercially available with MSI authorized to market, manufacture and distribute the 96-well plate format test kits. We anticipate completing the necessary validation procedures to qualify the 96 well plate format for regulatory use by public health officials. We are also developing a field test kit that can be used to detect DA levels in shellfish tissues above or below 20 ppm within 10 min after extraction. The test will require no laboratory equipment other than a homogenizer and can be used directly in the field by non-technical personnel, including shellfish harvesters and members of citizen monitoring groups and local volunteers.

In summary, the NOAA/MSI ELISA test kit provides an accurate, flexible and cost effective method for measuring DA in clam, mussel and scallop tissues, as well as in phytoplankton samples. The assay yields concentrations for DA that are indistinguishable from those obtained by HPLC. With further validation, the NOAA/MSI ELISA kit is expected to be approved as a regulatory method for making decisions concerning public health. The short assay (1.5-h) processing time, and relatively low cost, compared with HPLC analysis, mean that the ELISA can be used in more remote locations by environmental managers and public health officials to provide near real-time monitoring capacities.

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**LITERATURE CITED**


