Detecting paralytic shellfish toxins in oysters

Initial assessment of the AquaBC rapid test kit

Andreas Seger and Alison Turnbull

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Researcher Contact Details

Name:	Andreas Seger
Address:	15-21 Nubeena Crescent, 7053 Taroona, TAS
Phone:	03 6226 8324
Fax:	
Email:	Andreas.seger@utas.edu.au

FRDC Contact Details

Address:	25 Geils Court
	Deakin ACT 2600
Phone:	02 6122 2100
Email:	frdc@frdc.com.au
Web:	www.frdc.com.au

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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Executive Summary

This project trialled two rapid test kits for the rapid detection of paralytic shellfish toxins (PST) in oysters. The original project scope only included the AquaBC test kit, but a second test that become available towards the end of the project was also trialled to see if it could reliably detect PST. PST are produced by a range of marine microalgae and can rapidly accumulate to harmful levels in filter feeding bivalves, where they present a risk for human health and market access. Traditional monitoring of PST via advance analytical methods is costly and requires extensive technical expertise. In Tasmania and South Australia, this testing was previously supplemented by the use of a rapid PST test kit, not too dissimilar in operation to a COVID RAT test. This test (Neogen, Lansing, Michigan, USA) offered quick sample turnaround (<20min), at much reduced cost and could be run on farm. The test kit was previously used in Tasmania to reduce business risk, (i.e. batch testing of shellfish that can rapidly accumulate PST was conducted at harvest and prior to transport to market to reduce the risk of product recalls) and employed in South Australia for regulatory purposes (screening of low-risk product to reduce compliance testing costs). In 2023, Neogen unexpectedly ceased production of this PST test kit, and a suitable replacement therefore needed to be identified by:

1. Reviewing the range of microalgal species commonly encountered in Australian waters and the types of PST that they produce (referred to as the PST profile).
2. Identifying the performance criteria that a rapid test kit should meet.
3. Assessing two commercially available test kits against these criteria: AquaBC (Los Lagos, Chile) and SensoReal (Montreal, Canada).

Key microalgal species that recurrently bloom in Australian waters were identified as *Alexandrium catenella* and *Gymnodinium catenatum* in Tasmania and *Alexandrium minutum* and *Alexandrium pacificum* in New South Wales (NSW) and occasionally South Australia (SA). The types and relative proportions of different PST toxins produced by these microalgal species are an important consideration when assessing the suitability of rapid detection methods. A review of the available literature/data revealed that the major types of PST of concern in Australia include the highly toxic gonyautoxins (GTX1&4 and GTX2&3) and less toxic c-toxins during blooms of *Alexandrium* species. This contrasts with blooms of *G. catenatum,* where the PST profile predominantly consisted of decarbomylsaxitoxins (dcSTX), decarbomylgonyautoxins (dcGTXs), and c-toxins (C1&C2). Also important are saxitoxin and neosaxitoxin (STX and NEO), as both are highly potent and sometimes present.

To assess the suitability of each test kit for the Australian toxin profiles, we established the following performance criteria. A suitable test kit should:

1. Provide fast sample turnaround (preparation and analysis time) and be simple enough so that it could be used on farm.
2. Provide very low, or no false detections of PST.
3. Yield a positive result for all samples above the bivalve regulatory level (0.8 mg STX.2HCl equiv./kg) across all toxin profiles.
4. Have a high probability of detection (i.e. a high likelihood of providing a positive result) at PST levels above 0.4 mg STX.2HCl equiv./kg (i.e. at half the regulatory level).
5. Have a low probability of detection at low PST levels between 0.1-0.4 mg STX.2HCl equiv./kg.

Our initial trials showed that both the AquaBC and the SensoReal test kit require additional work before they can meet all five performance criteria. The AquaBC test kit proved suitable for on farm testing and met the first four criteria but proved overly sensitive in the 0.1-0.4 mg STX.2HCl equiv./kg range, particularly for samples containing higher proportions of GTX2&3 (100% probability of detection). While this may be suitable for regions where PST is infrequently observed and no trace amounts of PST persist between blooms (e.g. South Australia), it makes

it unsuitable for use where low background levels of PST are frequently observed (e.g. Tasmania). Vice versa, the SensoReal test kit proved not sensitive enough and will require some substantial method development before it will be suitable for use for all Australian toxin profiles. While this project was ending, two alternative testing approaches (Attogene and Toranelli Moana) were identified as promising alternatives. The Attogene test kit is of particular interest, as it is already available on the market and our New Zealand colleagues have obtained promising results with this kit for samples containing GTX rich PST profiles like those encountered in Australia.

Based on the currently available results and extensive discussion with various test kit manufacturers, we believe that there are multiple kits currently on the market that show promise. However, as it currently stands, additional pilot trials are required to select a suitable replacement for the Neogen rapid test kit. We recommend a dilution trial with the AquaBC test kit to see if the number of positive detections at lower PST concentrations could be reduced and to conduct some pilot trials with the Attogene kit. While these tests are being conducted, it would be worthwhile to include pilot tests with the Toranelli Moana detection module. This multipronged approach makes the most of the remaining PST positive shellfish tissues and ensures that the best test kit is selected for subsequent validation work. This would require additional quantities of PST positive material, which could be sourced from either natural blooms during the 2025 biotoxin season (if biotoxin activity occurs), or by capitalising on other biotoxin exposure experiments in 2026 to produce PST contaminated oysters.

Keywords

Paralytic shellfish toxins, marine biotoxin, detection, rapid test kit, bivalve shellfish, Pacific Oyster, *Magallana gigas*

Introduction

Reliable detection of marine biotoxins is a critical requirement for any effective biotoxin monitoring program for shellfish destined for consumption, requiring all analytical techniques to be properly validated. One family of marine biotoxins that requires ongoing monitoring in Australian shellfish (and other seafood species) are the paralytic shellfish toxins (PST). PST are a suite of toxins that each differ in their relative potencies due to slight differences in their chemical structure (e.g. neosaxitoxin is twice as toxic as saxitoxin). They are produced by certain species of microscopic microalgae and can rapidly (within days) accumulate in filter feeding shellfish and other seafood species during blooms of these microalgae. Should contaminated seafood be consumed in sufficient quantities by humans, these toxins can cause a series of neurological symptoms referred to as paralytic shellfish poisoning, which in severe cases can lead to respiratory arrest and death.

To effectively manage this risk, PST concentrations are routinely monitored in shellfish growing States by their respective shellfish quality assurance programs. Regular testing of shellfish flesh ensures that PST levels remain below the regulatory level of 0.8 mg saxitoxin dihydrochloride equivalents per kg of shellfish flesh (i.e. 0.8 mg STX.2HCl equiv./kg). This measure considers the differential toxicity of each PST analogue (analogue = type of PST), by calculating its toxicity relative to the saxitoxin dihydrochloride parent molecule using toxin equivalency factors (e.g. neosaxitoxin is twice as toxic as saxitoxin).

In Australia, PST producing blooms predominantly occur in Tasmania, although PST events have previously also led to repeated shellfish harvest closures in New South Wales and Victorian waters, with much less frequent PST detections reported from South Australian waters. In Tasmania, blooms of the PST producing dinoflagellates *Alexandrium catenella* and *Gymnodinium catenatum* occur almost annually. The duration and geographic extent of these blooms varies between years, with low background levels of PST (0.2-0.4 mg STX.2HCl equiv./kg) frequently encountered during the higher risk season (generally late summer/autumn for *Gymnodinium*, and May - December for *Alexandrium).* In New South Wales, the predominant PST producers belong to the genus *Alexandrium*. Blooms in this area have historically been infrequent, until in 2016 an unprecedented bloom of *A. pacificum* closed shellfish harvest areas in Twofold Bay (Eden) (Barua et al., 2020). Since then, different species of *Alexandrium* have been observed almost every year (Barua et al., 2020), with shellfish aquaculture and wild lobster fisheries being closed in 2022 due to biotoxin activity. PST detections in NSW are generally not as frequent as in Tasmania and are rarely reported for shellfish from South Australian and Victorian waters. However, historic records indicate the presence of PST producing microalgae in these jurisdictions (4.8 mg/kg STX equiv./kg in mussels during an *A. pacificum* bloom in Port Phillip Bay, VIC in 1993, and 27 mg STX equiv./kg from an *A. minutum* bloom in the Port River in Adelaide, SA, (Hallegraeff et al., 2021)).

Monitoring of PST is routinely conducted via liquid chromatography tandem mass spectrometry (LC-MS/MS), which requires advanced technical expertise and expensive laboratory equipment. In Tasmania and South Australia, this testing was supplemented using a rapid test kit for the detection of PST, produced by Neogen (Lansing, Michigan, USA). Just like a COVID rapid antigen test (RAT), this antibody-based test kit proved relatively simple to use and provided a qualitative result, indicating whether PST was present or absent. The quick sample turnaround and much reduced cost made this an ideal tool for on-farm decision making. Through extensive validation efforts, we learned that the probability of PST detection increased with the concentration of PST in shellfish flesh (Dorantes-Aranda et al., 2018; Turnbull et al., 2018). The probability of obtaining a positive result was much lower at background PST concentrations (<0.5 mg STX.2HCl equiv./kg) and increased with increasing PST concentration, consistently yielding positive results at PST concentrations closer to the regulatory level (i.e. positive results above 0.6 mg STX.2HCl equiv./kg). This gave farmers confidence that a negative result indicated low PST levels, and that harvest could proceed, while a positive result indicated that there was an increased likelihood that elevated PST levels were present and that the result should be confirmed with LC-MS/MS analysis.

Given the excellent performance of the Neogen test kit, the test was widely used in Tasmania to reduce business risk, (i.e. batch testing of shellfish that can rapidly accumulate PST was conducted at harvest prior to transport to reduce the risk of product recalls) and employed in South Australia for regulatory purposes (screening of low-risk product to reduce compliance testing costs).

However, in 2023, Neogen ceased production of this PST test kit, taking Australian, United Kingdom and New Zealand seafood safety managers & shellfish industries that had relied on this test kit off-guard. Subsequent conversations with Neogen have indicated that there is no desire to recommence production and a suitable replacement therefore needs to be identified. Several new companies are striving to fill the void in the market left behind by Neogen, including AquaBC (Los Lagos, Chile - https://www.aquabc.cl/business-consulting/), Sensoreal (Montreal, Canada - https://www.sensoreal.com/) and Attogene (Lincoln, Texas - https://www.attogene.com/shop/saxitoxin-psp-lateral-flow-kit-shellfish-rapid-test/?srsltid=AfmBOoraY4fzpfr3B6sAdHhljjnp_1BCEk4mzmmGUfLP8MjoNGCKlyhZ).

A key factor influencing the suitability of antibody-based rapid test kits is the PST profile present in the sample to be analysed. The term PST profile describes the relative concentrations of different PST analogues that might be present in each seafood sample. These profiles differ between toxic algal species, different seafood species and their tissues. Not all PST analogues are equally detected by the antibodies of different test kits (quantified as the % cross-reactivity). These cross-reactivities are critical for ensuring the test kit provides reliable detection across different combinations of PST analogues that might be present in shellfish.

From our previous efforts validating the Neogen rapid test kit, we have identified following five success criteria for selecting a suitable replacement. A suitable replacement should:

1. Provide fast sample turnaround (preparation and analysis time) and be simple enough so that it could be used on farm.
2. Provide very low, or no false detections of PST.
3. Yield a positive result for all samples above the bivalve regulatory level (0.8 mg STX.2HCl equiv./kg) across all toxin profiles.
4. Have a high probability of detection (i.e. a high likelihood of providing a positive result) at PST levels above 0.4 mg STX.2HCl equiv./kg (i.e. at half the regulatory level).
5. Have a low probability of detection at low PST levels between 0.1-0.4 mg STX.2HCl equiv./kg.

To determine if the AquaBC or Sensoreal rapid test kit are a suitable replacement for routine on farm monitoring of PST levels and a candidate for subsequent, more in depth validation efforts, we here provide an initial assessment of the test kits key performance parameters by:

1. Selecting a range of PST profiles containing the major analogues commonly encountered during Australian blooms and
2. Challenging the AquaBC and Sensoreal test kit against these profiles at various PST concentrations to provide an initial assessment of how these test kits compare to the above-described requirements.

Objectives

Objectives of the project – as agreed in the contract

1. Review & identify different PST profiles that may be encountered in TAS, SA and NSW oysters.
2. Challenge the AquaBC test kit against a range of different PST concentrations and profiles to provide an initial assessment of its suitability for detecting PST in Australian oyster tissues.

Method

Sample collection/selection

Oyster tissues collected during the 2024 Tasmanian and New South Wales biotoxin seasons were obtained from Analytical Services Tasmania/ShellMap and the New South Wales Department of Primary Industries and Regional Development (NSW DPIRD)/Symbio laboratories. As biotoxin activity was limited during 2024, additional PST positive samples were retrieved from the IMAS biotoxin library. Any samples that had been stored for >1 year were re-analysed via LC-MS to confirm PST concentrations and analogues present.

Rapid test kit trials

AquaBC

To conduct an initial suitability assessment of the AquaBC test kit, the test kit was challenged with common Australian PST profiles at a range of concentrations below and at the regulatory level. Firstly, to determine if there were any matrix specific effects of the blank matrix (PST confirmed to be below <0.10 mg STX.2HCl eq./kg), oyster tissues from 12 different growing areas were tested in triplicate. The remainder of this tissue was then pooled and used to dilute 10 different PST positive samples (5 from TAS, 5 from NSW) to yield PST concentrations of each profile at 0.2, 0.4, 0.6 and 0.8 mg STX.2HCl eq./kg. At each PST concentration, at least 5 sub-samples were independently extracted and analysed with the AquaBC test kit following the rapid extraction protocol provided by the manufacturer. This included extraction of toxins from 5 g of shellfish homogenate with 5 mL of a 70% Isopropyl and 5% acetic acid mixture through vigorous shaking for 30 seconds. The extract was subsequently passed through a paper coffee filter and 100 µL transferred to the buffer solution supplied with the test kit and shaken by hand for another 30 seconds. 100 µL of this buffer mixture was then added to the test strip cassette, incubated for 45 minutes and then read with the supplied test strip reader.

All samples were prepared by a single operator and analysed blind by a second operator.

Sensoreal

In addition to the AquaBC test kit, we also conducted some pilot tests with a second rapid test kit (Sensoreal, Canda) that became available towards the end of the project (not listed in the original scope). This test kit was challenged against two of the Tasmanian oyster samples described above at PST concentrations of <0.1, 0.2 and 0.8 mg STX.2HCl eq./kg. The tests were conducted in triplicate for each PST concentration for each of the toxin profiles and toxins were extracted following the protocol supplied by the manufacturer. 2 g of shellfish homogenate were added to 8 mL of the supplied buffer and incubated for 15 minutes, prior to filtering the mixture through a filter paper. 0.5 mL of the resulting filtrate was added to 1 mL of conversion solution provided with the test kit and incubated at room temperature for 40 minutes. 45 µL of this solution was then added to 1 mL of diluent provided with the kit and vortexed to mix. 70 µL of this diluted sample were then added to the conjugate tube and pipetted up and down to ensure that the lyophilised conjugate was dissolved. This solution was then incubated on a shaker table for a further 15 min, prior to transferring 70 µL onto the test strip cassette. After a further 15 minutes of incubation, the test result was obtained.

Results and Discussion

Toxin profiles

The type and relative concentration of different PST analogues present in a sample can significantly influence test kit performance, since the detection antibodies of lateral flow immunoassays generally detect some PST analogues more readily than others (Jawaid et al., 2015). It is therefore important to challenge any prospective test kit against the range of PST analogues that are likely to be encountered.

As part of these initial trials, several samples with different PST profiles from both Tasmania and New South Wales were therefore analysed (Figure 1). The major PST analogues in the NSW samples that were available for this pilot work include those previously reported for *A. pacificum* in NSW waters (Barua et al., 2020), containing high proportions of GTX1&4 and C1&2, with lesser contributions from GTX5 and GTX2&3. While no information on NSW *A. minutum* profiles in shellfish was found in the literature, previous investigations in South Australia indicated that this species produces predominantly GTX1&4 (Lippemeier et al., 2003; Negri et al., 2003), which was present in high levels in samples 1 and 3-5 from NSW).

The toxin profile of two of the available Tasmanian samples (TAS1 and TAS5), resembled PST profiles encountered in bivalve shellfish during *G. catenatum* blooms (Turnbull et al., 2018), with high proportions of dcSTX, dcGTXs and some c-toxins. The remaining three profiles (TAS2-4) more closely resembled those encountered in shellfish flesh during *A. catenella* blooms. However, the GTX2&3 content in these samples relative to GTX1&4 was much higher than that generally observed in the field (26-46% GTX 1&4, (Turnbull et al., 2018)), with no GTX1&4 found in samples TAS3-4. Nevertheless, high GTX1&4 levels were represented in four of the NSW samples.

Overall, the ten available samples provided a good variation of PST analogues commonly encountered in Australian shellfish, including those with higher toxicity (e.g. GTX1&4, GTX2&3) and those with lower toxicities, but higher abundance (e.g. c-toxins, dcSTX, dcGTXs). It should be noted that all samples contained c-toxins (either C1&2 and/or C2&4), which have not previously been reported during *A. minutum* blooms (Lippemeier et al., 2003; Negri et al., 2003). The implications of this for test kit sensitivity/detection rates are discussed in more detail below.

The ability for antibody based lateral flow immuno-assays to detect different PST profiles depends on the cross-reactivity of the antibodies. Antibodies are often raised against the parent molecule saxitoxin, which means that they tend to be very good at detecting saxitoxin (i.e. 100% cross-reactivity). These antibodies can also react to the presence of other PST analogues, but the extent to which they can do so differs between antibodies/test-kits. Furthermore, the toxicity of each PST analogue relative to saxitoxin also differs (see Table 1). For the test kit to be effective, we therefore require a suitable antibody with a cross-reactivity that considers the relative contribution of a given PST analogue towards total toxicity. For example, an antibody that had a high cross-reactivity (i.e. a high sensitivity) to c-toxins, would be problematic for detection of PSTs in Tasmanian shellfish samples collected during *A. catenella* blooms (samples TAS 2-4), since there are lots of c-toxins present, but they only contribute <10% of total toxicity due to their low relative toxicity (compare TEF for C1&2 of 0.1 to 0.6 for GTX2&3). For a test kit to effectively detect the presence of PST across a range of PST profiles, the cross-reactivities of the detection antibodies need to reflect the relative abundances and toxicities of PST analogues as closely as possible.

Some test kits, like that produced by Sensoreal, employ a conversion step in their extraction protocol, which aims to improve the detection of PST by converting all the analogues present to the readily detected, saxitoxin parent molecule. This would increase the sensitivity of the test across all PST profiles and may be suitable for areas where PST is very infrequently encountered (e.g. SA). However, for other states, like Tasmania, where low background levels of PST are commonly observed, this would mean that background levels of low toxicity analogues (e.g. C1&2) may increase the rate of positive detections, that would then need to be followed up with costly LC-MS/MS analysis to confirm.

Table 1 List of major PST analogues likely to be encountered in Australian waters. The relative toxicity of each major analogue compared to saxitoxin is provided. This is called the toxin equivalency factor (TEF). How PST cross-reactivities of a given antibody can vary between analogues is exemplified here for the no longer produced Neogen test kit.

Jurisdiction	**Algal bloom species**	**Major PST analogues**	**Relative toxicity (TEF)**	**Neogen antibody cross-reactivity (%)**
Tasmania	*Alexandrium catenella*	STX GTX 2&3 C1&2	1.0 0.6 0.1	100% 23.4% ± 10.4% 3.1% ± 1.2%
Tasmania	*Gymnodinium catenatum*	dcSTX dcGTX1&4 dcGTX2&3 c-toxins	0.5 0.5 0.4 0.1	55.6% ± 10.9% N/A 8.3% ± 2.7% 3.1% ± 1.2%
New South Wales	*Alexandrium pacificum*	GTX1&4 GTX 2&3 c-toxins	1.0 0.6 0.1	5.7% ± 1.5% 23.4% ± 10.4% 3.1% ± 1.2%
New South Wales	*Alexandrium minutum*	GTX1&4	1.0	5.7% ± 1.5%

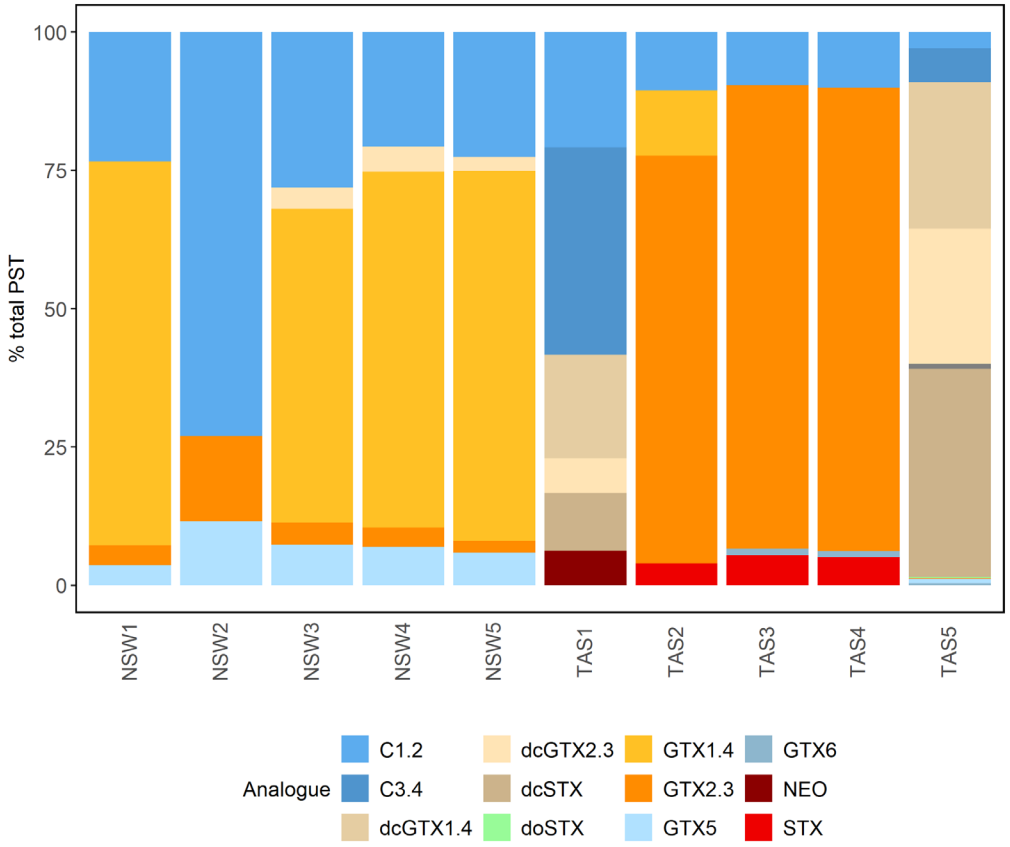

Figure 1 Toxin profiles for the oyster samples obtained from NSW (NSW1-5) and Tasmania (TAS1-5). Coloured bars indicate the relative percentage contribution of individual PST analogues to total toxicity. These samples were subsequently each made into a dilution series with negative oyster matrix (<0.1 mg STX.2HCl eq/kg) and tested with the AquaBC kit. The PST profiles TAS4 and 5 were also analysed using the Sensoreal test kit.

AquaBC performance

General considerations for operation – comparison to Neogen

The rapid extraction protocol for the AquaBC test kit was relatively straight forward and not too dissimilar to the Neogen protocol. Instead of water, the AquaBC kit uses a mix of 5% acetic acid (vinegar) and 70% isopropyl (rubbing alcohol) to extract the water-soluble toxins from the shellfish matrix. These chemicals are readily obtained and can be safely handled in a field/farm setting. Unlike the roller-based stomacher bag extraction for the Neogen, the AquaBC extract is filtered through a coffee filter and the filtrate added to the buffer provided with the assay. While the Neogen test included antibody coated test strips that were directly dipped into the sample extract/buffer mix, the AquaBC antibodies are contained within a testing cassette (similar to COVID tests). The entire cassette is then inserted into a reader, which proved less fiddly than inserting Neogen test strips into strip holder prior to reading the result.

The reader needs to be separately purchased (~$5,500) and the incubation period for the AquaBC test is longer (45 min) than for the Neogen (5 min). However, multiple samples can be run simultaneously in a staggered fashion. It is estimated that an experienced operator would be able to run 10 samples in an hour (providing shellfish are shucked and homogenised already). Other than the reader and the materials included with the test kit, additional equipment required includes a 100 µL pipette & tips, blender, beakers, coffee filters, filter holder, 1 m of bench space and access to power for the reader. The reader stores all sample information, which can be downloaded through a separate software packet (currently available free of charge through AquaBC).

The mode of detection is similar, in that the cassette/test strip contains two coloured bands: one strip as the control, and a second with the detection antibody. If PST is present, the colour intensity of the detection strip is much reduced. The AquaBC reader measures the ratio of the colour intensity of the detection strip relative to the control strip. This ratio is reported by the reader and used to qualitatively group the results into negative, slight positive and positive.

This output is shown for all samples in Figure 2, where the higher ratios were reported as negative results and the lower ratios as positives, while those in between were reported by the reader as slight positives. These results are discussed in more detail in the following section.

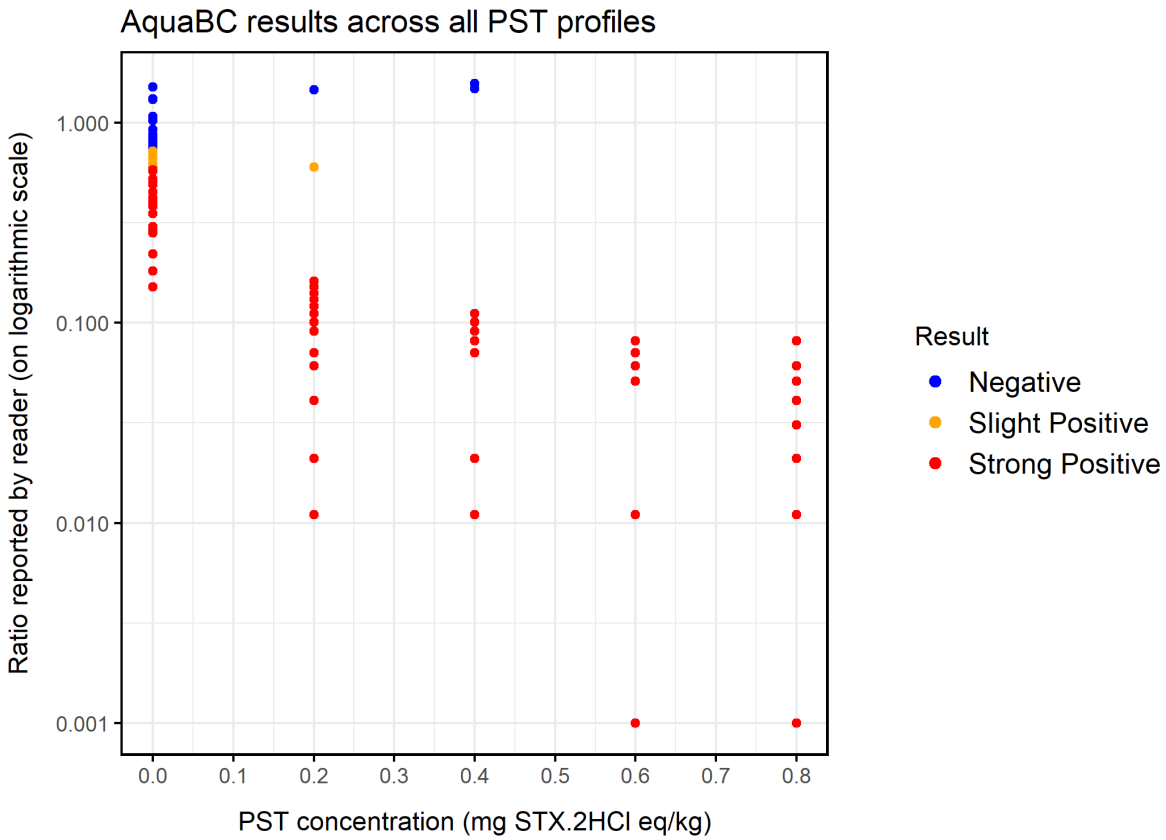

Figure 2 AquaBC test kit results across all PST profiles and concentrations tested (n=187 samples). Colours indicate negative (blue), slight positive (orange) and strong positive results (red) reported by the reader along with the ratio of the colour intensities of the detection and the control test strips (note logarithmic scale of y-axis).

AquacBC performance in blank oyster matrix (no PST)

For the AquaBC test kit to be effective, the rate of false positive detections in samples containing no or very low levels of PST, should be as low as possible i.e. the probability of detection is equal or close to 0. Of the 47 tests conducted across 14 different samples that were confirmed with chemical analysis to contain <0.10 mg STX.2HCl eq/kg (i.e. below the level of reporting for positive PST results), 16 tested negative, 7 slight positive and 24 returned a strong positive result. This means that almost half (POD of 49%) of the tests returned a false positive result. More detailed investigation revealed that the rate of false positives was dependent upon the sample itself. This suggests either the presence of matrix effects (e.g. different condition of oysters, chemical/nutrient profiles) that may have influenced the result, or detection of trace levels of PST in these samples (i.e. below the LC-MS level of reporting). Within a particular sample, test results differed between replicates (e.g. the “All combined” sample, see bottom right panel on Figure 3). While some variation in the measured ratio between replicates is to be expected due to e.g. slight differences in the extraction procedure, ideally, all blank oyster samples should have tested negative.

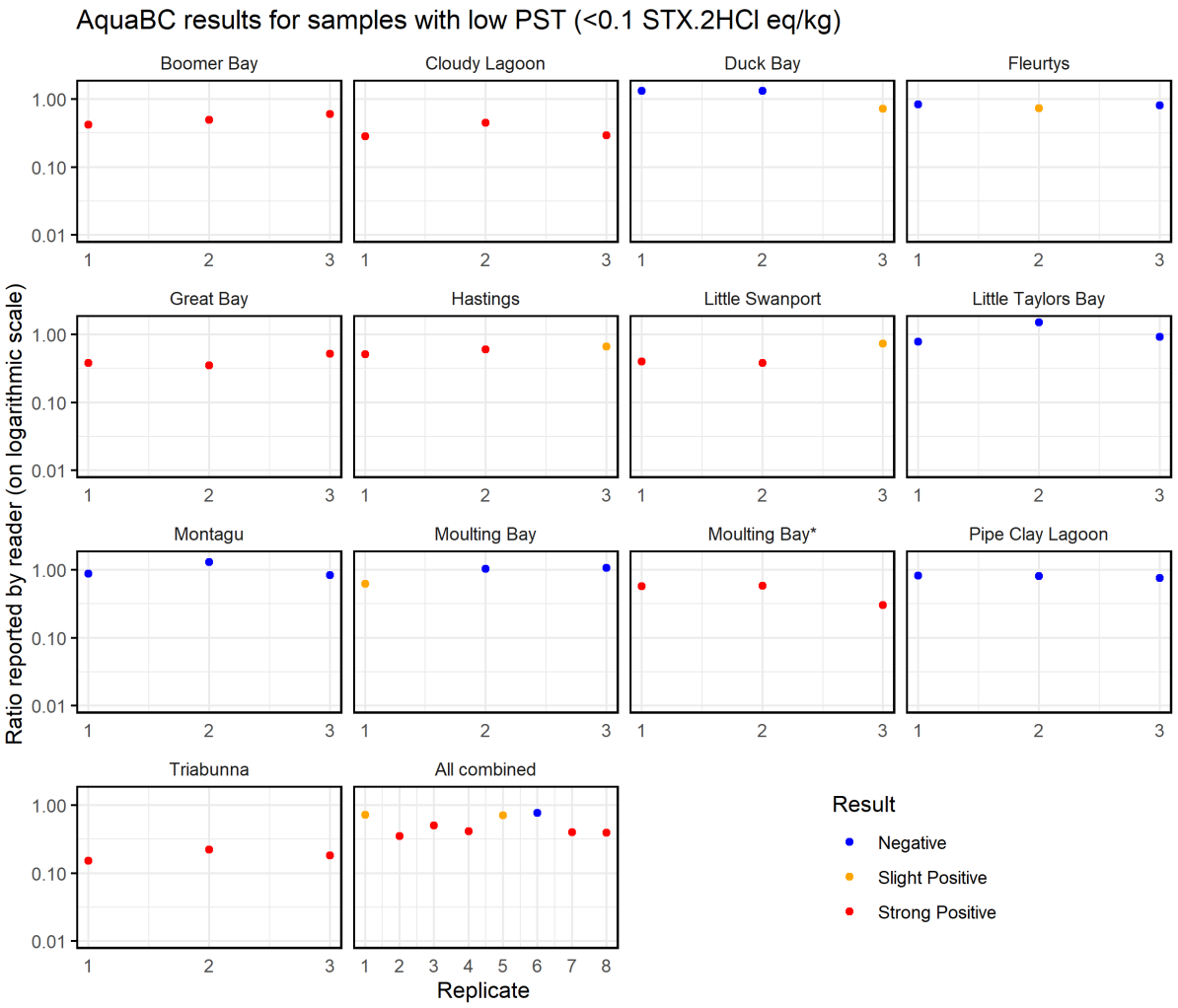

Figure 3 Screening of blank oyster matrix (13 oyster samples tested in triplicate) with the AquaBC test kit in samples where PST levels were confirmed to be below the LC-MS level of reporting (<0.10 mg STX.2HCl eq/kg). Colours indicate negative (blue), slight positive (orange) and strong positive results (red) reported by the reader along with the ratio of the colour intensities of the detection and the control test strips (note logarithmic scale of y-axis). The last panel provides the results for all 13 blank oyster samples pooled into one sample and then tested 8 times – this pooled tissue was used to dilute the PST positive samples.

AquaBC performance with different PST profiles

The ideal test kit would reliably detect the presence of PST above 0.4-0.5 mg STX.2HCl eq/kg and have a low probability of detection below this concentration, independent of the PST profile present. The AquaBC test kit proved overly sensitive, yielding positive results for almost all samples containing PST, with the exception of 1 slight positive (profile TAS2), 1 negative at 0.2 mg STX.2HCl eq/kg (TAS1) and 2 negatives at 0.4 mg STX.2HCl eq/kg (TAS3; see Figure 4). The reason for the much higher ratio in these two replicates of TAS3 is unknown, but highlights that PST detection at a given concentration can differ between PST profiles and is a probability of detection for a given sample, rather than an absolute value (i.e. 3/5 samples positive for TAS3 at 0.4 mg STX.2HCl eq/kg = a probability of detection of 0.6 or in other words a 60% chance of detection at this PST level).

It is noteworthy that the antibodies used in the AquaBC test kit are identical to those used in the previously marketed Jellet and NovaScotia test kits, both of which were shown to be highly sensitive to c-toxins. While it is tempting to attribute the high rate of positive detections to the presence of c-toxins in all the 10 profiles tested, the interpretation of the results is not as simple.

The ratio reported by the reader (i.e. colour intensity) relative to the different PST concentrations tested differed significantly between PST profiles (Figure 4). For NSW samples 1,3, 4 & 5 and TAS samples 1 and 5, the ratio decreased with increasing concentration, while in the other samples

(NSW 2, TAS 2, 3 &4), the ratio was already much lower at very low PST concentrations. For these samples, the observed ratios were already much lower at a PST concentration of just 0.2 mg STX.2HCl eq/kg and did not drop any further at higher PST concentrations. This indicates that the detection antibodies strongly reacted to these samples, essentially reducing the colour intensity of the detection strip to near zero (i.e. higher PST concentrations would not be able to reduce the colour intensity much further). This may be due to a higher sensitivity of the detection antibodies to GTX2&3, which was the only analogue that separated these samples from those where a more linear relationship between the ratio and PST concentration was observed. There are avenues to investigate to determine if this reaction could be reduced (e.g. smaller volumes of initial sample, dilution of sample) but all variations need to be tested to determine if they have other unintended consequences for the probability of detection at different PST concentrations.

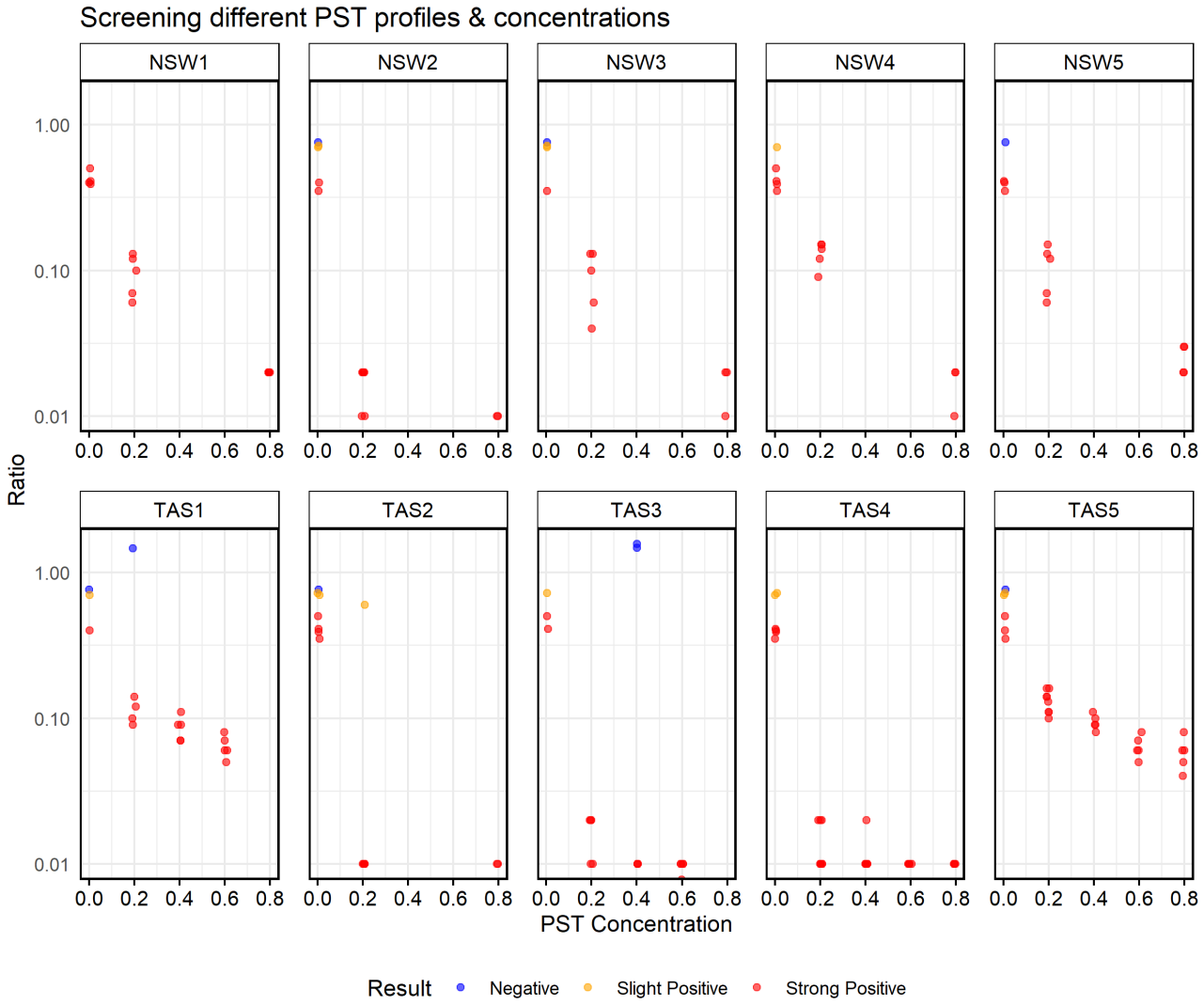

Figure 4 AquaBC test kit results for dilutions series of PST positive samples with different PST profiles representative of Tasmanian and NSW waters. Colours indicate negative (blue), slight positive (orange) and strong positive results (red) reported by the reader along with the ratio of the colour intensities of the detection and the control test strips (note logarithmic scale of y-axis). The dashed line indicates the fit of a linear regression (goodness of fit is indicated by the r^2 value). Note that the regression should be used as a general guide only, since it is not weighted for biased samples sizes (n=8 and 5 for samples with PST <0.1 and >0.2 mg STX.2HCl eq/kg, respectively).

Sensoreal performance

Sensoreal – general considerations for operation – comparison to AquaBC

When compared to the AquaBC test kit, the sample extraction procedure for the Sensoreal test kit required a few additional incubation/extraction steps that would make it unsuitable for field use in the current format. Like for the AquaBC test kit, the extraction procedure for the Sensoreal kit included passing the sample extract through a filter paper. This was followed by a conversion step that is unique to the Sensoreal test kit, where the addition of cysteine promotes the conversion of various PST analogues to the saxitoxin parent molecule. This conversion step (1 x 40min) and additional incubation periods (3 x 15 min) added to the overall analytical time (~1.5 hours). While an experienced operator might be able to run multiple samples in parallel, the number of shorter, subsequent incubation steps limits the number of samples that could be run in this fashion.

Like for the AquaBC test kit, the Sensoreal detection method is based on the addition of sample to a test strip cartridge. While the AquaBC cartridge is kept out of the reader until the final incubation period is complete, the Sensoreal cartridge is directly inserted into the reader and the sample then added to the cartridge. This currently restricts the number of samples that can be run to a single sample during the final 15-minute incubation window. However, Sensoreal has indicated that they are currently working on developing a reader that will be able to run multiple samples at once in a shorter period (5 min).

Sensoreal – performance with different PST profiles & concentrations

The Sensoreal test kit returned negative results for all three replicates of the PST negative oyster flesh (LC-MS/MS analysis = <0.1 mg STX.2HCl equiv./kg), i.e. it did not yield any false positive results. However, in its current format, this test kit did not appear sensitive enough to detect elevated concentrations of PST. All samples at 0.2 mg STX.2HCl equiv./kg tested negative, while at the regulatory level (0.8 mg STX.2HCl equiv./kg), 5 false negatives and only 1 positive result were obtained. In subsequent discussions, the manufacturer indicated that the sensitivity of the reader could be increased to provide a higher probability of detection at higher PST levels (0.8 mg STX.2HCl equiv./kg). We did indeed observe a pattern in the ratios returned by the Sensoreal reader for the high and low PST samples of the Tasmanian *Alexandrium* profile (TAS4), but not for those contaminanted with decarbomyl toxins, such as found during *Gymnodinium* blooms (TAS5). This is unlikely to be resolved through a reader sensitivity adjustment and indicates that the Sensorial test kit would require some additional method development before it could detect all PST profiles commonly encountered in Tasmania, where *Gymnodinium* blooms commonly occur.

Table 2 Probability of detecting the presence of PST with the Sensoreal test kit in two different Tasmanian PST profiles at different PST concentrations (0.2 and 0.8 mg STX.2HCl equiv./kg). Each PST profile/PST concentration combination was analysed in triplicate.

	PST concentration (mg STX.2HCl equiv./kg)		
PST profile	0	0.2	0.8
TAS4	0%	0%	33%
TAS5		0%	0

Summary of test kit performance against suitability criteria

As it currently stands, the AquaBC and Sensoreal test kits both require additional method development to meet all the five performance criteria identified above. The performance of the test kits against these criteria are summarised in Table 3, and discussed in more detail below.

Table 3 Comparison of the performance of the AquaBC, Sensoreal and the no longer available Neogen rapid test kits against the performance criteria defined in this work. Tick marks or crosses indicate whether a given test kit met the performance criterium. Question marks indicate where the potential exists to adjust reader sensitivities to alter performance.

Performance criteria A suitable rapid test kit for the detection of PST should:		**Neogen**	**AquaBC**	**Sensoreal**
1.	Provide fast sample turnaround and be simple enough so that it could be used on farm	✓ Test is suitable for on farm use	✓ Test suitable for on farm use	✘ Extraction protocol would need refinement (currently requires laboratory, ~2 hours to result)
2.	Provide very low, or no false positive detections of PST	✓ Yes	✓ Sensitivity of reader could be adjusted to eliminate/reduce false positive detections	✓ Correctly identified all PST negative samples
3.	Yield a positive result for all samples above the bivalve regulatory level across all toxin profiles	✓ Yes	✓ Yes	✘ ? Requires adjustment of reader sensitivity & further R&D
4.	Have a high probability of detection (i.e. a high likelihood of providing a positive result) at PST levels above 0.4 mg STX.2HCl equiv./kg (i.e. at half the regulatory level)	✓ Yes	✓ Yes	✘ ? Requires adjustment of reader sensitivity & R&D.
5.	Have a low probability of detection at low PST levels between 0.1-0.4 mg STX.2HCl equiv./kg	✓ Yes	✘ The test would need to be modified to distinguish between high and low PST concentrations.	? Would need to be confirmed after reader sensitivity & method development has been completed.

AquaBC

Criteria 1: Provide fast sample turnaround and be simple enough so that it could be used on farm

The AquaBC rapid extraction method was deemed suitable for use in the field and proved similar to the Neogen test kit in terms of time and effort required. No specific laboratory equipment nor overly hazardous chemicals were required (rubbing alcohol and vinegar for extraction). The final incubation step before the test can be read takes 45 minutes, which is considerably longer the 5 min required by the Neogen.

Criteria 2: Provide very low, or no false positive detections of PST

In its current state, the AquaBC test kit proved overly sensitive, providing a high number (49%) of positive results for samples where the test should ideally have returned negative results (PST was confirmed with LC-MS/MS to be below 0.1 mg STX.2HCl equiv./kg in these samples). However, AquaBC has indicated that the sensitivity of the reader could be adjusted. This involves reprogramming the reader to adjust the ratio above and below which the reader provides a negative/positive result. For example, if the reader was reprogrammed to provide positive results above a ratio of 0.1, the reader would have correctly identified all samples with PST <0.1 mg STX.2HCl equiv./kg, meeting criteria 2 (0% POD if no PST is present).

Criteria 3: Yield a positive result for all samples above the bivalve regulatory level across all toxin profiles

The AquaBC test kit returned positive results for all samples above the bivalve regulatory level (0.8 mg STX.2HCl equiv./kg) across all toxin profiles containing the major toxin analogues analysed here. All these samples returned a ratio of less than 0.1 on the reader, which means that should the reader sensitivity be adjusted to meet criteria 2, the test would still return positive results for all samples at the bivalve regulatory level and continue to meet criteria 3.

Criteria 4: Have a high probability of detection (i.e. a high likelihood of providing a positive result) at PST levels above 0.4 mg STX.2HCl equiv./kg (i.e. at half the regulatory level)

The reader successfully provided a high probability of detection at PST levels of 0.4 mg STX.2HCl equiv.kg (91% POD) and above (100% POD). Even if the sensitivity/ratio of the reader were adjusted to 0.1, the reader would have still returned a POD of 81% at 0.4 mg STX.2HCl equiv./kg, and 100% POD for higher PST levels, thus continuing to meet criteria 4.

Criteria 5: Have a low probability of detection at low PST levels between 0.1-0.4 mg STX.2HCl equiv./kg.

The AquaBC test kit returned a high number (98% POD) of positive results at PST concentrations < 0.2 mg STX.2HCl equiv./kg. By adjusting the ratio/sensitivity of the reader to 0.1, the detection frequency was reduced to 62% at this concentration. Ideally, this number would be much lower (as close to 0% POD as possible), to avoid providing positive results at low background PST levels, which are commonly encountered in Tasmania.

Sensoreal

Criteria 1: Provide fast sample turnaround and be simple enough so that it could be used on farm

The Sensoreal test kit protocol would need to be optimised to meet this criterion. Several incubation steps, including a 45 min conversion step, prolong the analysis (~1.5 hours to result) and various test kit components and pipetting steps require a laboratory environment. As it currently stands, this test would not be suitable for on farm use.

Criteria 2: Provide very low, or no false positive detections of PST

The Sensoreal test kit met this criterion and correctly provided negative results for all three samples with PST <0.1 mg STX.2HCl equiv./kg (confirmed by LC-MS/MS).

Criteria 3: Yield a positive result for all samples above the bivalve regulatory level across all toxin profiles

The sensitivity of the Sensoreal test kit would need to be increased to meet this criterium. With the current settings, the test only detected the presence of PST in 17% of samples at the bivalve regulatory level (noting that only two profiles representing PST commonly encountered during *Alexandrium* and *Gymnodinium* blooms were tested, with no positive detections for the decarbomyl rich *Gymnodinium* profile at the regulatory level).

Criteria 4: Have a high probability of detection (i.e. a high likelihood of providing a positive result) at PST levels above 0.4 mg STX.2HCl equiv./kg (i.e. at half the regulatory level)

As described for criteria 3, the sensitivity of the Sensoreal test kit would need to be increased to obtain a high likelihood of a positive result at levels above 0.4 mg STX.2HCl equiv./kg.

Criteria 5: Have a low probability of detection at low PST levels between 0.1-0.4 mg STX.2HCl equiv./kg.

The Sensoreal test kit did not detect any PST at levels between 0.1-0.4 mg STX.2HCl equiv./kg. It is currently unknown whether this low rate of detection would remain true if the reader sensitivity could be increased to meet criteria 3 and 4.

Alternative test kits – Attogene

As this project was ending, we were made aware of another rapid PST test kit (Attogene, Austin, United States). Experiences from our New Zealand colleagues show that this test kit in its current format underestimated the presence of PST in Pacific Oysters contaminated with GTX rich PST profiles. However, a distinct difference in the ratios for PST negative, low PST and high PST samples indicates that the reader software may be able to be modified to provide more accurate results. We have been in conversations with the company and have received instructions on how to fine-tune the Detekt reader software. In addition, Attogene has offered to provide free test kits for evaluation.

Alternative detection approaches – Moanelli Torana

A small start up company in New Zealand has been developing aptamer-based detection modules for paralytic shellfish toxins. Unlike the lateral flow immuno-assays described above, this approach uses specially designed detection molecules to detect the presence of specific PST analogues. We met with the developers to discuss the latest progress. The company currently has trial modules that can detect & quantify GTX1&4, STX, GTX2&3 and neoSTX in an aqueous mixture and is currently working to trial it in shellfish flesh. The test promises quantification of individual PST analogues, but several further development and validation steps will be required, including development of detection molecules for other frequently detected PST analogues, tissue specific toxin extraction procedures that may influence detection and quantification, as well as a user-friendly interface. While the company indicated that the development process could be sped up through additional investment, it may be sometime before a commercial product will be available. We have provided the company with a list of Australian PST analogues to consider in their development and are continuing to look at how we can support the development/validation of these techniques (e.g. through the provision of PST contaminated mussel/oyster tissues – see recommendations below).

Implications & further development

AquaBC

The AquaBC test kit was found to reliably detect the presence of PST across different toxin profiles, but appeared overly sensitive, yielding a high rate of false positives (>49%). While these false positives could be eliminated by reprogramming the reader software, the test kit could not distinguish between low (<0.4 mg STX.2HCl equiv./kg) and high PST levels (>0.4 mg STX.2HCl equiv./kg), particularly in samples containing high GTX2&3. This does not satisfy the fifth performance criteria of a "low probability of detection at low PST levels." While this may be suitable for regions where PST is very infrequently observed and no trace amounts of PST persists between blooms (e.g. South Australia), it makes it unsuitable for use where low background levels of PST are frequently observed (e.g. Tasmania). In subsequent discussions, AquaBC has indicated that they would consider the development of new antibodies more suited to Australian PST profiles. However, this would appear to be some time away (likely years), as the company is currently focused on the development of Amnesic and Diarrhetic shellfish toxins and may require additional investment to further pursue development of new PST detection antibodies. A more immediate modification for the AquaBC test kit could be to introduce an additional dilution step during the extraction procedure. This may improve test kit performance by reducing the number of positive results obtained at slightly elevated PST concentrations (between 0.2-0.4 mg STX.2HCl equiv./kg). The extent of the dilution would have to be experimentally determined using PST positive oyster material to ensure continued reliable detection of PST near the bivalve regulatory level.

Sensoreal

In its current format, the Sensoreal test kit will not be suitable for on farm detection of PST due to a complex sample extraction/testing protocol and lack of sensitivity towards the Australian PST profiles tested. The test kit met the performance criteria of low detection rates in PST free or low PST samples, but it's low detection rate (0-33% POD) at the regulatory level is concerning. While the results indicate that the sensitivity of the test kit might be able to be adjusted for the detection of GTX rich toxin profiles found during *Alexandrium* blooms, the test could not distinguish between non-toxic controls and high concentrations of toxins associated with *Gymnodinium* profiles (rich in decarbomyl toxins). The company recognises that the Sensoreal test kit currently does not react to decarbomyl toxins and would need to conduct further R&D to cover this. The company is prepared to look at simplifying the extraction technique, but this will require experimentation. Considerable method development is likely required to ensure that the test kit is suitable for use in Tasmanian waters, where *Alexandrium* and *Gymnodinium* blooms frequently occur.

Alternative testing options

The results obtained by our New Zealand colleagues with the Attogene test kit appear promising and show that this test kit may be able to differentiate between no, low and high PST concentrations. Attogene has already provided us with instructions on how to adjust the reader sensitivity and is willing to provide test strips free of charge. There is sufficient PST positive material for both Tasmanian *Alexandrium* and *Gymnodinium* profiles to provide a first indication of the suitability of the test, but a financial variation to this project would be required to conduct additional PST testing and cover staff salaries for testing & reporting. This would also open the opportunity to trial the aptamer-based test that Monelli Toana have been developing. This test currently can detect the most toxic PST analogues (STX, GTXs and NeoSTX) and promises quantification of individual PST analogues. Should the detection platform be found suitable for use in shellfish tissues, this would eliminate the dependence on antibody based cross-reactivities. The company is currently looking to test their product on PST positive shellfish tissues to establish proof of concept and attract investors for further R&D.

PST positive material

PST positive material representing the full range of toxin profiles commonly encountered in Australia is critical to testing the performance of any rapid test kit. This project was able to capitalise on PST positive samples stored in the IMAS biotoxin library and those from recent NSW blooms that were held by Symbio laboratories. While there are some PST positive tissues left that are immediately available for additional small-scale trials, further material will be required to validate/refine testing approaches and train farmers. Potential opportunities to collect additional PST contaminated samples for these efforts include monitoring and storage of PST positive samples from the field, but also add-on components to existing biotoxin exposure experiments that could generate additional PST positive materials.

Recommendations

While we obtained some promising results, both the AquaBC and the SensoReal test kit require additional work before they can meet all five performance criteria. At the same time, two alternative testing approaches (Attogene and Toranelli Moana) have been identified as promising alternatives. While Toranelli Moana is at an early stage of development, the approach appears promising and could readily be supported as part of additional trials with the AquaBC and the Attogene test kit. The latter is of particular interest, as it is already available on the market and our New Zealand colleagues have obtained very promising results with this test kit for samples containing similar, GTX rich PST profiles. For the Sensoreal test kit, the additional R&D work required will be too extensive at this point. The test kit would need to be modified to make it suitable for the detection of Australian toxin profiles and the extraction/testing procedure would need to be significantly altered to make it suitable for on farm use.

Based on the currently available results and extensive discussion with various test kit manufacturers, we think that there are multiple kits currently on the market that show promise. Further pilot work is required for some, and we have yet to test others to identify a suitable test kit for full validation that will be required before field use of the test kit (includes measures of repeatability, probability of detection curve and selectivity). We recommend the following activities to identify the most promising test kit & testing capabilities:

1. Conduct pilot trials with the Attogene test kit using both *Alexandrium* and *Gymnodinium* profiles. This should include adjustment of the reader sensitivity and re-testing of extracts to ensure suitability for Australian conditions. Rationale: good pilot results with GTX rich profiles by NZ colleagues (like Australian *Alexandrium* profiles), ability to adjust sensitivity, fast sample turnaround (30 min), company agreed to supply test strips free of charge.
2. Conduct additional trials with the AquaBC test kit to see if the high rate of positive detections at lower PST concentrations can be reduced. Rationale: AquaBC test kit satisfied all 4 other performance criteria, company is approachable, test was found suitable for on farm use.
3. Test extracts prepared as part of 1. and 2. with the pilot aptamer detection module produced by Tonelli Moana. Rationale: readily included in above trials, proof of concept will help company attract funds for additional R&D, analogue specific quantitation has potential to replace reliance on antibodies with various cross-reactivities.
4. Maintain a watch & act on all Australian biotoxin activity through the states' ASQAP programs to capitalise on opportunities to collect & store additional PST positive materials. Rationale: critical for future validation/training purposes.
5. In the absence of PST blooms in the 2025 biotoxin season, consider investing into existing aquaculture biotoxin trials to obtain PST contaminated tissues critical to test kit trials/training. An upcoming opportunity may exist in mid-2026 during planned aquaculture trials with PST producing *Alexandrium* and *Gymnodinium* microalgae. Rationale: these types of trials present unique opportunity to obtain large volumes of PST positive material with minimal effort/cost (i.e. it usually takes 4-6 weeks to ramp up algal production, which would not need to be funded if conducted as an add-on to an existing project). More PST

positive materials are required for future validation efforts once a suitable replacement test kit has been identified.

6. Once a suitable test kit has been identified, a full validation will be required to ensure that key performance criteria are met (including repeatability, probability of detection curve and selectivity).

Extension and Adoption

The project was presented to the Oysters Australia board by project co-investigator Alison Turnbull in an online meeting and the NSW and SA shellfish quality assurance programs were approached via email to contribute PST profile data and positive oyster tissues. The NSW DPIRD (through Symbio Laboratories) kindly provided several PST positive samples that allowed for the AquaBC test kit to be screened against additional PST profiles. Project lead Andreas Seger presented the project to shellfish quality managers from all Australian jurisdictions at the Australian Shellfish Quality Assurance Program meeting on the 13th of November 2024 (University of Technology Sydney).

Throughout the project, we have kept and are continuing to keep in regular contact with overseas colleagues in the United Kingdom (Centre for Environment, Fisheries and Aquaculture Science) and New Zealand (Cawthron Institute), who are also interested in rapid PST testing, along with the Tasmanian Abalone Council. In addition to the AquaBC test kit, the project team has met several times with Canadian based company SensoReal. While not included in the initial project scope, we conducted some additional trials with the test as part of this work. A summary of the project progress was provided to OA via email on the 11th of March 2025 and the project extended by ~ 1 month to include feedback from New Zealand colleagues and manufacturers of additional test kits that became available throughout the duration of this project. We met several times with Attogene (USA) and Tonelli Moana to include their feedback into this report and provide detailed next steps.

Appendices

- A1 - List of researchers and project staff (boat skippers, technicians, consultants)
- A2 - References

A1 – List of project staff

Name	Position
Andreas Seger	Lead investigator
Alison Turnbull	Co-investigator
Riana Bell	Technician
James Brady	Technician

A2 - References

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