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Comparative performance of four immunological test kits for the detection of Paralytic Shellfish Toxins in Tasmanian shellfish

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ABSTRACT

Blooms of the toxic dinoflagellate *Alexandrium tamarense* (Group 1) seriously impacted the Tasmanian shellfish industry during 2012 and 2015, necessitating product recalls and intensive paralytic shellfish toxin (PST) product testing. The performance of four commercial PST test kits, Abraxis™, Europroxima™, Scotia™ and Neogen™, was compared with the official AOAC LC-FLD method for contaminated mussels and oysters. Abraxis and Europroxima kits underestimated PST in 35–100% of samples when using standard protocols but quantification improved when concentrated extracts were further diluted (underestimation ≤18%). The Scotia kit (cut off 0.2–0.7 mg STX-diHCl eq/kg) delivered 0% false negatives, but 27% false positives. Neogen produced 5% false negatives and 13% false positives when the cut off was altered to 0.5–0.6 mg STX-diHCl eq/kg, the introduction of a conversion step eliminated false negatives. Based on their sensitivity, ease of use and performance, the Neogen kit proved the most suitable kit for use with Tasmanian mussels and oysters. Once formally validated for regulatory purposes, the Neogen kit could provide shellfish growers with a rapid tool for harvesting decisions at the farm gate. Effective rapid screening preventing compliant samples undergoing testing using the more expensive and time consuming LC-FLD method will result in significant savings in analytical costs.

1. Introduction

In recent years recurrent blooms (up to 300,000 cells/L) by the Paralytic Shellfish Toxin (PST) producing dinoflagellate *Alexandrium tamarense* (Group 1) have seriously impacted the Tasmanian shellfish industry. An initially undetected bloom event in October 2012 led to product recalls with an estimated economic loss of ~US$24 million dollars (Campbell et al., 2013). The regulatory action limit or permissible concentration of PST toxins in shellfish is 0.8 mg STX-diHCl eq/kg shellfish meat (0.8 mg STX eq/kg from now on). During 2015 closures of oyster and mussel farms which lasted for up to 4 months, PST levels were instigated and reached up to 32 mg STX eq/kg, resulting in four documented hospitalizations that occurred after individuals consumed wild mussels (i.e. recreational harvesting) from an affected area with public health warning signs. The current system for shellfish testing by the Tasmanian Shellfish Quality Assurance Program (TSQAP) requires shipping samples to an accredited Sydney laboratory leading to delays (4–12 days) for shellfish growers. The AOAC Official Method AOAC.2005.06 (pre-column oxidation, Pre-COX) using liquid chromatography with fluorescence detection (LC-FLD or Lawrence method; Lawrence et al., 2005) is the designated regulatory method for PST in shellfish in Australia. The method is highly specific and sensitive, providing a complete toxin profile and concentration of each PST analogue. However, it has been claimed that the method overestimates gonyautoxin 1&4 (GTX1&4) and neosaxitoxin (NEO), and underestimates gonyautoxin 2&3 (GTX2&3) and sulfocarbamoyl C1&2 compared to AOAC Official method

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2011.02 (post-column oxidation, PCOX) (Turner et al., 2014a). Immunological PST test kits, which were first trialled in the early 2000s (Jellett et al., 2002; Laycock et al., 2000), have the advantage of being sensitive, fast, easy to use and cheaper than HPLC-based analytical methods, and ideally allow farmers to perform tests on site to guide harvesting decisions. However, due to significant variability in PST toxin profiles of different *Alexandrium* species and geographic populations, as well as widely different potency of PST analogues, the applicability of different commercial test kits for local product testing requires careful consideration. Most available kits target saxitoxin (STX), but have low cross-reactivity for GTX1&4 and GTX2&3. The latter are common in Australian shellfish products as well as shellfish in Great Britain (Turner et al., 2014b).

Enzyme-linked immunosorbent assays (ELISA), such as the Abraxis™ and Europroxima™ kits, are quantitative tests that allow the user to calculate the concentration of PST toxins (as mg STX eq/kg) using a STX standard curve. These protocols require laboratory experience to avoid high user errors. By contrast, lateral flow immunoassays (LFIA) are qualitative tests that deliver positive or negative results based on a predetermined cut off limit. Scotia Rapid Test™ (formerly Jellett Rapid Test; Jellett et al., 2002) has a detection limit of ~0.2–0.7 mg STX eq/kg, whereas Neogen™ states that it has a cut off of 0.8 mg STX eq/kg. LFIA kits are more user friendly and simpler to use than ELISA kits, while laboratory experience is not essential. Different commercial immunological tests exhibit highly variable cross-reactivity to different PST analogues (Table 1). These cross-reactivity profiles do not fully correlate with the toxicity of individual toxins as determined by the mouse bioassay and the toxicity equivalency factors applied in total toxin determination of the LC-FLD method. Therefore, commercial test kits must be shown to be fit for purpose with geographical toxin profiles prior to implementation within testing regimes.

In the present study the performance of four commercially available immunological PST test kits for shellfish testing were evaluated during a major *Alexandrium tamarense* bloom event on Tasmania's East Coast, Australia, between July and November 2015.

2. Materials and methods

2.1. Shellfish samples

Sixty nine shellfish samples, including mussel *Mytilus galloprovincialis* and Pacific oyster *Crassostrea gigas*, which originated from 12 farms along the East Coast of Tasmania, Australia were used. Samples (homogenates from whole organisms) were stored at −20 °C and analysed within 1 month after harvesting.

2.2. Liquid chromatography analysis

Advanced Analytical Australia (AAA), the certified laboratory that TSQAP uses for phycotoxin analysis, determined PST toxin concentration using the AOAC.2005.06, LC-FLD or Lawrence method. Screen and confirmation (when >0.4 mg STX eq/kg were found) analyses of the method were performed.

PST toxins were extracted from 5 g of shellfish homogenate using 3 mL of 1% acetic acid. The mixture was placed in a boiling water bath for 20 min, mixed, allowed to cool and centrifuged at 3600 × *g* for 10 min. The supernatant was recovered and the pellet resuspended in 3 mL 1% acetic acid, mixed and centrifuged again. Both supernatants were mixed and made up to 10 mL with water. A sample clean-up was performed using a SPE C18 cartridge and screen testing was performed after periodate oxidation of samples. Standards, samples and PST positive certified reference matrices were oxidised with the inclusion of a matrix modifier. The matrix chosen for the matrix modifier reflected the predominant shellfish in the run. Oxidation using the matrix modifier circumvents the need to apply recovery factors for differing shellfish matrices. A further confirmation analysis was performed after peroxide oxidation of C18 cleaned extracts. All results were converted to mg STX-diHCl eq/kg using EFSA's toxicity equivalency factors (EFSA, 2009) (mentioned as mg STX eq/kg). Subsamples analysed by AAA were returned to IMAS for use in the PST screening with the rapid test kits.

Table 1
Cross-reactivity (mole %) of four immunological test kits as specified by the manufacturers.

PST analogue	Quantitative		Qualitative	
	Abraxis	Europroxima	Neogen[a]	Scotia[b]
STX	100	100	100	100
NEO	1.3	1.4	129	26
GTX2&3	23	5.6	23	100
GTX1&4	<0.2	<0.1	6	1.8[c]
C1&2	nd	0.2	3	nd
GTX5	23	26.2	23	62
dcSTX	29	19.2	56	100
dcNEO	0.6	0.5	28	nd
dcGTX2&3	1.4	0.2	8	15

nd = not determined.
[a] Jawaid et al. (2015).
[b] Formerly Mist Alert and Jellett (Jellett et al., 2002; Laycock et al., 2000).
[c] If an extra step involving hydrolysis conversion of GTX1&4 to NEO is performed, this cross-reactivity can be increased to 26%.

2.3. Test kits

2.3.1. Quantitative tests

2.3.1.1. Abraxis™. Abraxis test kits (52255B, lot number 15B5951) were stored at 4 °C until analysis. PST toxin quantification was performed according to the manufacturer's protocol.

2.3.1.1.1. Extract preparation. A subsample of 10 g of shellfish homogenate was mixed with 10 mL of 0.1 M HCl (modified version of the AOAC.959.08 method, extraction protocol as per the mouse bioassay) and placed in a boiling water bath for 5 min, allowed to cool down and centrifuged at 3500 × *g* for 10 min. Supernatants were recovered and pH adjusted to 3.0, and diluted in 1× sample diluent (1:1000). Initially, all 69 samples were considered as blind samples and analysed as per the standard test protocol (i.e. 1:1000 dilution). For a second analysis, 15 of these samples were further diluted (i.e. 1:10 or 1:100) based on the known toxin concentration (LC-FLD by AAA) in order to bring them within the working range of the calibration curve.

The Abraxis kit can operate with an alternative extraction method using 80% methanol (MeOH). For this purpose the 15 samples mentioned above (i.e. with extra dilution performed) were tested. Shellfish homogenate (1 g) was mixed with 6 mL of MeOH for 1 min using a Vortex mixer, centrifuged at 3000 × *g* for 10 min and the supernatant transferred into a clean tube. MeOH (2 mL) was added to the pellet, mixed and centrifuged. Both supernatants were combined and made up to 10 mL with MeOH. Similar to the HCl extracts, the MeOH extracts were analysed using the standard test protocol dilution (1:100) and also with an extra dilution (i.e. 1:10 or 1:100) as required.

2.3.1.1.2. Test protocol. A volume of 50 μL of STX standards (provided at 0, 0.02, 0.05, 0.10, 0.20 and 0.40 ng mL^{-1}) and samples (in HCl or MeOH) was transferred into the 96-well coated plate in duplicate, followed by 50 μL of enzyme conjugate and 50 μL of antibody. The microplate was mixed and incubated for 30 min at