

# Biotoxin Test Kits: Cefas Investigations



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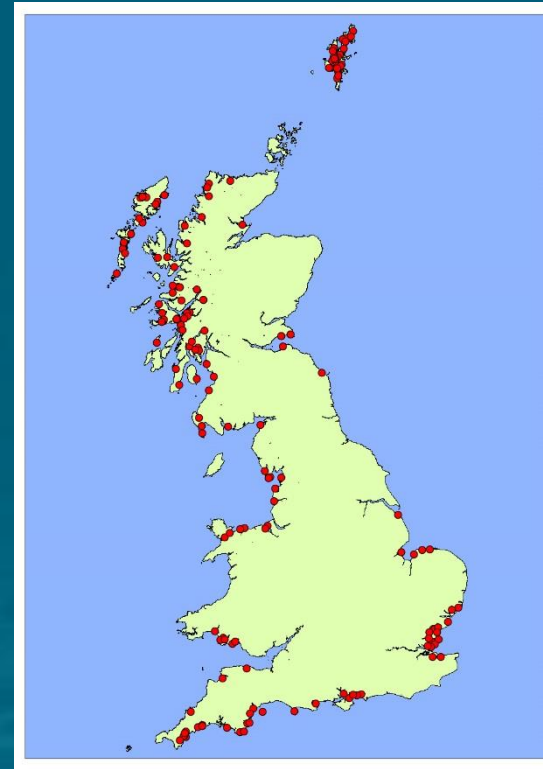
# Background: Toxin Monitoring

Official Control Flesh monitoring programme

Samples collected from monitoring points within commercially active, classified production and relaying areas

End Product Testing

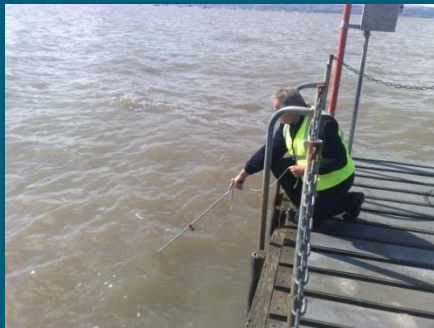
- 1) Commercial laboratory testing – using Official Control Testing Methods
- 2) Commercial laboratory – using rapid tests
- 3) Own end-product testing (lab, office or field)



•>200 sites

•>3,500 samples

•Covering all of GB



# EPT Method Choice

- High expense of running instrumental chemical analysis for EPT
- But - importance of ensuring rapid test methods are appropriate:
  - Accuracy
  - Sensitivity
  - Cross-reactivity appropriate
  - Specificity / matrix effects
  - Linearity / detection range
  - Rates of false –ve / +ve
  - Portable / practical to use?
- Important to conduct testing & validation to determine all the above

# Cefas work

- Assessment of commercial RTK assays
- No funding, so work conducted in spare time
- Done so to provide industry and regulators with useful data describing:
  - Test kit performance
  - Comparative data with official methods
- Without test kit validation and evidence for applicability to UK samples – commercial methods are useless to UK industry

# LFIA

# ELISA

## Test formats



- Neogen
- Scotia (ex-Jellett)



- Abraxis
- Beacon
- BiooScientific
- Europroxima
- R-Biopharm
- Zeu Lab

# Specific tests studied

- PSP
  - Abraxis
  - Beacon
  - BiooScientific
  - Europroxima
  - Neogen
  - R-Biopharm
  - Scotia

- DSP
  - BiooScientific
  - Neogen
  - Scotia
  - ZeuLab

- ASP
  - Beacon
  - BiooScientific
  - Europroxima
  - Neogen
  - Scotia

Different tests have different quoted sensitivities – makes comparisons tricky...

# Study samples

- Shellfish from Official Control Testing
- Scottish, English and Welsh origin
- Variety of monitoring points
- Range of different species where possible
- Between 2011-2015
- Different levels of toxicity:
  - “Negative” samples – no toxins by LC methods
  - “Positive” samples – containing toxins by LC
  - Variable toxin concentrations

# Results

- Summary - details on posters

## Definitions used

- False negative = RTK negative or  $< 0.5$  MPL when LC result  $>$  MPL
- False positive = RTK positive or  $> 0.5$  MPL when LC  $<$  LOQ

## Official methods

- ASP – LC-UV
- PSP – LC-FLD
- DSP (LT) – LC-MS/MS

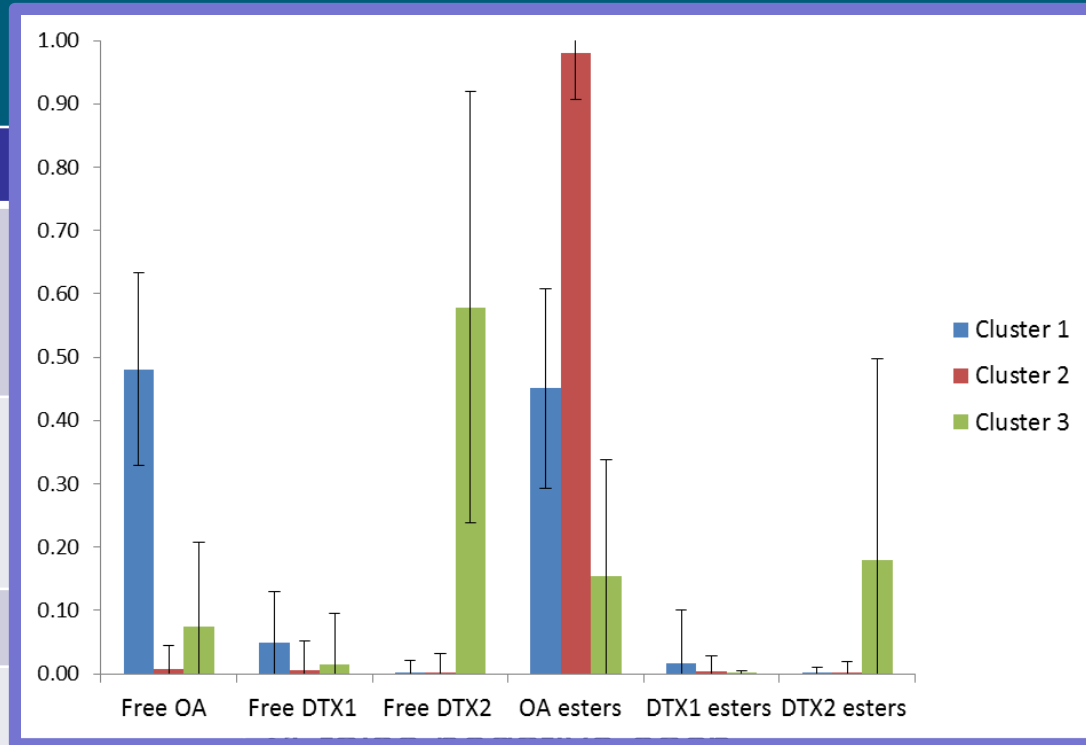


# DSP – Key Points

## (135 samples)

Supplier	Kit type	Brief summary of findings
ZeusLab	PP2A	<ul style="list-style-type: none"> <li>• Only kit to show no false negative results</li> <li>• Overall over-estimation in toxicity</li> <li>• Some false positives – mostly in oyster species</li> </ul>
BiooScientific	ELISA	<ul style="list-style-type: none"> <li>• Better correlation with quantitative results</li> <li>• 1 false negative</li> <li>• No false positives</li> </ul>
Neogen	LFIA	<ul style="list-style-type: none"> <li>• Both LFIAs showed high % agreement with LCMS</li> <li>• No false positives</li> <li>• 1 false negative each</li> </ul>
Scotia	LFIA	
General Points		

# DSP – Key Points (135 samples)



## Supplier

ZeuLab

BioScientific

Neogen

Scotia

General Points

- Agreement with LCMS worse when samples contain high levels of DTX2
- DTX2 is found commonly in DSP +ve shellfish
- Lower cross reactivity to DTX2 in kits

# PSP – Qualitative Tests

## (160 samples)

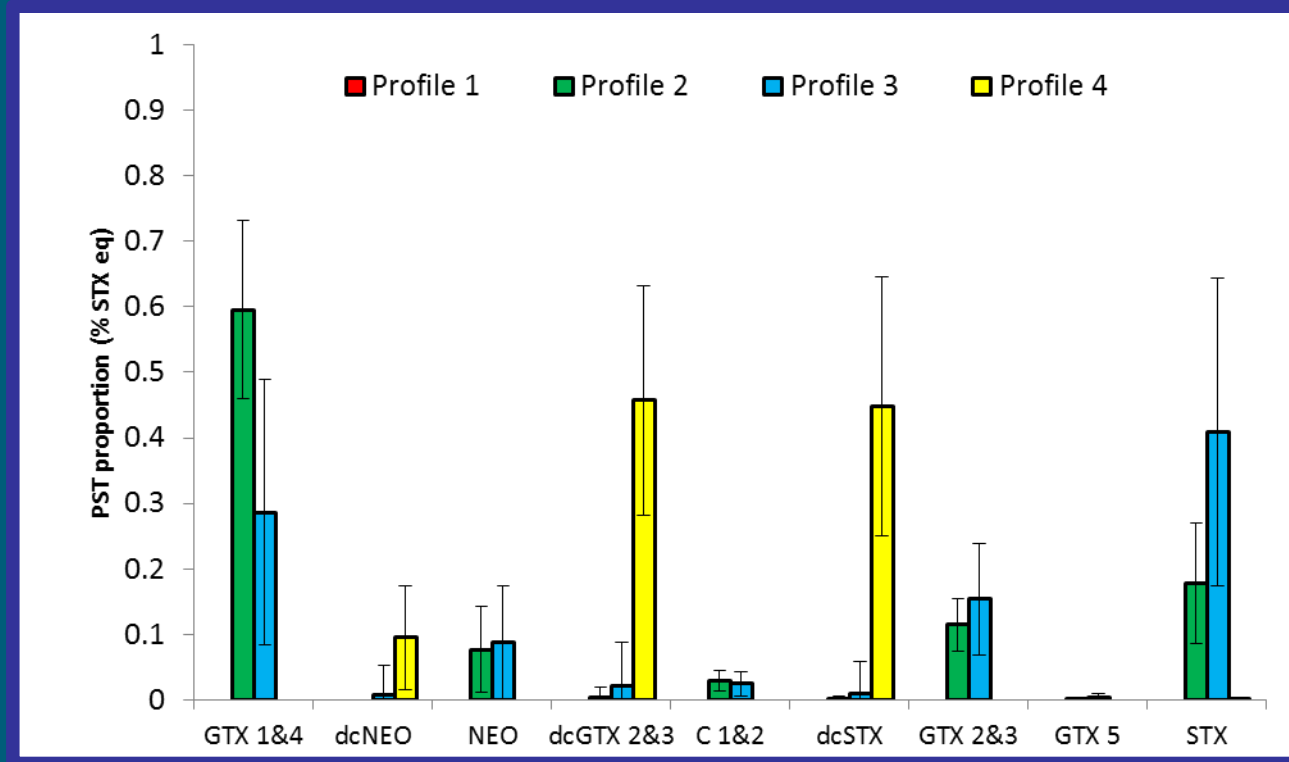
Toxicity by LC-FLD	Neogen	Bioo	Europroxima	Beacon	R-Biopharm	Abraxis
<b>Not detected</b>	100% Neg	100% < 400	100% < 400	100% < 400	100% < 400	100% < 400
<b>&lt; 400</b>	5% Pos	0% > 400	0% > 400	72% > 400 28% > 800	5% > 400 0% > 800	78% > 400 0% > 800
<b>400 - 800</b>	43% Pos	83% > 324 <sup>a</sup>	93% > 77.4 <sup>b</sup>	96% > 400	98% > 400	96% > 400
<b>&gt; 800</b>	95% Pos	92% > 324 <sup>a</sup>	98% > 77.4 <sup>b</sup>	100% > 400	98% > 400	100% > 400

- No false positives
- Low false negatives

# PSP – Quantitative tests

- Beacon, Abraxis & R-Biopharm
  - Beacon visible over-estimation
  - Abraxis/R-Biopharm – good correlation up to MPL
- Bioo & Europroxima
  - Narrow linear range – different dilutions required to make these useful tools

# Importance of GTX1&4



- More negative RTK results with higher levels of GTX1&4
- Scotia PSP LFIA\* improves this with use of additional conversion step

# ASP results

	Neogen	Scotia
%RTK positive samples >MPL (20 mg/kg)	100%	100%
% RTK false positive on samples <1 mg/kg by LC-UV	0%	0%
%RTK positives from shellfish >1mg/kg	29%	27%
% RTK positive > LOD*	100%	81%
% RTK positive < LOD*	10%	3%

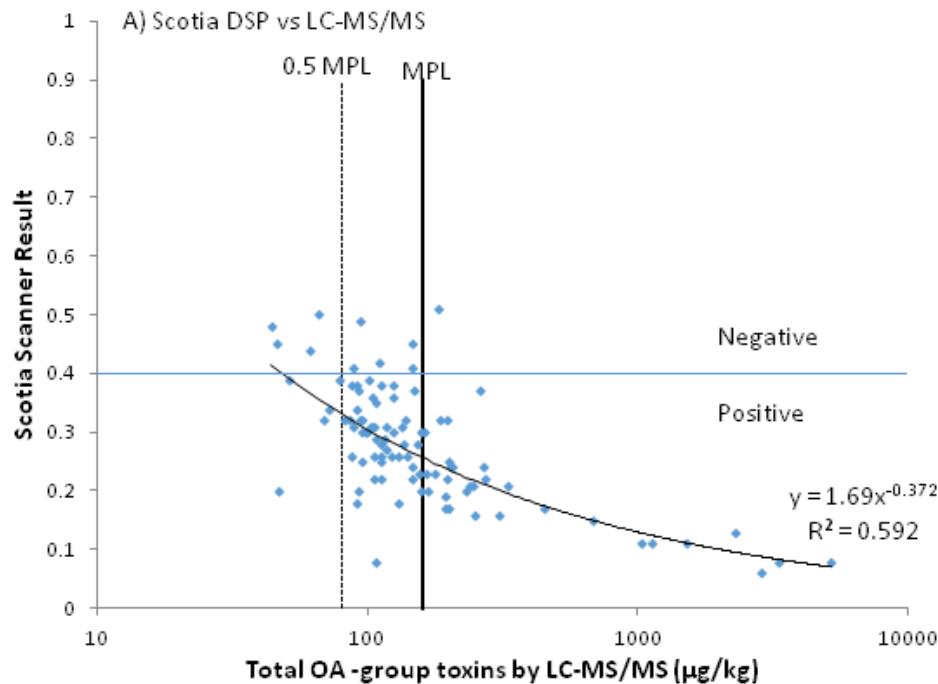
\*Neogen LOD = 20 mg/kg; Scotia LOD = 10 mg/kg

- No false negatives
- No false positives
- Test sensitivities appropriate for use
- ASP Elisa linear range unsuitable without additional dilutions

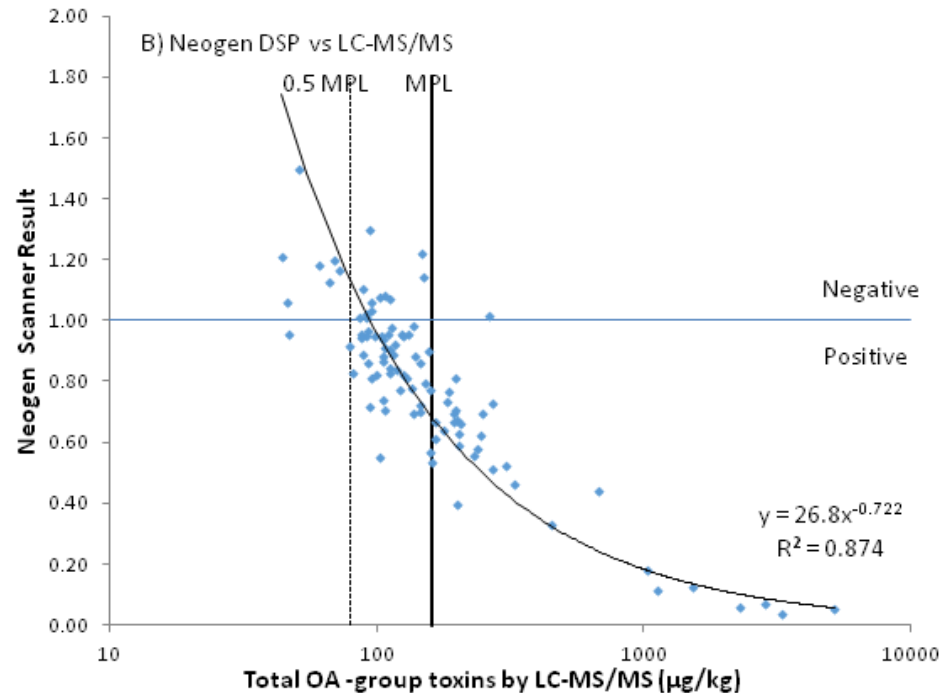
# Overall - RTK interpretation

Supplier	Kit type	Brief summary of findings
ZeuLab	PP2A	<ul style="list-style-type: none"> <li>•Provides quantitative result</li> <li>•Therefore indicates toxicity / toxin concentrations well as qualitative pos/neg decision</li> <li>•ASP ELISA – additional sample dilutions required to achieve meaningful results in relation to reg limit</li> </ul>
BiooScientific	ELISA	
Abraxis	ELISA	
Beacon	ELISA	
Europroxima	ELISA	
R-Biopharm	ELISA	
Scotia	LFIA	<ul style="list-style-type: none"> <li>•Pos/neg result both visually and with scanner</li> <li>•“Scan value” is provided – enables assessment of toxicity level. <b>Very useful – but use with care!</b></li> </ul>
Neogen	LFIA	<ul style="list-style-type: none"> <li>•Post/neg result only (scanner only)</li> <li>•Scanner does have “value” hidden – showed potential for providing indication of toxicity – <b>not accessible</b></li> </ul>

# Example: LFIA Scan Value



- Highly informative number
- Useful for risk management
- Only Scotia provide this formally





# Overall Summary

- In general – good qualitative indication of toxicity in shellfish from most kits
- Variable accuracies of quantitation
  - Linear range inappropriate for some ELISA
- Low false +ve – for most kits
- Low false –ve – for most kits
- Scan value from LFIA very useful
- In combination with portability – LFIA powerful and flexible tools
  
- Issues need investigation

# Acknowledgements

- FSS and FSA for allowing the use of shellfish samples for RTK testing
- Supply of test kits by Abraxis, Beacon, BiooScientific, Neogen, R-Biopharm, Scotia

