Monitoring & Management #23

DEVELOPMENT OF AN IMMUNOASSAY FOR AUTONOMOUS, SUBSURFACE DETECTION OF PARTICULATE MICROCYSTINS IN LAKE ERIE

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Rationale & Objectives

Timely detection and monitoring of harmful algal bloom (HAB) development and toxicity are of growing importance, especially for freshwater systems that supply drinking water to municipalities. The urgent need to advance detection capabilities was highlighted by the 'do not drink' advisory issued for over 400,000 Toledo, OH residents in August 2014.

MODIS satellite image of Lake Erie during a *Microcystis* bloom in the western basin (Sept., 2013)

The Environmental Sample Processor (ESP) is an autonomous, in-water instrument capable of assessing concentrations of potentially toxic HAB species and their toxins in near real-time. Although the ESP has been deployed extensively in marine coastal waters, this technology has not been utilized in freshwater systems to monitor potentially toxic cyanobacteria and cyanotoxins.

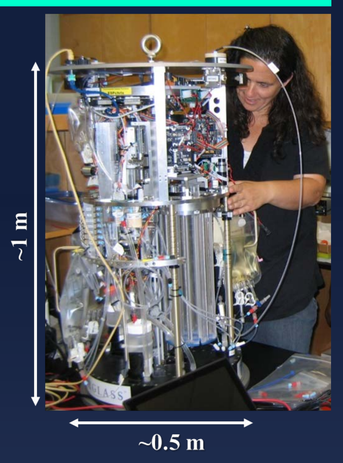
Given the current regulatory focus on drinking water contamination by microcystins (MCs) and the need for advanced warning of increasing toxin levels, ***our overall aim is to deploy ESP technology for particulate MCs detection in Lake Erie***.

Objectives of Current Research & Development:

1) develop & validate particulate MC extraction protocol (≥ 95% recovery)
2) develop & calibrate a MC competitive ELISA (cELISA) with 'in-water' sub-ng/mL limit of detection, taking into account sample matrix effects
3) conduct preliminary validation of ESP MC cELISA versus Abraxis ELISA kit

Environmental Sample Processor (ESP) Platform

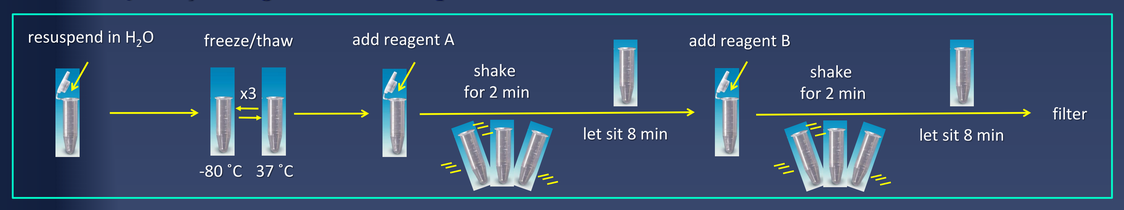
- ESP, designed by the Monterey Bay Aquarium Research Institute, is available commercially from McLane Research Laboratories
- robotic, electromechanical instrument deployed primarily sub-surface on a stationary mooring
- acquires, processes, analyzes samples for molecular-based detection and measurement of organism and metabolites (e.g., toxins) concentrations
- transmits data to remote sites in near real-time



Microcystin Extraction Protocol Development

- aim to achieve MC recovery similar to freeze/thaw + Abraxis QuikLyse™ 'gold standard' currently used routinely by NOAA-GLERL
- protocol logistics/chemistry compatible with ESP fluidics & functionality
- tested Abraxis QuikLyse™ vs. two modifications vs. various aqueous methanol percentages + 0.01% Tween 20 (with heating)

Abraxis QuikLyse™ 'gold standard' protocol



- ✓ ***Abraxis QuikLyse™ Modification A:*** no freeze/thaw; add reagents A & B together; let sit 10 min; filter
- ✓ ***Abraxis QuikLyse™ Modification B:*** no freeze/thaw; add reagent A; let sit 10 min; repeat for reagent B; filter
- ✓ ***AqMeOH:*** 5, 20, 50% aqMeOH + 0.01% Tween 20; heat 10 min, 60 °C; filter

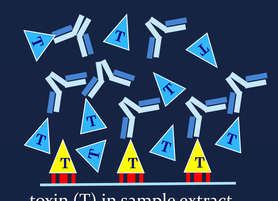
Extraction Protocol	Avg % recovery
Abraxis QuikLyse™ , modification A	13
Abraxis QuikLyse™, modification B	103
5% MeOH/0.01% Tween 20	101
20% MeOH/0.01% Tween 20	113
50% MeOH/0.01% Tween 20	97

Summary of Results

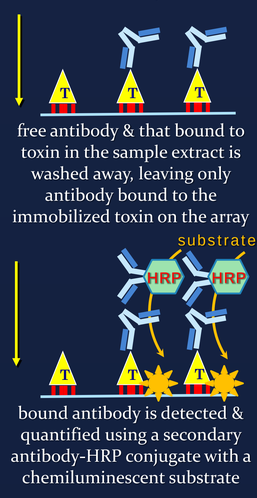
- **all protocols except QuikLyse 'Mod A' yielded acceptable recovery rates**
- ***50% MeOH/0.01% Tween 20 selected* based on prior use for ESP algal toxin extraction protocols (i.e., DA, PST)**

Microcystin ESP cELISA Development

Microcystin cELISA Schematic



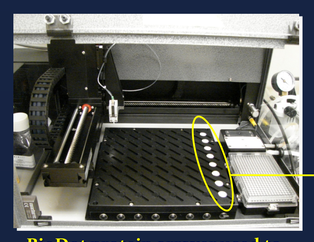
toxin (T) in sample extract competes with toxin immobilized on membrane surface for antibody in solution



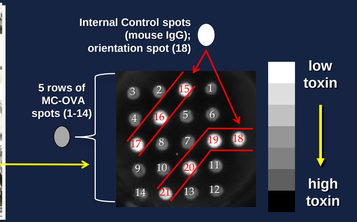
free antibody & that bound to toxin in the sample extract is washed away, leaving only antibody bound to the immobilized toxin on the array

bound antibody is detected & quantified using a secondary antibody-HRP conjugate with a chemiluminescent substrate

Printing & Development of Microcystin cELISA Arrays



BioDot protein arrayer used to print MC-OVA protein conjugate



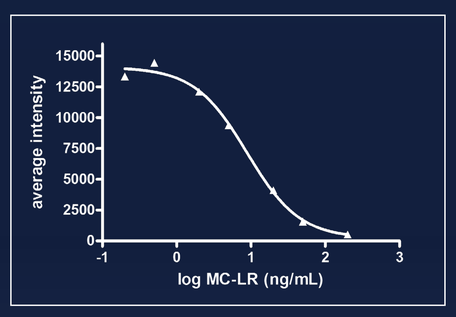
MC cELISA array map/image

ESP benchtop mimic

Microcystin cELISA Details

1) formatted as a competitive assay (left); signal intensity inversely proportional to toxin concentration in extract
2) employs commercial, 'ADDA-specific' monoclonal antibody for comparability with Abraxis MC ADDA ELISA response, independent of MC variants in sample
3) membrane array (above middle) spotted with MC-ovalbumin conjugate & mouse IgG chemistry controls
4) using ESP benchtop mimic (above right), optimize antibody & conjugate dilutions to maximize dynamic range, sensitivity, magnitude of linear response

MC cELISA Calibration & Preliminary Validation



MC cELISA calibration in presence of matrix (10⁶ *Microcystis* cells/mL extract)

- assay half-maximal response or EC_{50} is 8.8 ng/mL (in extract)
- range of quantitation (assay linear range; EC_{80}-EC_{20}) is 2.9 – 27.3 ng/mL; 'in extract' limit of quantitation (LOQ) ~3 ng/mL

Cyanobacterial Sample	Abraxis Kit (ng/mL)	ESP cELISA (ng/mL)	% Recovery
Microcystis aeruginosa (LB2385)	3.5	< LOQ	--
Anabaena cf. *lemmermanii* (AL02)	< LOQ	< LOQ	--
Planktothrix agardhii (SAG6.89)	5.6	4.9	88

Summary of Results

- **calibration of MC cELISA in matrix increased the assay EC_{50} by ~ 2-fold from 3.8 ng/mL (data not shown) to 8.8 ng/mL; assay linear range is ~ 1 order of magnitude**
- **estimated MC cELISA limit of quantitation (LOQ) for 0.2 L sample vol. on ESP ~0.03 ng/mL, taking into account 2 mL extraction volume used on ESP**
- **preliminary validation of MC cELISA agreed with Abraxis MC ADDA ELISA for 2 of 3 samples; sample LB2385 value was within 15% of cELISA LOQ and will be re-examined**

Conclusions & Future Work

- MC cELISA calibrated 'in matrix'; LOQ for 0.2 L sample on ESP ~50-fold less than EPA 10-Day Drinking Water Health Advisory (2015) level of 1.6 µg/L
- Preliminary validation of ESP MC cELISA vs. Abraxis MC ADDA ELISA showed general agreement, but much more extensive sample set is required
- Continue to optimize MC-OVA concentrations & antibody dilutions with aim of increasing assay sensitivity and magnitude of linear response
- Transition MC extraction and cELISA from benchtop to core ESP instrument; conduct initial field trials of MC cELISA in western Lake Erie on GLERL ESP & custom shallow-depth mooring during summer 2016

Acknowledgements: The authors gratefully acknowledge Drs. C. Elliott & K. Campbell (Queen's University Belfast) for preparation of MC-OVA conjugate. Funding provided by Great Lakes Restoration Initiative, Cooperative Inst. for Limnology & Ecosystem Research, OAR-Great Lakes Environmental Research Lab., NOS-Ctr. for Coastal Environmental Health & Biomolecular Research.