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Quantification of saxitoxin in human blood by ELISA

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Abstract

Saxitoxin (STX) is a potent marine toxin that causes paralytic shellfish poisoning (PSP) which can result in significant morbidity and mortality in humans. Low lethal doses, rapid onset of PSP symptoms, and brief STX half-life *in vivo* require sensitive and rapid diagnostic techniques to monitor human exposures. Our laboratory has validated an enzyme-linked immunosorbent assay (ELISA) for quantitative detection of STX from 0.020 to 0.80 ng/mL in human whole blood and from 0.06 to 2.0 ng/mL in dried human blood which is simple, sensitive, rapid, and cost-effective. To our knowledge, this is the first validated method for the quantitation of saxitoxin in whole blood. Microsampling devices were used in sample collection which allows for standardized collection of blood, stable storage, and cost-efficient shipping. Quality control precision and accuracy were evaluated over the course of validation and were within 20% of theoretical concentrations. No detectable background concentrations of STX were found among fifty whole blood and dried blood convenience samples. Additionally, ten spiked individual whole blood and dried blood samples were tested for accuracy and precision and were within 20% of theoretical concentrations. Gonyautoxins 2&3 (GTX2&3) cross-reacted with this ELISA by 21%, but all other structurally related PSP toxins tested cross-reacted less than two percent. While clinical diagnosis or treatment of PSP would be unaffected by GTX2&3 cross-reactivity by ELISA, to accurately quantify individual PSP toxins, these results should be coupled with high performance liquid chromatography mass spectrometry measurements.

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Ethical statement

This paper represents experiments carried out under the standard procedures of scientific ethics at the Centers for Disease Control and Prevention. All authors have read the manuscript, agree to its publication in *Toxicon*, and agree that it has followed the rules of ethics presented by Elsevier's Ethical Guidelines for Journal Publication. The material beins submitted has not, in whole or in part, been published elsewhere, nor is it being considered concurrently by any other publishers. In addition, all authors have been personally and actively involved in a substantive role leading to the publishing of this article.

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Keywords

Saxitoxin; ELISA; Dried blood; Microsampling devices

1. Introduction

Saxitoxin (STX) is a potent member of the paralytic shellfish poisoning (PSP) class of toxins which are produced by certain species of marine dinoflagellates and freshwater cyanobacteria (Cusick and Sayler, 2013; Maki et al., 2010). Shellfish bioaccumulate STX during red tide blooms; human exposures to STX typically occur from eating contaminated shellfish (Huot et al., 1989). Once ingested, STX binds to and blocks the pores of voltage-gated sodium channels, inhibits ion conductance across the plasma membrane of neurons and muscle cells, and results in PSP (Cusick and Sayler, 2013). Symptoms of PSP usually occur within 2–12 h post-consumption of contaminated meat and range from tingling or numbness to paralysis, respiratory distress, and circulatory failure (Ainsbury et al., 2011). STX is categorized as a Schedule 1 chemical warfare agent by the Chemical Weapons Convention of 1993 (Gad, 2014) and is on the U.S. federal Select Agent list (2005). LD_{50} values in mice range from 8 to 263 μg/kg depending on the route of exposure, and the estimated lethal dose in humans is 0.3–1 mg (Gad, 2014). Despite monitoring programs which ban harvesting seafood with an excess of 80 μg STX equivalents/100 g of tissue, there are an estimated 2000 worldwide human cases of PSP reported yearly, with a mortality rate of 15% (Etheridge, 2010; Van Dolah, 2000). Limited case reports suggest that the half-life of STX is less than 10 h in serum and 20.4 h in urine (DeGrasse et al., 2014; Gessner et al., 1997). Because of its potency and threat to human health along with its short-half life *in vivo*, there is a need for rapid and sensitive diagnostic techniques to monitor human exposures and confirm diagnoses.

Several methods are currently available for the detection of STX. The most common method is the mouse bioassay which measures the presence of active toxin by injecting mice with extracts from contaminated shellfish and monitoring the mortality rate. This method has several drawbacks including the use of live mice, high detection limit (40 μg STX/100 g shellfish), variable quantitative results, inability to distinguish between PSP toxins, and high labor and assay costs (Humpage et al., 2010). More specific methods for STX detection include high performance liquid chromatography fluorescence (HPLC-FL) and HPLC-MS. These methods overcome several of the drawbacks of the mouse bioassay such as sensitivity and selectivity (Bragg et al., 2015; Humpage et al., 2010; Oshima, 1995); however they require expensive equipment and specialized training (Humpage et al., 2010).

Biochemical assays also have many advantages over the mouse bioassay. Immunoassays encompass receptor-binding assays which exploit the ability of cellular receptors to bind an analyte of interest and enzyme-linked immunosorbent assays (ELISAs) which use antibody interactions with their target and cross-reactive antigens for detection. The receptor binding assay for STX is a rapid, reproducible, and more cost-effective means for detection of STX than the mouse bioassay, but can only detect down to 5 ng/mL, relies on rats for the source of sodium channels, uses radioactive materials, and cannot distinguish between PSP toxins

(Doucette et al., 1997). In contrast, ELISAs are highly sensitive, cost-effective, rapid, and require only minimal training to perform (Ainsbury et al., 2011; Reverte et al., 2014). Because ELISAs use pattern recognition of epitopes for detection, they may have the ability to detect multiple toxin analogs or derivatives if a common recognition site is shared (Reverte et al., 2014).

Currently, several commercial STX ELISA kits marketed for qualitative or quantitative detection of STX in fresh and brackish water samples as well as shellfish extracts can quantitate STX as low as 0.02 ng/mL, making them extremely sensitive tests (Sato et al., 2014). The kit used in this study utilizes polyclonal antibodies which recognize STX in a direct competitive ELISA format for detection. The method presented here evaluated this ELISA kit for the detection of STX in human whole blood and dried blood on microsampling devices. Whole blood allows for rapid analysis of samples while dried blood is advantageous since it is stable at room temperature and reduces shipping costs compared to cold chain transport of whole blood or blood products. Microsampling devices bypass hematocrit biases in quantitation that can be problematic in dried blood spot subpunches and are compatible with automated processes which would limit human error during analysis (Spooner et al., 2015). The net benefit of the ELISA presented here is a method that is accurate, rapid, cost-efficient, and sensitive which would complement HPLC-MS/MS analysis as a pre-screening measure for specific quantitation of STX in human blood.

2. Materials and methods

2.1. Materials

Certified reference material saxitoxin dihydrochloride (66.3 μmol/L in 0.003 M hydrochloric acid (HCl)), gonyautoxins-2 and -3 (GTX2&3, 157.6 μmol/L in 0.003 M HCl), gonyautoxins-1 and -4 (GTX1&4, 80.0 μmol/L in 0.003 M HCl), neosaxitoxin (NEO, 65.6 μmol/L in 0.003 M HCl), and decarbamoylgonyautoxins-2 and -3 (dcGTX2&3,142 μmol/L in 0.003 M HCl) were obtained from the National Research Council Canada (Halifax, NS) and were stored in glass vials at −80 °C. Pooled and individual whole blood was purchased from Tennessee Blood Services (Memphis, TN). The acquired samples were from anonymous, random individuals representative of a general population, thus this work did not meet the definition of human subjects research as specified in 45-CFR 46.102 (f). Synthetic blood was purchased from Immunalysis (Pomona, CA).

2.2. Preparation of calibrators and quality control (QC) samples

The quantitative range of STX in whole and synthetic blood was evaluated from 0.0020 to 4.0 ng/mL. The optimal range was determined to be 0.020–0.80 ng/mL. For ELISA validation, calibrators were prepared at the following concentrations: 0.80, 0.50, 0.20, 0.10, 0.050, and 0.020 ng/mL in pooled and synthetic blood. QCs were prepared at 0.30 and 0.080 ng/mL in pooled, synthetic, or individual blood samples. Whole blood calibrators were prepared fresh daily throughout the validation whereas the synthetic blood calibrators and QCs were prepared at the beginning of the validation, frozen at −20 °C in single-use aliquots, and thawed as needed. The calibrators for the cross-reactivity study were evaluated

from 0.0050 to 250 ng/mL by diluting STX, NEO, GTX2&3, GTX1&4, and dcGTX2&3 reference materials in pooled blood.

The quantitation range for STX in dried blood on microsampling devices was evaluated from 0.020 to 500 ng/mL. The optimal quantitation range was determined to be 0.060–2.0 ng/mL. Calibrators covering this range were prepared at the following concentrations for intraday and interday validation: 2.0, 1.6, 0.80, 0.40, 0.20, 0.10, and 0.050 ng/mL and QC solutions were prepared at 1.2 and 0.16 ng/mL in pooled or individual human blood.

The tips of 10 μL Mitra® microsampling devices purchased from Neoteryx (Torrance, CA) were dipped into each blood solution until approximately two seconds after the absorptive tip turned completely red according to the manufacturer's recommendations. The tips were then removed from the blood solutions, placed back into the plastic storage clamshell, and allowed to dry a minimum of two hours or overnight at room temperature. The calibrators and QC samples were stored at room temperature in plastic storage containers containing desiccant packs purchased from Control Company (Houston, TX) until ready to use.

2.3. Saxitoxin ELISA

Saxitoxin (PSP) ELISA (part number 52255B), microtiter plate kits were purchased from Abraxis (Warminster, PA). All reagents of the ELISA kits were brought to room temperature before starting the following procedure. Microsampling devices containing each concentration of toxin-spiked or blank whole blood were removed from the plastic applicator and dropped into the wells of the ELISA plate. Fifty microliters of the enzyme conjugate solution followed by 50 μL of the polyclonal rabbit antibody solution were added to each well of the plate. The ELISA plate was covered with an adhesive seal and placed on an Eppendorf ThermoMixer C (Hauppauge, NY) for one hour with 30 s intervals of shaking at 800 rpm followed by two minute intervals of no shaking. Contents of each well were removed, and the wells were washed four times with 300 μL wash buffer. One hundred microliters of the substrate solution was added to each well of the plate, covered with an adhesive seal, placed on a ThermoMixer C, and shaken for one minute at 600 rpm. The plate was then incubated covered, at room temperature for 30 min. One hundred microliters of the stop solution was then added to each well of the ELISA plate, and the absorbance at 450 nm was read immediately on a Biotek PowerWave HT microplate spectrophotometer (Winooski, VT). Absorbances for each calibrator were divided by the absorbance of the unspiked matrix blank to generate the $\%B/B_0$. The $\%B/B_0$ values for each calibrator were plotted against theoretical concentrations and fit to a 4-parameter fit curve using the Biotek Gen5 v2.04 software. QCs and spiked individual blood samples were interpolated from the generated curve.

The protocol for the liquid blood ELISAs were identical to the protocol for the microsampling device ELISA except for the following modifications. Fifty μL of calibrator or QC were added to each well of the ELISA plate instead of the microsampling device. After addition of the enzyme conjugate and antibody solutions, the ELISA plate was incubated on a ThermoMixer C, but was only shaken for 30 s followed by 30 min of no shaking at room temperature. The remaining steps of the protocol were identical to the microsampling device ELISA.

2.4. Cross-reactivity ELISA

Duplicate calibration curves for STX, NEO, GTX2&3, GTX1&4, and dcGTX2&3 were run according to the ELISA protocol above. Standard curves of each analyte were obtained by fitting calculated averages to a four-parameter function using Biotek Gen5 v2.04 software. Midpoints (M) from each standard curve were used to calculate cross-reactivities of each analyte with respect to STX (%cross-reactivity = $[M_{STX}/M_{analyte}] \times 100$) as described previously (Zeck et al., 2001).

2.5. Hematocrit measurement

Hematocrit levels were determined for pooled and individual whole blood samples after centrifuging at 12,000 rpm for 5 min in a LW Scientific M24 micro-hematocrit centrifuge (Lawrenceville, GA). Reported hematocrit levels were determined by taking the average of triplicate measurements of each sample (Hempen et al., 2015).

3. Results

3.1. Working range determination for STX in pooled human blood and synthetic blood

Synthetic blood and pooled blood calibrators and QCs were both evaluated in this study to determine which would be the most appropriate matrix for analysis. Synthetic blood would offer a more convenient option for analysis as these calibrators and QCs could be prepared ahead of time, aliquoted, and frozen whereas pooled blood calibrators and QCs would have to be prepared at the time of analysis due to stability issues over time. Pooled blood however offers the advantage of being matrix matched to the samples which would be analyzed during exposures. Sigmoidal calibration curves (Fig. 1A) were generated using the STX ELISA kit over the range of 0.0020–4.0 ng/mL STX in pooled blood or synthetic blood to determine the working quantitation range of the assay. Linear regression analysis plotting theoretical spiked STX concentrations vs. calculated STX concentrations reproducibly showed good correlation ($R^2 > 0.99$) for both the pooled blood and synthetic blood samples between 0.010 and 1.0 ng/mL (Fig. 1B). The EC_{50} (effective concentration giving 50% of the maximum response) was 0.053 and 0.055 ng/mL for pooled and synthetic blood, respectively.

3.2. Pooled and synthetic blood STX ELISA method performance

Interday accuracy and precision for STX calibrators and QCs were characterized by evaluating a minimum of 20 cumulative measurements for % accuracy and RSD. Measurements were taken over the course of four weeks using three different analysts, with no more than two sets of calibrators and QCs per day. Percent accuracies for each interday calibrator and QC sample in pooled blood ranged from 96 to 107% and RSDs were between 8.1 and 17%. Percent accuracies for each interday calibrator and QC sample in synthetic blood were between 93 and 114% and RSDs ranged from 3.4 to 15% (Table 1A). Intraday accuracy and precision for STX QC samples were characterized by evaluating five replicate samples for % accuracy and RSD over the course of two independent experiments. Percent accuracies for QCs in pooled blood were both 100% and RSDs ranged from 8.1 to 9.0% for each sample (Table 1B). Intraday % accuracy in synthetic blood ranged from 94 to 100%

and RSDs fell within 4.5–10%. These data meet the criteria outlined by the U.S. Food and Drug Administration for ligand binding assays (U.S. Department of Health and Human Services, 2013).

Further characterization of the ELISA method was carried out by spiking ten individual whole blood convenience samples with 0.30 ng/mL STX, and 0.080 ng/mL STX. These spiked samples were analyzed for accuracy and precision against calibrators prepared in pooled whole blood or synthetic blood. The mean % accuracy in pooled blood using pooled blood calibrators for the 0.30 ng/mL calibrator was 110% and the 0.080 ng/mL calibrator was 113%. The RSD for the high spike was 14% and 11% for the low spike (Fig. 2A). The mean % accuracy in synthetic blood was 83% for the 0.30 ng/mL sample and 63% for the 0.080 ng/mL sample. The RSD for both spiked levels was 15% (Fig. 2B). No bias was found between individual sample calculated concentrations and hematocrit levels (data not shown), although samples 7 and 8 were more viscous than the other eight samples tested (personal observation). Fifty individual whole blood convenience samples were also analyzed for background levels of STX. These samples were used to calculate the method limit of detection according to the guidelines outlined by the NCCLS (Tholen et al., 2004). Among the fifty samples analyzed, no values measured exceeded the calculated limit of detection of 0.019 ng/mL. These results indicate that accurate quantitation can be achieved in individual samples using this assay and that unexposed samples should not proTduce false positive results when operating in the 0.020–0.80 ng/mL reporting range.

3.3. Dried blood on microsampling devices STX ELISA method performance

In cases where there are suspected exposures to STX in large populations or in areas that are not in close proximity to sites that can analyze exposure samples, it is necessary to ship samples for exposure analysis. To address these scenarios, we evaluated this ELISA kit's ability to quantitate STX in human blood that was dried on microsampling devices. The working range of this ELISA was determined the same way as for liquid pooled and synthetic blood. Good correlation ($R^2 > 0.99$) between calculated and spiked STX concentrations was observed within the working range of 0.06–2.0 ng/mL. This range was higher than the whole blood working range because of the smaller sample volume collected on the microsampling devices (10 μL vs. 50 μL). The interday accuracy and precision of STX-spiked calibrators and QCs were evaluated over the course of 21 cumulative measurements over the course of three weeks and three different analysts. No more than two sets of calibrators and QCs were run on any given day. Interday accuracies of calibrator and QC samples ranged from 88 to 110% and RSDs ranged from 8.7 to 19% (Table 2A). STX QC samples (1.2 and 0.16 ng/mL) were also characterized for intraday accuracy and precision by evaluating five samples within one experiment. Intraday percent accuracies ranged from 81 to 100% and RSDs were less than 5% for each sample (Table 2B). All accuracy and precision data for interday and intraday measurements met the U.S. Food and Drug Administration's guidelines for ligand binding assays (U.S. Department of Health and Human Services, 2013).

Further evaluation of this method was performed by analyzing 10 individual blood convenience samples spiked at both 1.2 ng/mL and at 0.16 ng/mL STX and dried on

microsampling devices. The ability to accurately and precisely quantitate STX in these samples was evaluated by interpolation from calibrators prepared in pooled human blood and dried on microsampling devices. The average % accuracy of these spiked samples ranged from 94 to 117% and the average RSDs were between 9.3 and 11% (Fig. 3). No correlation was found between individual sample hematocrit levels and calculated STX concentrations (data not shown). Fifty unexposed individual dried blood samples were used to calculate the limit of detection by the NCCLS method (Tholen et al., 2004). The limit of detection was calculated to be 0.06 ng/mL, and zero out of the fifty unexposed samples tested exceeded this level. Together, these results confirm that we can accurately and precisely quantitate 0.060–2.0 ng/mL STX in dried human blood samples.

3.4. ELISA cross-reactivity with other PSP toxins

Cross-reactivity was evaluated in pooled whole blood using PSP toxins structurally related to STX. The toxins evaluated were GTX1&4, GTX2&3, NEO, and dcGTX2&3. All toxins were evaluated up to 50 ng/mL, nearly 1000 fold higher than the STX EC_{50}. Cross-reactivities of less than 1% were detected with NEO and GTX1&4 and 1.51% for dcGTX2&3, while GTX2&3 was approximately 21% cross-reactive (Fig. 4). This cross-reactivity data shows that samples containing other structurally related PSP toxins will produce positive quantitation results to varying extents.

4. Discussion

STX exposures affect an estimated 2000 people each year resulting in approximately 300 fatalities worldwide making it a significant public health concern (Cusick and Sayler, 2013). There is currently very little published data on STX levels in clinical specimens. Two reports of STX exposures identified STX levels of up to 51 nM (15.3 ng/mL) in serum and 3.4 μM (1017 ng/mL) in urine during acute exposure (DeGrasse et al., 2014; Gessner et al., 1997). Because onset of symptoms occur quickly (within 1–2 h of exposure) and the half-life of STX is short (less than 10 h in serum and 20.4 h in urine), it is important to develop methods that are rapid, accurate, and sensitive to detect STX in clinical samples and confirm diagnosis.

Based on the available exposure information, the above ELISA is relevant to detect STX in clinical samples as it can consistently and accurately quantitate STX down to 0.02 ng/mL in pooled blood and 0.06 ng/mL in dried blood. To our knowledge, this is the first validated method for quantitation of STX in whole blood. When compared with analytical methods for detection of STX in other clinical matrices, this method is approximately 50 times more sensitive in whole blood and more than an order of magnitude more sensitive in dried blood than currently developed LC/MS-MS methods for quantitation of STX in urine and approximately 200 times more sensitive in whole blood and 66 times more sensitive in dried blood than previously reported LC-MS methods for serum (Bragg et al., 2015; DeGrasse et al., 2014; Johnson et al., 2009). Because of this method's high sensitivity compared with other techniques, it would be advantageous to add as a screening measure of PSP exposures so as not to report out false negatives.

While the use of synthetic blood calibrators would be ideal since they could be prepared ahead of time and frozen, quantitation of STX-spiked individual samples analyzed with synthetic calibrators were less accurate than the same samples quantitated with pooled blood calibrators and tended to show an underestimation bias (Fig. 2B and D). This is likely due to matrix differences between pooled and synthetic blood. Individual blood samples 7 and 8 which had lower % accuracy than the other samples were noticeably more viscous than the other samples analyzed (personal observation). This could be a contributing factor to the reduced accuracy for these samples as the competitive reaction might be somewhat restricted with increased viscosity (Fig. 2A and B). Although this ELISA has a limited quantitative range, it could serve as a useful means to qualitatively screen for PSP exposures close to point of care. The developed ELISA also facilitates fast turnaround of test results as 100 samples could be analyzed within two hours after sample collection or two hours post-dry time while an estimated 100 samples would take approximately 15 h by current LC-MS/MS methods. A third advantage of this ELISA is its cost-effectiveness. All materials for this ELISA can be easily purchased directly from their respective vendors. The only instrumentation required is a microplate spectrophotometer, which is inexpensive and common in most laboratories. The use of the microsampling devices in this ELISA allows for standardized sampling and because dried blood is stable at room temperature, shipping charges are much less than shipping refrigerated liquid blood. Finally, this method requires no blood processing or sample clean-up before analysis making it extremely user-friendly and simple to perform.

The minimal cross-reactivity of the polyclonal antibody with GTX2&3, GTX1&4, NEO, and dcGTX2&3, make the quantitation results most useful as a screen for STX exposures, but would produce negative results for many other PSP toxin exposures. Development of an accurate and sensitive immunoassay for detection of all PSP exposures would be ideal, but would likely require the incorporation of multiple antibodies with higher reactivity towards other PSP toxins. For accurate identification and quantification of individual PSPs, the results generated from this test should be coupled with another analytical technique such as HPLC-FL or HPLC-MS for confirmation. The net benefit of adding this screen before confirmation is the time and cost-savings associated with the ELISA coupled with its high sensitivity compared with LC-MS/MS.

In conclusion, this work demonstrates an accurate, sensitive, rapid, and simple quantitative ELISA method that can detect STX in liquid blood or dried human blood samples on microsampling devices. This method offers significant complementary benefits to shellfish monitoring programs as it can provide human exposure data and detect STX at much lower levels than currently developed LC-MS methods, is rapid to perform, requires no expensive instruments or advanced training, and offers a cost-effective means for collecting and shipping samples.

Abbreviations:

STX	saxitoxin
PSP	paralytic shellfish poisoning

GTX2&3	gonyautoxins 2&3
GTX1&4	gonyautoxins 1&4
NEO	neosaxitoxin
dcGTX2&3	decarbamoyl gonyautoxins 2&3.

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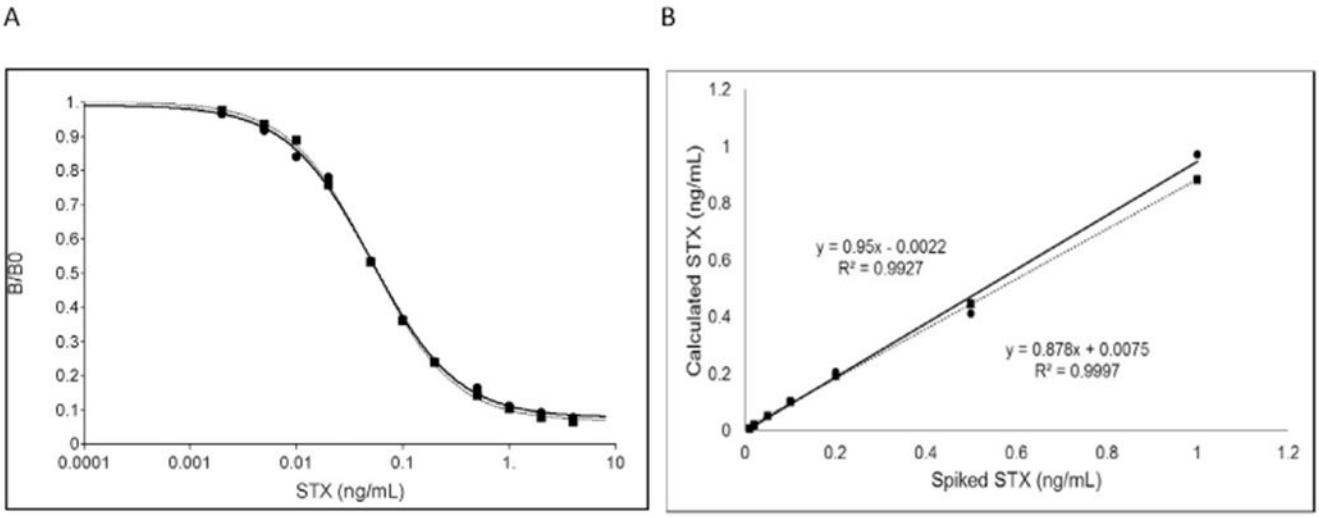
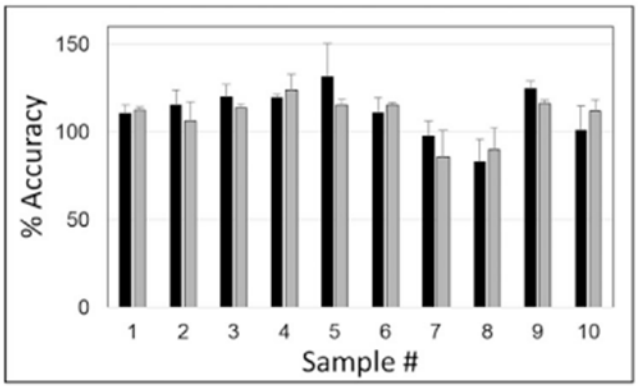


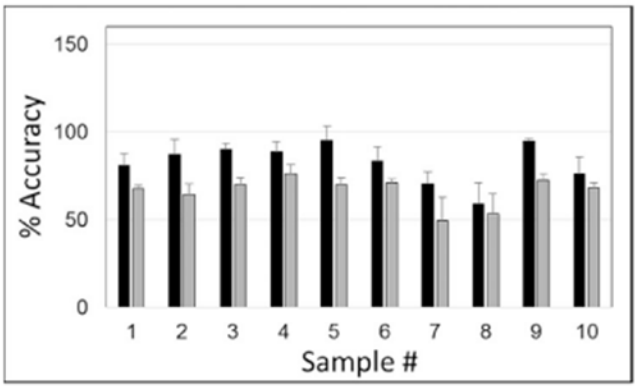
Fig. 1.

(A) Calibration curve for STX. Each point on the curve represents the mean of duplicate samples and is representative of two independent experiments. (B) The working range was assessed by performing linear regression on a plot of theoretical spiked STX concentrations against STX concentrations interpolated by the assay (n = 2) and is representative of two independent experiments. Solid line, spiked pooled blood; Dashed line, synthetic blood.

A



B



C

Saxitoxin (ng/mL)	Mean (ng/mL)	RSD[1]	% Accuracy[2]
0.30	0.33	14	110
0.080	0.087	11	109

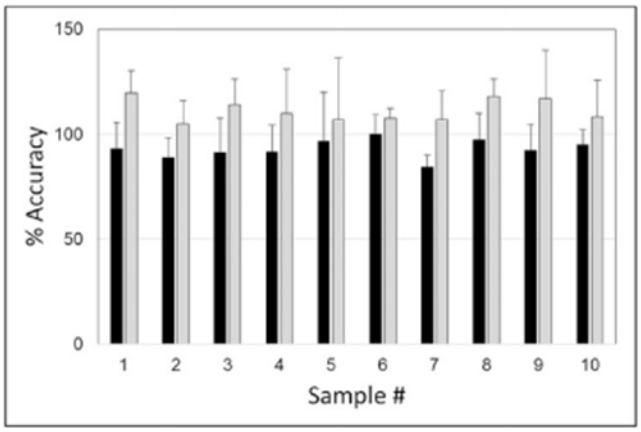
D

Saxitoxin (ng/mL)	Mean (ng/mL)	RSD[1]	% Accuracy[2]
0.30	0.25	15	83
0.080	0.053	14	66

Fig. 2.

Accuracy and precision for 10 individual whole blood convenience samples each spiked at 2 concentrations with STX and interpolated from a (A) pooled blood standard curve or a (B) synthetic blood standard curve. 0.3 ng/mL STX, black bar; 0.080 ng/mL STX, gray bar. Error bars reflect the standard deviations from 2 replicate experiments. Spiked convenience sample mean, average % accuracy, and average RSD interpolated from (C) whole blood calibrators or (D) synthetic blood calibrators from 2 replicate experiments. [1]Standard deviation/average × 100%. [2]Measured concentration/theoretical concentration × 100%.

A



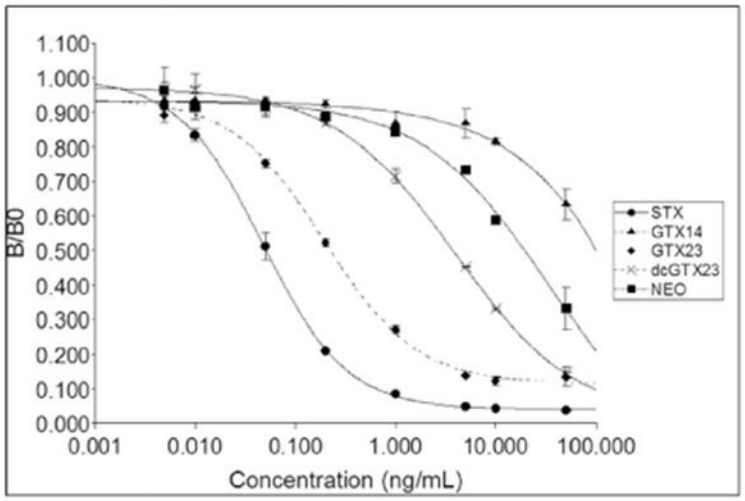
B

Saxitoxin (ng/mL)	Mean (ng/mL)	RSD[1]	% Accuracy[2]
1.2	1.4	11	117
0.16	0.15	9.3	94

Fig. 3.

(A) Accuracy and precision for ten individual whole blood convenience samples each spiked at two concentrations with STX. 0.16 ng/mL STX, black bar; 1.2 ng/mL STX, gray bar. Error bars reflect the standard deviations from three replicate experiments. (B) Mean, % accuracy, and RSD averages from three replicate experiments. [1]Standard deviation/average × 100%. [2]Measured concentration/theoretical concentration × 100%.

A



B

Analyte	% Cross-reactivity [1]
STX	100
NEO	0.090
GTX2&3	21.2
GTX1&4	0.025
dcGTX2&3	1.51

Fig. 4.

(A) Standard ELISA curves for STX and structurally related PSP toxins. Each point on the curve represents the mean of duplicate samples ± the standard deviation and is representative of 2 independent experiments. (B) Cross-reactivities of PSP toxins based on the standard curves. [1]Midpoint STX/midpoint PSP analyte × 100%.

Table 1

(A) Interday accuracy and precision for pooled (n = 21) and synthetic blood (n = 22) STX spiked calibrators and quality control (QC) samples. (B) Intraday accuracy and precision for pooled (n = 5) and synthetic blood (n = 5) STX spiked QC samples.

Saxitoxin (ng/mL)	Pooled Mean (ng/mL)	Pooled RSD[a]	Pooled % Accuracy[b]	Synthetic Mean (ng/mL)	Synthetic RSD[a]	Synthetic % Accuracy[b]
(A)						
0.80	0.84	15	105	0.91	15	114
0.50	0.48	9.2	96	0.48	6.5	96
0.30[c]	0.32	15	107	0.28	13	93
0.10	0.098	8.1	98	0.10	3.4	100
0.080[d]	0.078	17	98	0.077	5.8	96
0.020	0.020	15	100	0.020	7.5	100
(B)						
0.30[c]	0.30	9.0	100	0.30	10	100
0.080[d]	0.080	8.1	100	0.075	4.5	94

[a] Standard deviation/average × 100%.

[b] Measured concentration/theoretical concentration × 100%.

[c] QH.

[d] QL.

Table 2

(A) Interday accuracy and precision for STX spiked dried blood (n = 21) calibrators and QC samples. (B) Intraday accuracy and precision for STX spiked dried blood (n = 5) QC samples.

Saxitoxin (ng/mL)	Mean (ng/mL)	RSD[a]	% Accuracy[b]
(A)			
2.0	2.2	9.9	110
1.2[c]	1.2	12	100
0.80	0.78	8.7	98
0.20	0.19	10	95
0.16[d]	0.14	18	88
0.050	0.052	19	104
(B)			
1.2[c]	1.2	4.6	100
0.16[d]	0.13	4.2	81

[a] Standard deviation/average × 100%.

[b] Measured concentration/theoretical concentration × 100%.

[c] QH.

[d] QL.