Is this green stuff toxic? Tell me now and not in 3 days please!

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The Problem

• Analytical methods for cyanotoxins take too long
  – 3 – 5 days for HPLC determination of PSPs
  – Need to transport samples to lab for analysis
    ➢ Not an operationally useful timeframe

• High capital set-up & staff time costs
The Ideal Solution

- Rapid
- Sensitive
- Reliable
- Field-based
- Fool proof
Project Aim

- To assess applicability of various commercially available rapid antibody-based assays for cyanotoxins, especially PSPs

1. Assays that are able to be done in the field
   - Simple, robust, no specialised equipment, yes/no result

2. Rapid lab-based assays
   - Higher skill level, specialised equipment, more sensitive, precise, quantitative
Assays Assessed

Saxitoxins (PSPs)  •  *Jellett* Rapid Test for PSPs

Microcystins  •  *Envirologix* Test-tube kit for microcystins
  •  *Abraxis* Strip Test for microcystins

Cylindrospermopsin  •  ????????
Assay Principle: Antibody binding

Advantages
• Specific for each toxin type
• Range of toxin analogues can be detected
• Assays can be made to be very sensitive (no sample concentration)
• Rapid (minutes)

Disadvantages
• Response varies between toxin structural analogues (cross-reactivity)
• Result does not necessarily correlate with toxicity (or any other assay method!)
• Other chemicals or salts in water may affect toxin binding
Cross-reactivity versus Toxicity

Cross-reactivity
(binding affinity rel. to STX)

- STX = 100%
- GTX2/3 = 23%
- C1/2 = 2%
- dcGTX2/3 = 0%

Relative Toxicity

- STX = 100%
- GTX2/3 = 42%
- C1/2 = 2.7%
- dcGTX2/3 = 70%
Assessment criteria

• No false negatives
• Detection of the range of toxin analogues found in SA (esp. PSPs)
• Accuracy (how defined?) with mixtures of analogues
• Reproducibility over replicate tests of known samples
  – (standards and cultures, field samples where available)
• Limit of detection
• Lack of interference from other compounds in cyanobacterial extracts or raw water
• Practicality and ease of use
Jellett Rapid Test for PSPs
JELLETT RAPID TEST
for Marine Biotoxin testing...

How it works...

1. Add 100 μL Shellfish extract (to black line of dispenser) to brown buffer vial

2. Mix buffer and extract together by inserting the dispenser in the buffer tube and squeezing dispenser bulb three (3) times

3. Fill dispenser up to the black line
   Empty dispenser contents into the sample hole

Wait 35 min for results

JELLETT RAPID TEST have given the identical results as the mouse bioassay test with 1000's of shellfish samples tested around the world
Control (non-toxic)

Positive (toxic)
Abraxis strip test for microcystins
Algal Toxin Strip Test (Microcystins) Source Drinking Water

1. Collect Sample
   - Collect 1 to 2 mL of sample.

2. Transfer/QuikLyse™*
   - Using the graduated pipette provided, transfer 1 mL of SAMPLE to the lysis vial containing the dried lysis reagent.
   - Cap and shake for 2 minutes.
   - Let rest for 8 minutes.

3. Add Reagent Paper/QuikLyse™**
   - Using the forceps provided, add 1 reagent paper to the lysis vial.
   - Cap and shake for 2 minutes.
   - Let rest for 8 minutes.

4. Transfer
   - Using the pipette provided, add 7 drops of SAMPLE to the conical, flip-top tube.
   - (The conical, flip-top tube contains dried reagents.)

5. Shake and Incubate
   - Close the conical, flip-top tube and shake for 30 seconds.
   - Incubate for 20 minutes.
   - (Dried reagents will dissolve, turning the sample purple.)

6. Test
   - Insert test strip into conical, flip-top tube with arrow pointing down. (Sample pad down.)
   - Incubate for 10 minutes.

7. Dry
   - Remove test strip. Lay flat and allow to continue developing for 5 minutes.

8. Interpret
   - ppb
   - 5.0
   - 2.5
   - 1.0
   - 0.5
   - 0

<table>
<thead>
<tr>
<th>CONTROL LINE</th>
<th>TEST LINE</th>
<th>INTERPRETATION</th>
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</thead>
<tbody>
<tr>
<td>NO CONTROL LINE PRESENT</td>
<td>NO TEST LINE PRESENT</td>
<td>INVALID RESULT</td>
</tr>
<tr>
<td>CONTROL LINE PRESENT</td>
<td>NO TEST LINE PRESENT</td>
<td>&gt;5 ppb</td>
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<tr>
<td>CONTROL LINE PRESENT</td>
<td>WEAK INTENSITY</td>
<td>BETWEEN 0 AND 5 ppb</td>
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For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Streamwood Drive, Warminster, PA 18974 Phone: 215-357-5911 Fax: 215-357-6232 www.abraxiskits.com
ABRAXIS Dilution Series 24/2/09.

5216 NEAT

1/3

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Overall Conclusions

- Speed & simplicity of Jellett good (LOD an issue)
- Envirologix, Abraxis ~OK but could be improved
- Useful to provide on site decision support for field staff & plant managers
- Cross-reactivity an issue – ideally need to know which toxin analogues to expect
- Sample preparation protocols important to minimise risk of false negatives
- Should not be relied upon for compliance testing
Acknowledgements

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