

Brevetoxin test kits: initial assessment of performance and possible usage scenarios

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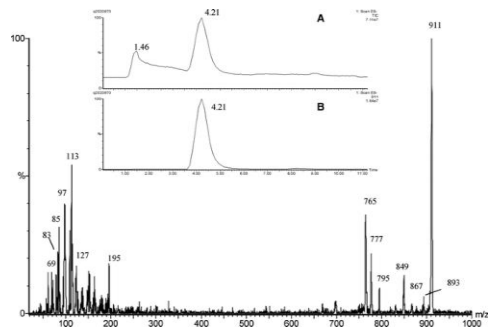
SafeFish - National Seafood Safety & Market Access Program

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Toxin detection techniques

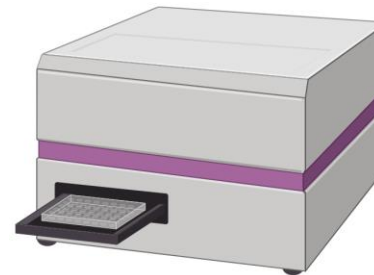
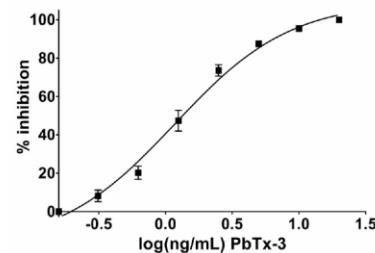
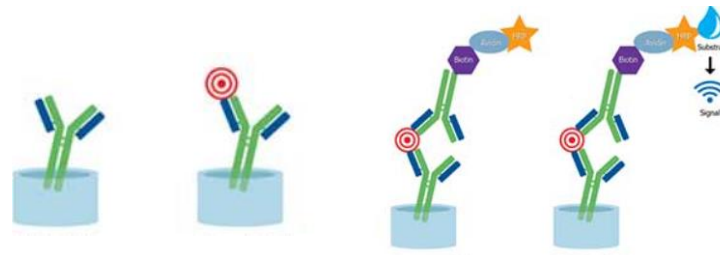
Chemical confirmatory analysis LC-MS/MS



Wang et al. 2004 *Anal. Bioanal. Chem.*

Cost: ~ \$800-1,000 per sample

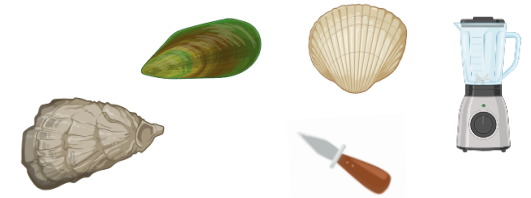
Enzyme-linked immuno-sorbent (ELISA) or receptor binding assays (RBA)



Need to be validated for Australian usage scenarios

Cost: <\$100 per sample,
dependent on use

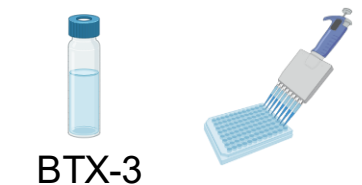
1. Sample homogenisation



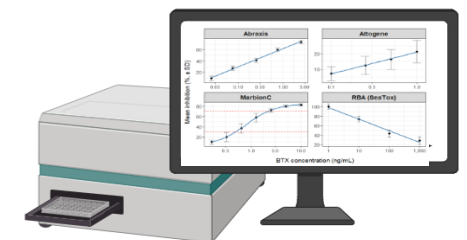
2. Brevetoxin extraction



3. Microplate assay



4. Quantification



What is involved in validating a test kit?

Initial comparison:

- Understand toxin profiles & concentrations in different seafood matrixes:
Pipis, mussels, abalone, Pacific Oysters
- Shortlisted candidate kits:



SeaTox: MARBIONC & RBA

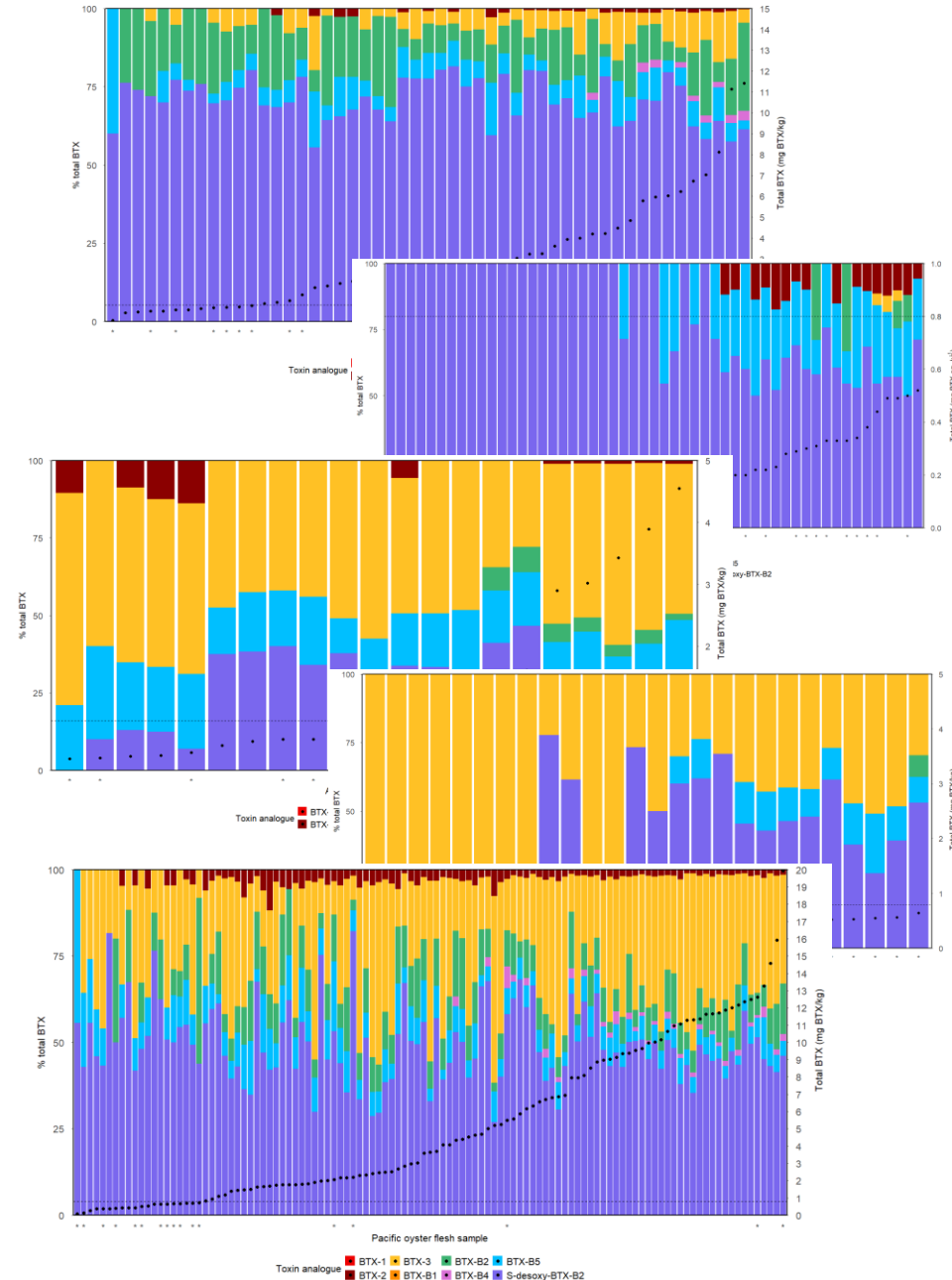


Attogene



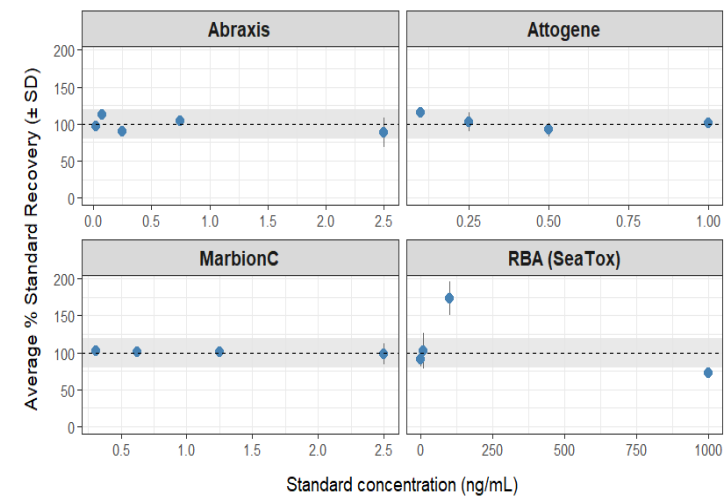
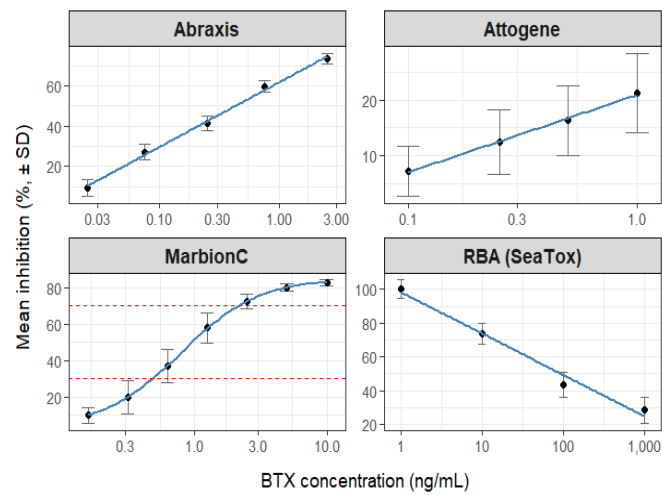
Gold Standard Diagnostics: Abraxis

- Conducted initial trials to identify best candidate(s):
 - Usability
 - Assay performance: reliability of results
 - Likelihood of false positive detections where no BTX is present
 - Likelihood of false negatives where BTX is present
 - Comparison to reference method (LC-MS/MS)
- Usage scenario: how can these kits be best used?



Assay performance

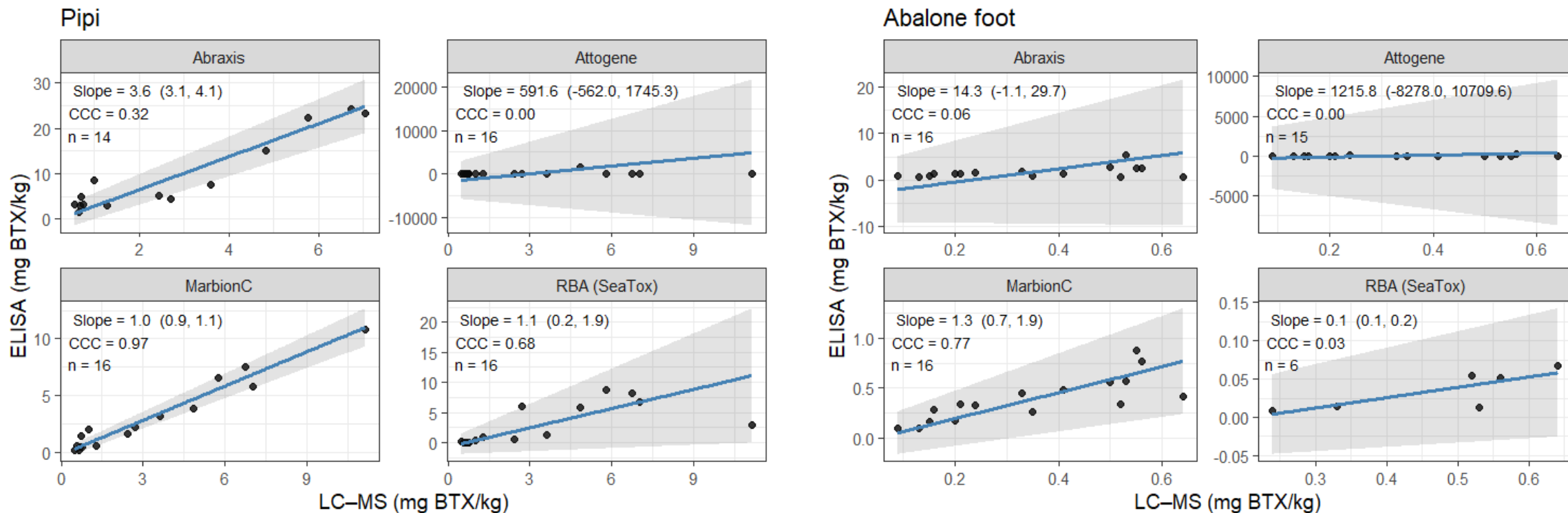
- Dynamic range
- Standard recovery
- %CV
- Linearity of dilution
- Cost-estimate



Likelihood of false positives

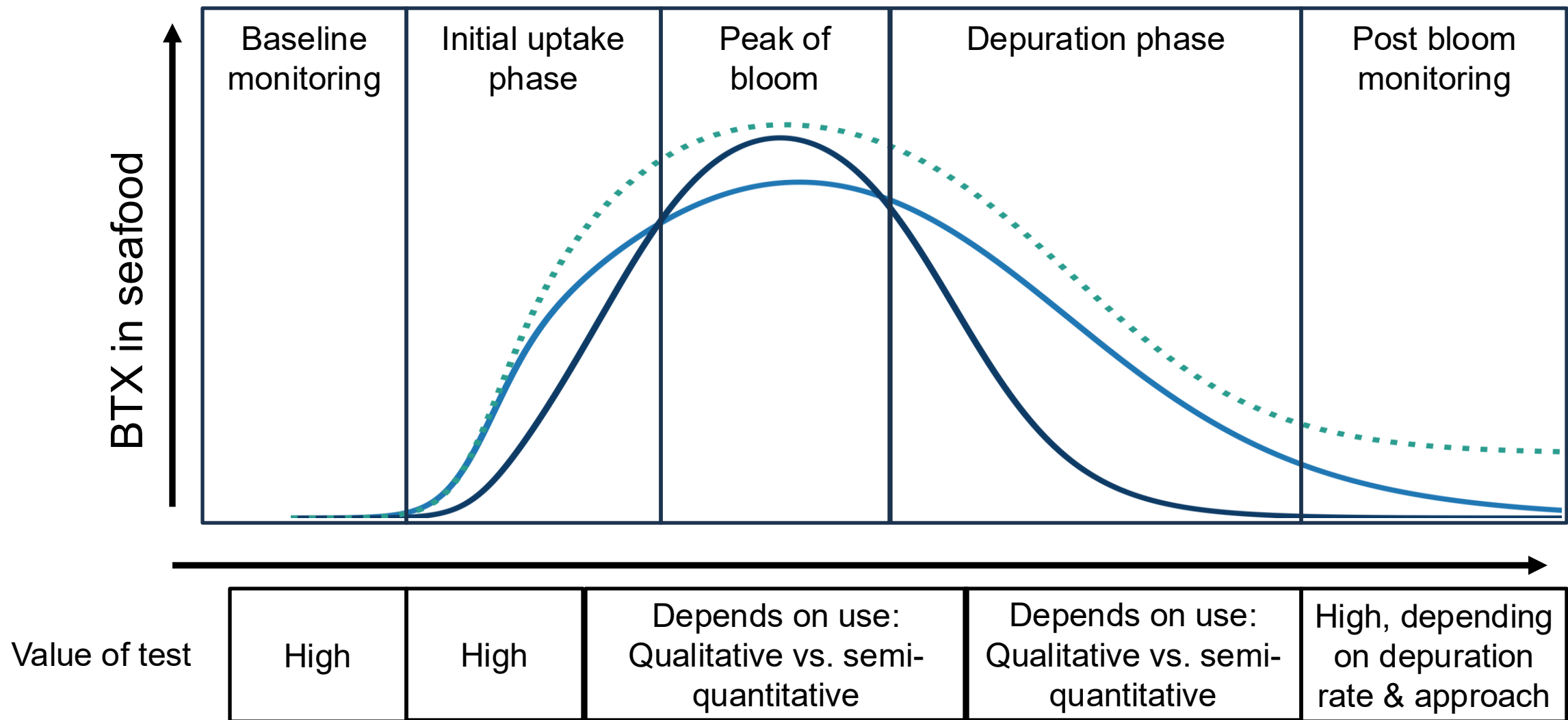
	MARBIONC	Attogene	Abraxis	RBA
Provide very low, or no false positive detections (negative samples)	No BTX detects	Several false positives (0.02-0.9 mg/kg)	Low-mid level detections (0.001-0.3 mg/kg)	Low-mid level detections (0.006-0.2 mg/kg)

Relationship between test kits & LC-MS/MS



- BTX detected in all samples where LC-MS/MS indicated BTX >0.1 mg BTX/kg
- MARBIONC kit proved most consistent across matrixes, providing estimates that agreed well with LC-MS/MS
- Other test kits did not perform well in samples tested as part of this study

Value during bloom



Conclusions & next steps

- Validation for specific scenario important & complex
- MARBIONC clearly performed best for the samples tested here
- Semi-quantitative approach would offer highest cost & time savings (if properly validated)
- Additional samples required to build confidence, particularly for semi-quantitative use
- Extend of additional testing depends on use (business risk vs. regulatory)
- Working with SASQAP, SARDI and industry partners to identify more detailed usage scenarios and validation steps



Full report available from SafeFish website