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Environmental Measurements Methods

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sample
Microcystin
Cylindrospermopsin

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ABSTRACT

Harmful algal blooms in freshwater systems are increasingly common and present threats to drinking water systems, recreational waters, and ecosystems. A highly innovative simple to use, portable biosensor system (MBio) for the rapid and simultaneous detection of multiple cyanobacterial toxins in freshwater is demonstrated. The system utilizes a novel planar waveguide optical sensor that delivers quantitative fluorescent competitive immunoassay results in a disposable cartridge. Data are presented for the world's first duplex microcystin (MC) / cylindrospermopsin (CYN) assay cartridge using a combination of fluorophore-conjugated monoclonal antibodies as detector molecules. The on-cartridge detection limits of 20% inhibitory concentration (IC_{20}) was 0.4 µg/L for MC and 0.7 µg/L for CYN. MC assay coverage of eight important MC congeners was demonstrated. Validation using 45 natural lake water samples from Colorado and Lake Erie showed quantitative correlation with commercially available laboratory-based enzyme linked immunosorbent assays. A novel cell lysis module was demonstrated using cyanobacteria cultures. Results show equivalent or better performance than the gold-standard but more tedious 3X freeze-thaw method, with > 90% cell lysis for laboratory cultures. The MBio system holds promise as a versatile tool for multiplexed field-based cyanotoxin detection, with future analyte expansion including saxitoxin, anatoxin-a, and marine biotoxins.

1 INTRODUCTION

Harmful Algal Blooms (HABs) caused by cyanobacteria are increasing in frequency and duration and constitute a public health threat in addition to causing substantial economic losses, ecological damage, and food supply concerns[1]. Recent events in the United States demonstrated significant impacts on drinking water systems (Toledo, 2014[2]), recreational waters (Florida, 2016[3]), and public health (Utah, 2016[4]). The World Health Organization (WHO) and the US Environmental Protection Agency (EPA) have established guidelines for toxin levels in drinking water. The WHO has established a guideline that drinking water should have less than 1 μg/L of microcystin-leucine-arginine (MC-LR) including both free and intracellular toxin[5]. In 2016 the US Environmental Protection Agency (EPA) developed health advisories for toxins from cyanobacteria and recommended ≤0.3 μg/L for MCs and ≤0.7 μg/L for cylindrospermopsin (CYN) for children under 6 years of age and ≤1.6 μg/L of MC and ≤3.0 μg/L of CYN for anyone above 6 years of age[6, 7]. The US EPA also recently released draft Ambient Water Quality Criteria (AWQC) / Swimming Advisory levels at 4 and 8 μg/L for MC and CYN[8], respectively. Similarly, in the EU the European Commission has issued a proposal for a recast of Directive 98/83/EC to have a revised Directive on the quality of water intended for human consumption whereby MC-LR is included as a chemical parameter with a parametric value of 1 μg/L[9].

Fluorometric detection of phycocyanin and chlorophyll pigments can provide useful information on algal bloom composition and biomass, but these measurements do not indicate whether a bloom is producing toxin, which can vary with species, strain or sub-population[10], growth stage, and environmental conditions[11]. Toxin detection is generally based on laboratory methods, which means samples must be collected, packaged, and shipped to reference laboratories for testing. Laboratory methods for cyanobacterial toxin detection[12] include Enzyme-Linked ImmunoSorbent Assay (ELISA)[13-16], protein phosphatase inhibition assay[17-21], liquid chromatography / mass spectrometry (LC/MS)[22-26], micro-sphere flow cytometry[27], and mouse bioassay[28, 29]. Field-portable strip tests are available, but the strip tests are qualitative, only test for one toxin at a time, and require several user steps. As a result, there is a

significant and unmet need for a rapid, easy-to-use, field-portable system for real time monitoring of HAB toxins offering high performance, semi-quantitative results and multiplexed detection comparable to state-of-the-art laboratory methods. MBio Diagnostics is developing a cost-effective platform technology designed to meet these needs.

MBio uses a novel method of combining planar waveguide illumination, fluorescence imaging, and microarray technology to deliver a multiplexed fluorescence immunoassay. The core technology is based on simple disposable cartridges analyzed on a portable reader. The research community has been using the MBio technology to develop assays for multiplexed HAB toxin detection in freshwater and marine systems[24, 30-33]. McNamee *et al.* demonstrated a 5-plex toxin assay that included a combination of freshwater (MC, CYN) and marine biotoxins (saxitoxin, domoic acid, okadaic acid)[30]. Murphy *et al.* demonstrated sensitive, quantitative, reproducible MC detection using recombinant antibodies on the platform[32]. Devlin *et al*. combined a novel sample preparation method with MC detection[24]. Additional research has demonstrated toxin and other small molecule detection in marine samples[31], fish tissue[33], and milk matrices[34].

This study builds on that body of work and advances the platform by significantly simplifying the assay workflow whilst maintaining quantitative assay performance. The aim of this study is to demonstrate a one-step, 10-minute, duplex MC/CYN assay, which can be combined with a simple, 10-minute field-portable mechanical lysis method, enabling rapid, on-site detection of dissolved or total toxins. Together, these methods allow for the first demonstration of a field-portable, semi-quantitative duplex assay.

1.1 MBio Cartridge and Reader

The MBio platform is a novel, patent-protected system that uses planar waveguide and microarray technology to deliver a sensitive, multiplexed fluorescence assay in a simple fluidic cartridge. Fig. 1 provides a schematic of the core elements of the system, which is referred to as LightDeck® technology. A solid-state diode laser (639 nm) is used as the illumination source. The cartridge integrates

a laser light-coupling lens into the injection molded plastic waveguide, which couples and launches the laser light down the plastic substrate. The multimode waveguide generates an evanescent illumination field at the solid assay surface. By printing a spatial array of capture spots on the assay surface of the waveguide, the system enables multiple spot assays to be run simultaneously in every cartridge. Evanescent field illumination makes the system relatively insensitive to the solution phase components above the assay surface. This enables assays to be conducted in the presence of complex sample matrices without requiring wash steps. The simplicity of the MBio LightDeck® technology makes the system robust yet inexpensive.

Simultaneous MC and CYN competitive immunoassays are run in each single-sample fluidic cartridge, illustrated schematically in Fig. 2. Toxin-protein conjugates are printed on the assay surface of the waveguide in a two-dimensional array using non-contact microarray printing. The detection reagent is a lyophilized mixture of fluorescently labeled antibodies against MC and CYN that is rehydrated with the sample. By directly conjugating the primary detection antibodies with fluorophore, a one-step, multiplexed immunoassay is enabled. There is no need for anti-species detection antibodies, enzyme

conjugates, washes, color detection or stop solutions. Further, the lyophilized detection reagent contains a proprietary mix of salts, buffers, and blocker molecules that mitigate the effects of variability in raw water samples. Finally, lyophilizing the detection reagent improves the long-term packaged stability.

To measure the toxin contained in a filter or extracted sample, add it to the lyophilized detection reagent, aspirate/dispense, immediately transfer to the MBio cartridge, and insert the cartridge into the reader. The reader will automatically perform the assay by releasing the sample mixture down a fluidic channel. In the absence of toxin in the sample, the antibodies bind to the MC- and CYN-conjugate spots on the surface yielding bright spots on the microarray. When toxin is present, antibody binding to the conjugate spots is competitively inhibited and the fluorescence signal is reduced. Custom software performs all spot finding and image analysis and converts image data into a quantitative output.

Cartridges are factory-calibrated; unlike ELISA, users do not have to generate calibration curves with each sample run. Calibration curves for each cartridge lot are loaded into system software, which allows a single cartridge to deliver a quantitative (μg/L) output for both MC and CYN. This factory calibration accounts for small variations in spot intensity between lots. Packaged cartridges are shelf-stable and remain calibrated for months. The quantitative result is accurate for the MC-LR and CYN congeners. The assays, however, do not differentiate between congeners and the quantitative output depends on congener cross-reactivity, discussed in more detail below.

1.2 Portable Lysis Module

Algal cells that produce intracellular toxins must be lysed to determine total toxin (dissolved and particulate). Freezing and thawing the sample three times (3X freeze thaw) is the gold standard laboratory method, but this is not a field-portable option and is time-consuming when running single samples in the lab. Devlin *et al*. described a simple, effective method that combined glass beads with a milk frother for mechanical cell lysis[24]. MBio has significantly improved this method by eliminating all filtration and pre-concentration steps and modifying the mixing head to improve lysis efficacy. The result is a battery-operated mechanical lysis module that efficiently lyses 5 mL raw water samples in 10 minutes without any hands-on time from the operator. The method is simple and environmentally friendly, as there are no lysis chemicals required.

2 MATERIALS AND METHODS

2.1 Materials

Antibodies and Toxin-Protein Conjugates. The MC and CYN competitive immunoassays use proprietary murine monoclonal antibodies (mAbs) sourced from Xenobics Limited (Belfast, Northern Ireland). For microcystin, the immunogen was an intact MC-LR conjugated to bovine serum albumin[35]. Although the antibody was raised against the LR variant, clone selection was based in part on cross-reactivity against other MC congeners. The antibody to cylindrospermopsin was raised to

131 cylindrospermopsin conjugated to keyhole limpet hemocyanin as the immunogen[13]. For the one-step
132 assay format, the MC and CYN mAbs were directly labelled with the fluorescent dye CF®640R (Biotium)
133 using NHS chemistry. Purification was performed with size exclusion chromatography spin columns to
134 remove excess unbound dye (Amicon, Zeba). The purified dye-labeled antibody was then diluted to a
135 stock concentration in a storage buffer (1x PBS, 10 mg/mL BSA, 0.02% v/v Tween-20) containing
136 preservative (Proclin 300).

137 Microcystin- and cylindrospermopsin-ovalbumin (OVA) conjugates used for array printing were
138 purchased from Xenobics.

139 *Toxin Reference Standards*. Certified reference materials dissolved in methanol were purchased
140 for MC-LR, MC-RR, nodularins (NODR), dM MC-LR and CYN from the National Research Council of
141 Canada (NRCC). MC-LA, MC-LF, MC-YR, and MC-LW were purchased from Enzo Life Sciences and
142 were reconstituted in methanol according to the product data sheets. Further dilutions of all reference
143 materials were performed with reagent water.

144 *Water, Buffers, and Other Reagents*. The term reagent water refers to RNase-free, DNase-free,
145 protease-free bottled laboratory water sourced commercially from Sigma Aldrich or ThermoFisher
146 Scientific. Phosphate buffered saline (PBS), Tween-20, Saponin, Triton 100-X, Polymyxin B, Lugol's
147 iodine, and bovine serum albumin (BSA) were purchased from Sigma Aldrich.

148 **2.2 Algal Culture**

149 Two strains of *Microcystis aeruginosa* (LB 2385, LB 2063) and one strain of *Cylindrospermopsis*
150 sp. (LB 942) were purchased from the Culture Collection of Algae at the University of Texas at Austin
151 (UTEX). One strain of *Cylindrospermopsis* sp. (CS506) that produces CYN was supplied by Bowling
152 Green State University. *Microcystis* LE3 cultures were provided by the National Center for Coastal
153 Ocean Science (NOAA, Charleston, SC). All glassware and media were autoclave sterilized prior to use.
154 Inoculation and all culture handling procedures were performed in a sterile Class II biosafety cabinet.
155 Cultures were maintained at 20 °C ± 2 °C on a 16:8 h light:dark cycle (light levels were $0.3x10^{16}$

quanta/sec/cm^2) in sterile BG-11 medium purchased from UTEX or WC medium. Every three weeks, 2 mL of culture were withdrawn and added to 200 mL of medium and allowed to grow for between 3-6 weeks prior to use in experiments. The exponential growth phase occurs for approximately 1 week before entering a stationary phase.

2.3 Lake Water Samples

Lake water samples were collected from several sources in Colorado (USA) and from locations on Lake Erie (USA) during July and August 2017. Colorado water samples were sourced with the cooperation of water utilities in the cities of Boulder, Aurora, Thornton, and Westminster. [It should be noted that none of the water sources used for drinking water in Colorado had detectible toxins in this study.]. Through cooperation with the City of Northglenn, Colorado, samples from an active bloom in a retention pond were collected. Other samples with active blooms were collected from unused storage ponds in Thornton. Samples were also collected from an active bloom in a private pond in Boulder with permission. Lake Erie samples were collected at sampling stations near Toledo, Ohio in collaboration with the University of Toledo, and from sampling stations in Sandusky Bay in collaboration with Bowling Green State University.

All samples were collected in glass containers. Colorado lake water samples were measured on the day of collection as fresh samples, then aliquots were frozen at -20 °C for later testing. Lake Erie samples were shipped frozen in glass containers.

2.4 Sample Preparation (Lysis)

Cell lysis via 3X freeze-thaw was performed per EPA Method 546[36]. For the MBio portable lysis module, a 5 mL natural water or culture sample was added to a clean 1-ounce glass jar containing 5 g of 0.1-0.15 mm glass beads. The sample was then agitated with a custom-designed battery operated mixer for 10 minutes. After the motor stops, beads immediately settle to the bottom of the sample. Sample for the assay was withdrawn via pipet transfer from the layer of liquid above the beads and below the foam generated by frothing, and immediately transferred to the tube containing dried reagents, mixed with the

reagents, and then added to the cartridge. This method is a simplification of the previously published method[24], where filtration and concentration steps were used. The simplified method presented here is one that can be easily performed in the field.

Cell lysis efficiency was reported as percent lysis and was measured with light microscopy and manual cell counts were performed with a hemocytometer. Samples were mixed 1:1 with Lugol's iodine before loading the hemocytometer. Cells in the central 25x25 squares in the hemocytometer were counted for a total volume of 0.1 μL. If fewer than 400 cells were counted in this volume, additional squares were counted to improve the counting statistics. With 400 counted cells, the statistical error was 5%.

2.5 Array Printing and Cartridge Assembly

MBio waveguides were injection-molded using a proprietary low autofluorescence polymer resin. Waveguides were activated with a custom plasma-silanization process prior to array printing. The fluidic channel was defined using a pressure-sensitive adhesive (PSA) gasket. Toxin-protein conjugates were printed to the waveguides using a non-contact arrayer (Scienion S11). Printed waveguides were washed, a blocker-stabilizer was applied, and an injection-molded plastic upper component was bonded via a pressure sensitive adhesive gasket with a pneumatic press to form the cartridge. Cartridges were packaged in barrier pouches with desiccant prior to use.

2.6 MBio Assay Workflow

100 μL of the prepared sample were added to the kit microtube containing dried reagents and mixed via pipet aspirate/dispense five times to ensure reagent rehydration. The mixed sample was then immediately transferred to the inlet port of the cartridge and the cartridge was inserted into the reader. The MBio reader automatically read the cartridge barcode, confirming assay type, authenticity, and expiration date. The user entered a sample name into the software interface and pressed "start" to initiate the measurement. The reader automatically released the sample-antibody mixture into the detection chamber containing the capture array. The assay was timed automatically, and after 10 minutes the

proprietary software converted the array images into quantitative measured concentrations. In summary, the user mixed the sample and reagents, loaded the cartridge, inserted it into the reader, entered the sample name, and started the program. Hands-on assay time was less than one minute.

2.7 Reference MC and CYN ELISAs

As a reference, two competitive enzyme-linked immunosorbent assay (ELISA) kits were purchased from Abraxis: Microcystins/Nodularins (ADDA) (P/N 520011OH) and Cylindrospermopsin (P/N 522011). The kits were used in accordance with the manufacturer's instructions. The ADDA ELISA is the reference immunoassay used in US EPA Method 546[36].

3 RESULTS AND DISCUSSION

3.1 Assay Analytical Performance

When developing the MC and CYN assays on the MBio platform, several parameters were used to "tune" the assay sensitivity. Assay time, toxin-protein print concentration, and detection antibody concentration all influence the assay curve, with antibody concentration providing the strongest effect. For this demonstration, an IC_{50} of 1 µg/L for the MC and CYN assays was targeted. By centering the assay's detection range on 1 µg/L, a sensitive assay relevant for field testing with various applications (source waters, recreation, etc.) was delivered. It is important to note that assay sensitivity could be further adjusted up or down by titration of the detection antibody concentration.

Analytical performance was based on the analysis of serial dilutions of certified reference materials into reagent water. The output intensities were normalized to the intensity at zero toxin (B_0) and are plotted in linear-log plots of B/B_0 versus toxin concentration. 4-parameter logistic fits were used to define max, min, and 50% inhibitory concentration (IC_{50}). Fig. 3a provides a standard curve for the MC assay generated using MC-LR (NRCC standard) diluted into reagent water. IC_{50} is 1.1 µg/L and dynamic range reported as IC_{20} to IC_{80} was 0.4 to 3.1 µg/L. Fig. 3b provides a standard curve for the CYN assay generated using CYN (NRCC standard) diluted into reagent water. IC_{50} is 1.4 µg/L and dynamic range reported as IC_{20} to IC_{80} was 0.7 to 2.7 µg/L.

231 **3.2 Microcystin Congener Coverage**

232 Although MC-LR is the congener used in most safety guidelines[37], there are over 100 known
233 congeners[38] of microcystin, most with unknown toxicities. To be useful as a field microcystin test, the
234 MBio assay must show good coverage across a range of MC congeners. To test coverage of a range of
235 common toxins, standard curves for 8 different congeners were run on the MBio assay. MC-LR, MC-
236 RR, and MC-YR were chosen because they are the most common and toxic variants[39] followed by MC-
237 LA, MC-LW, and MC-LF[40]. Nodularins, produced by *Nodularia*[41], are structurally similar to MC.
238 [Dha^7]-microcystin-LR (dM MC-LR) is a cyclic peptide toxin also produced by cyanobacteria. Results
239 (see Fig. 4) show that although the IC_{50} was not the same for all congeners, the MBio assay provides
240 reasonable coverage for the 8 variants tested.

241 Using the same congener dilutions as on the MBio assay, calculated cross-reactivities on the ADDA-
242 ELISA were 100%, 131%, 167%, and 53% for NRCC standards MC-LR, dm MC-LR, NODR, and MC-
243 RR, respectively. Cross-reactivities for the Enzo toxins MC-LA, MC-LF, MC-YR, and MC-LW were
244 133%, 96%, 96%, and 100%, respectively. Since the MBio MC mAb was developed with a MC-LR
245 conjugate as the immunogen, a different cross-reactivity profile is expected relative to an immunoassay
246 directed specifically against the ADDA moiety[42, 43]. Variable cross-reactivities for all currently available
247 immunoassays (including MBio and the commercial ELISAs) could lead to either under-estimating or
248 over-estimating toxin concentrations depending on congeners in the sample[44]. By selecting an MC mAb
249 with reasonable congener cross-reactivity and standardizing against the highly toxic MC-LR congener,
250 MBio has developed a rapid, portable assay that should be broadly applicable for different toxin
251 producing blooms. Knowledge of congeners present in the sample, for example from LC/MS/MS data,
252 could be used to refine quantitative accuracy of the immunoassays. In particular, MBio software has the
253 ability to adjust calibrations if the dominant MC congener in the sample is known.

254 MBio did not test a range of CYN congeners. 7-deoxy-CYN is a known congener, but has low toxicity
255 compared to CYN. Although not tested on the MBio platform, the CYN antibody used in this study has

been shown to have low cross reactivity to 7-deoxy-CYN.[13] This is a potential advantage in a field testing platform where the utility is with toxic variant detection.

3.3 Cylindrospermopsin Producing Cell Culture

Since natural water samples containing CYN were difficult to obtain at the time of this study, a CYN producing cell culture (CS506) was diluted and measured on the MBio platform and compared to the same dilution as measured on ELISA. 3X freeze thaw was used to lyse the cell culture prior to measurement. There was direct correlation between the measured concentrations with one dilution measuring 1.8±0.1 µg/L on the MBio platform and 1.91±0.04 on ELISA. Similarly, another dilution measured as 0.6±0.1 µg/L on the MBio platform and 0.61±0.02 µg/L on ELISA.

3.4 Sample Preparation (Lysis)

The MBio mechanical lysis module was demonstrated using cultures of three cyanobacterial strains (UTEX 2385, UTEX 2063, and UTEX 942) and results are shown in Table 1. Microscopy was used to quantify cell lysis. Table 1 results show that cell lysis efficiency of the MBio module was equivalent or superior to the standard three freeze-thaw method for these cultured cells. In addition, MC toxin levels were measured before and after cell lysis with the toxin producing *Microcystis* cultures UTEX 2385 and LE3 and the measured toxin levels increased significantly after cell lysis. For example, prior to lysis the LE3 cell culture measured MC at 44 µg/L and after lysis it was 410 µg/L.

Since natural blooms have cell morphology and colony structure different from that encountered in laboratory cultures, it was important to test the lysis method with naturally occurring algae. Scum samples from a storage pond containing *Microcystis, Anabaena, and Aphanizomenon* were collected and measured on the day of collection. No toxin was detected prior to lysis. After 10-minute lysis on the MBio system, MC concentration was 25 µg/L. The same sample run after 3x freeze-thaw gave 22 µg/L MC. Cell counts were performed prior to lysis, after lysis with the MBio mechanical cell lysis method, and after 3X freeze thaw. Results are presented in Table 2 and demonstrate that the mechanical method is equivalent to 3X freeze thaw.

3.5 Comparison of Natural Lake Water Samples Measured with MBio and ELISA

Natural water samples were run on the MBio MC/CYN duplex assay and in parallel on the ADDA-ELISA and CYN-ELISA assays. Nine samples from Colorado drinking water reservoirs showed no detectable toxin on either assay. This is an important first step in establishing specificity of the MBio assay

in natural samples. Two scum samples from a retention pond with an active bloom were phenotypically identified to contain mixed colonies of *Microcystis aeruginosa* and *Anabaena*. Both samples showed very high MC toxin levels on MBio and ADDA-ELISA. 20-fold dilutions were required to bring samples into quantitative range. Good correlation was observed between MBio and ELISA. Scum sample 1 yielded 16 μg/L total MC on MBio and 20 μg/L total MC on ADDA-ELISA. Scum sample 2 yielded >80 μg/L total MC on both MBio and ADDA-ELISA. A second pond bloom was phenotypically suggestive of *Euglena* and *Peridinium*, neither

of which are expected to produce MC or CYN. Neither toxin was detected on the MBio or ADDA-ELISA assays. Importantly, this result demonstrates that despite the high cell load of 4×10^9 cells/L, the rapid, no-wash MBio assay did not report false positives for toxin.

Thirteen samples from stations across the western basin of Lake Erie were run on the MBio MC/CYN assay and in parallel on the ADDA-ELISA and CYN-ELISA. These sample were shipped frozen to MBio, and a 3X freeze-thaw protocol per US EPA Method 546[36] was run prior to splitting the samples onto the respective platforms. None of the samples showed detectable CYN on either MBio or the commercial ELISA. Table 3 provides a summary of MC results. Samples that were expected to contain significant toxin levels were measured with a 1:10 dilution. Sample 7M, week 0 was not expected to contain the significant toxin levels, so it was only run neat at the time of the study. These data show good quantitative correlation between the MBio assay and the ADDA-ELISA (R^2=0.78).

Twenty samples from stations across Sandusky Bay were run on the MBio MC/CYN system and parallel ADDA-ELISA and CYN-ELISA assays. Again, none of these samples showed detectable CYN on either assay. MC results are provided in Table 4. Data show that while there is overall correlation between MBio MC/CYN and ADDA-ELISA (e.g, low toxin to high toxin, R^2=0.85), MBio shows lower total MC relative to the ADDA-ELISA assay. The Sandusky samples were tested at the same time as the western Lake Erie samples, so the offset is not expected to be the result of laboratory or operator differences. Sandusky Bay is expected to have a diverse bloom profile, with *Microcystis aeruginosa*, *Planktothrix*, and other toxin producers possible (personal communication, George Bullerjahn). An explanation for the lower total MC concentrations could therefore be the result of different congener reactivities for MBio MC/CYN system relative to the ADDA-ELISA. In the absence of specific congener profiles and quantification, it cannot be determined *a priori* which measurement is the most accurate. We also note that there is discussion in the literature that the ADDA-ELISA could inherently over-report total MC concentrations given the sensitivity to free ADDA due to degraded toxin[44, 45].

Results presented in this manuscript demonstrate that a rapid, highly sensitive multiplex assay has been developed for the semi-quantitative, simultaneous screening of MC and CYN cyanotoxins. Monitoring programs for HAB biotoxins are increasingly becoming a necessity because of the potential dangers to human health and significant economic impacts. As monitoring in other areas such as food safety moves towards the implementation of methods based on performance criteria and harmonization of standards that are fit-for purpose and adaptable in suitability for end users, there is scope for alternative procedures and technologies. The MBio platform has shown the potential to offer next generation HAB toxin monitoring with a multiplex assay system that goes beyond current state-of-the-art detection and has the flexibility to incorporate additional biotoxins of concern without modifying the workflow. The next step would be a full comparison with state of the art LC-MS methods, but the drawbacks would be in the limited selection of analytical standard congeners available for a full quantitative comparison.

Safety. MBio staff are trained in the safe handling of biohazards. Toxin-producing algal cultures were handled in a certified biosafety cabinet in MBio's Biosafety Level 2 laboratory. Undiluted toxin stocks were handled in a chemical fume hood.

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Notes

MBio Diagnostics, Inc. is a commercial organization. SRB, PP, and MJL are employees of MBio Diagnostics.

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5 Figures

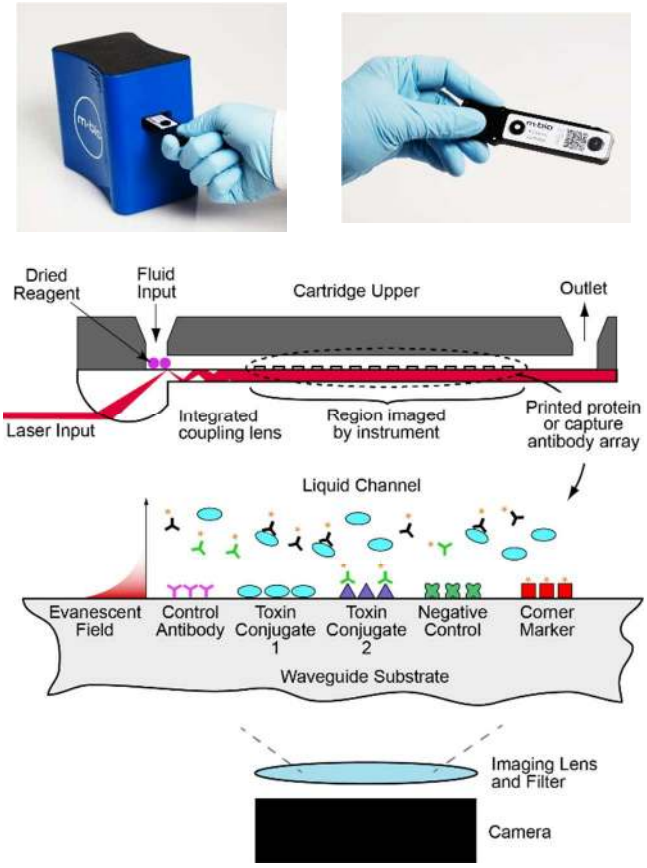

Figure 1. (*Top*) MBio reader and cartridge. (*Bottom*) Schematic of LightDeck® technology elements.

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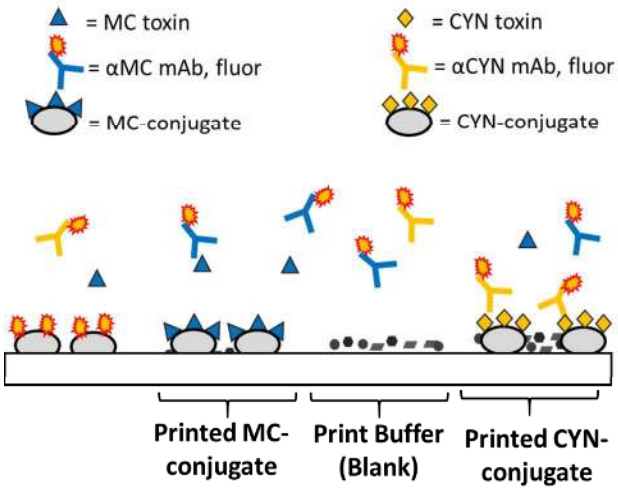

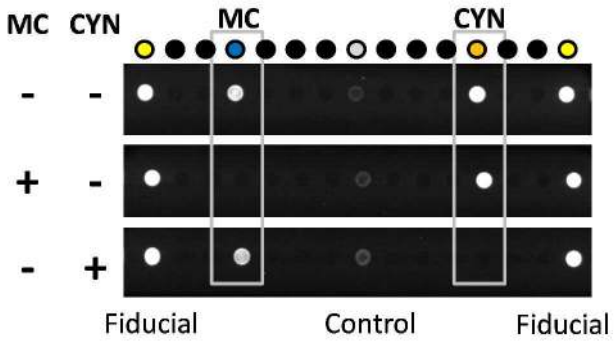

Figure 2. (*Top*) Cross-sectional schematic of the competitive immunoassay array concept. Not all array elements are represented. (*Bottom*) Representative array images for different assay conditions. A 1x15 spot array is printed. MC and CYN toxin-conjugate spots are indicated. Other spots in the array are print buffer blanks and process / fluidics controls. Array spots are ~0.6 mm diameter on a 1.25 mm spot-to-spot grid spacing.

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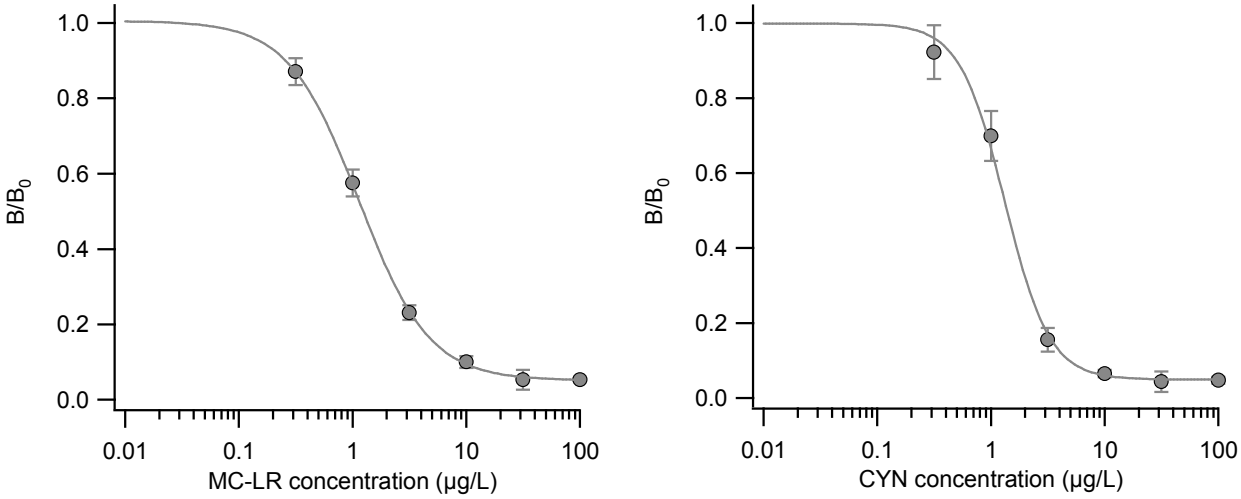

Figure 3. MC and CYN standard curves generated using certified reference materials from the NRCC in reagent water using the 10-minute MBio MC/CYN assay cartridge. Each point is the average of 3 replicates. Error bars are ± one standard deviation. Data are plotted with a 4-parameter logistic fit.

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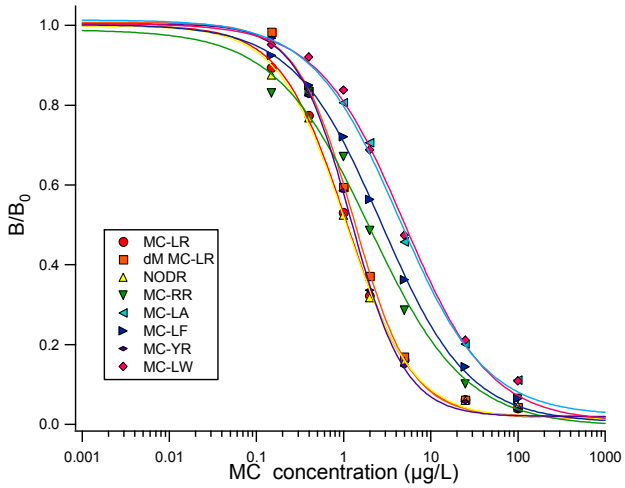

Congener	MBio
MC-LR	100%
dM MC-LR	83%
NODR	100%
MC-RR	53%
MC-LA	28%
MC-LF	40%
MC-YR	83%
MC-LW	26%

Figure 4. Microcystin congener standards run in serial dilutions on the MBio MC/CYN cartridge. Each symbol is the average of triplicate cartridges. Four parameter logistic fits were applied to each congener dilution series.

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6 Tables

Table 1. Comparison of Lysis Methods

Organism:	*Microcystis*[1]		*Cyl.*[2]
Identifier:	UTEX 2385	UTEX 2063	UTEX 942
Cell Conc. (cells/μL) x10^4	2.1	1.6	1.2
Method	**Percent Lysis**		
3X Freeze-Thaw	62%	94%	76%
MBio Mechanical	84%	95%	99%

[1] *Microcystis aeruginosa*

[2] *Cyl. = Cylindrospermopsis* sp.

Table 2. Comparison of Lysis Methods with Natural Water Samples

Organism:	*Aphanizomenon*	*Anabaena*	*Microcystis*[1]
Cell Conc. (cells/μL) x10^4	1.3	0.3	24
Method	**Percent Lysis**		
3X Freeze-Thaw	99.8%	98.4%	98.7%
MBio Mechanical	99.2%	99.5%	99.9%

[1] *Microcystis aeruginosa*

Table 3. Microcystin Detection, Lake Erie

Sample Name	**MBio (μg/L)**	**ADDA ELISA (μg/L)**
GR1, Week 0	< 0.6	0.2
GR1, Week 4	2.4±0.1	2.1
MB20, Week 0	< 0.6	0.2
7M, Week 0	> 4.0	> 5.0

7M, Week 1	< 0.6	0.2
4P, Week 0	< 0.6	< 0.15
4P, Week 1	< 0.6	0.4
CRIB, Week 1	< 0.6	0.5
CRIB, Week 4	2.0±0.1	1.8
8M, Week 1	< 0.6	0.3
MB18 Week 4*	6.4±2.0	6.7
8M, Week 4*	4.5±0.2	6.4
Buoy (EW 5), Week 4*	4.8±1.0	7.9

* These samples were diluted 1:10 to bring into assay range; reported results correct for the dilution. Standard deviations of the results on the MBio assay are reported for three replicates samples within the quantitative range.

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Table 4. Microcystin Detection, Sandusky Bay

Sample Name	**MBio (μg/L)**	**ADDA ELISA (μg/L)**
EC Station 1163, Week 3	1.5±0.2	2.1
EC Station 1163, Week 5	< 0.6	1.8
ODNR 1, Week 1*	2.7±0.2	6.5
ODNR 1, Week 3*	2.8±0.6	5.4
ODNR 2, Week 1	1.8±0.1	2.8
ODNR 2, Week 3*	3.3±0.6	6.1
ODNR 2, Week 5*	2.5±0.1	6.0
ODNR 4, Week 1	0.6±0.4	0.7
ODNR 4, Week 5	2.0±0.2	4.1
ODNR 6, Week 1	2.0±0.1	3.1

ODNR 6, Week 3*	2.6±0.9	6.2
ODNR 6, Week 5*	2.9±0.3	6.2
Channel Bells, Week 1	1.0±0.2	1.3
Channel Bells, Week 3	< 0.6	0.3
Channel Bells, Week 5	< 0.6	0.5
Buoy 2, Week 1	2.4±0.2	4.8
Buoy 2, Week 3*	2.1±0.1	5.2
Buoy 2, Week 5	1.6±0.1	3.2
Edison Bridge, Week 3*	2.6±0.7	6.9
Edison Bridge, Week 5*	2.5±0.6	6.5

* These samples were diluted 1:10 to bring into assay range; reported results correct for the dilution. Standard deviations of the results on the MBio assay are reported for three replicates samples within the quantitative range.

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