Portable System for Early Detection of Harmful Algal Bloom Toxins

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SUMMARY

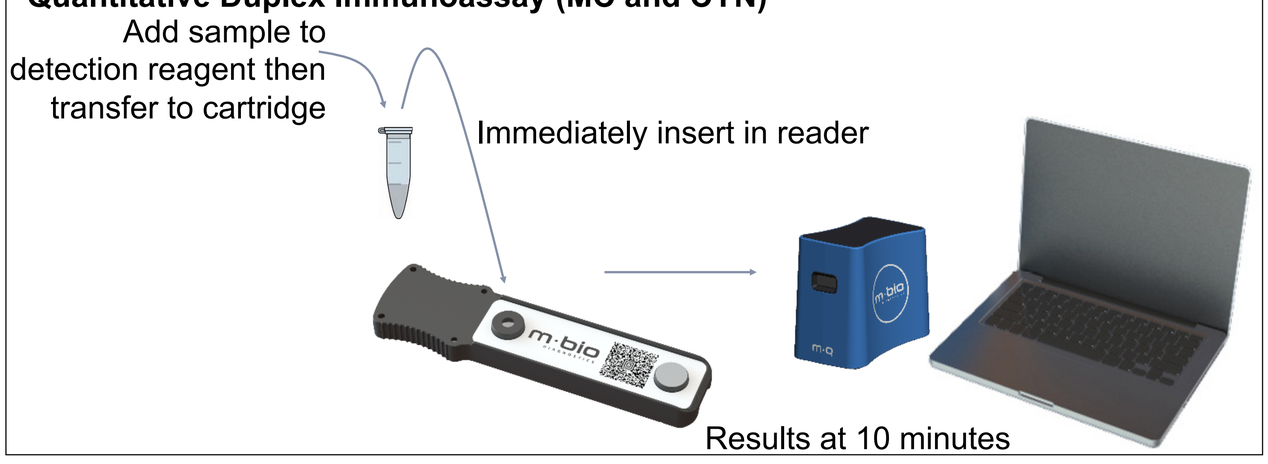
- **Duplex microcystin (MC) and cylindrospermopsin (CYN) direct toxin detection assay with quantitative results**
- **Field portable lysis method**
- **Can go from bloom to cell lysis and detection in 20 minutes**
- **Portable, low cost system**

SYSTEM FEATURES:

- **Multiplexed** – 6 or more (depending on assay)
- **Rapid** – 10 minutes assay plus 10 minutes lysis
- **Quantitative** – fluorescence immunoassay
- **Sensitive** – 0.5 ng/mL detection limit on MC
- **Robust** – field-ready technology
- **Versatile** – insensitive to debris in water sample

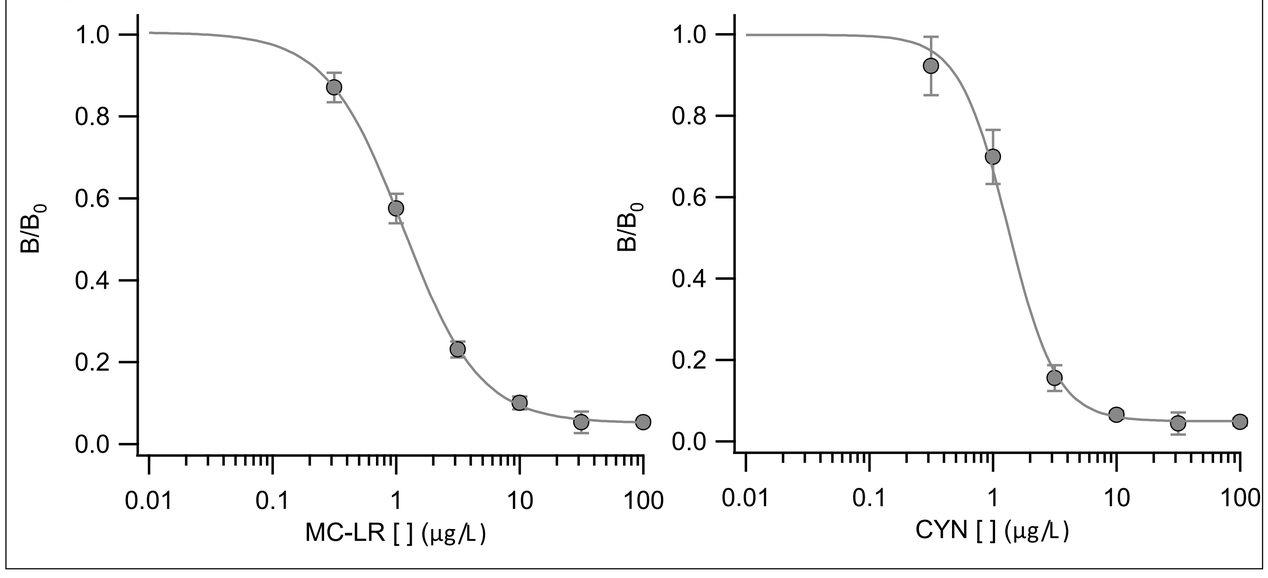
GEN 1 ASSAY FORMAT

Quantitative Duplex Immunoassay (MC and CYN)



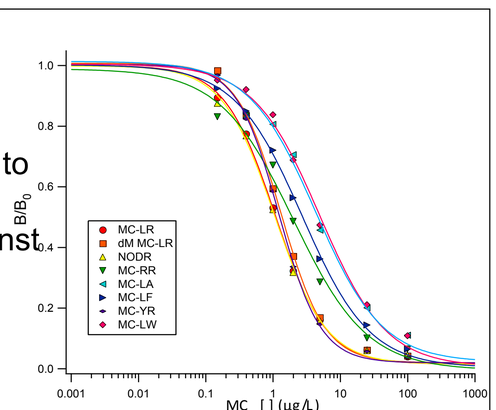
CALIBRATION / ANALYTICAL PERFORMANCE

- Factory calibrated which lasts for at least 6 months at room temperature and 12 months refrigerated
- Allows for the easy measurement of one or a few samples without the user measuring calibration curves
- Typical LOD is 0.5 µg/L for MC and 0.7 µg/L for CYN
- Typical standard curves for calibration are shown below



CONGENERS

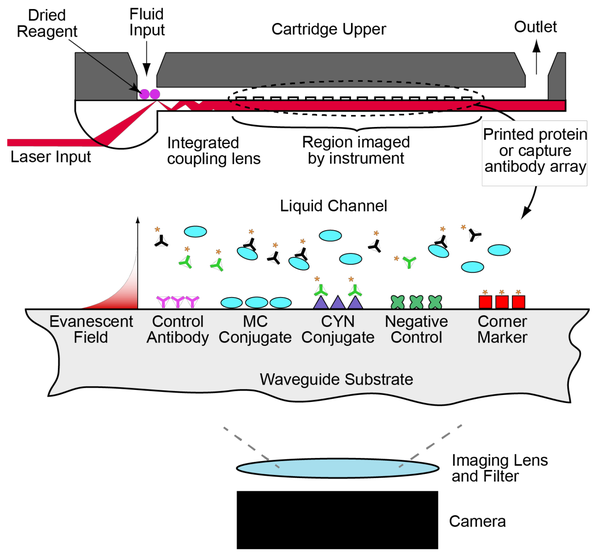
- Goal is detection of total microcystin
- Proprietary monoclonal antibodies
  - Immunogen was an intact MC-LR conjugated to a carrier
  - Clone selected based on cross-reactivity against other congeners
- Dilution series performed with each of the congeners
- Certified Reference Materials
  - MC-LR, MC-RR, Nodularins, dM MC-LR
- Other Congeners
  - MC-LA, MC-LF, MC-YR, MC-LW

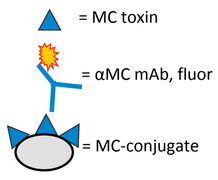


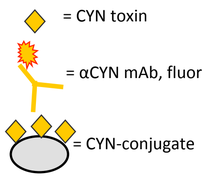
See Reference 1

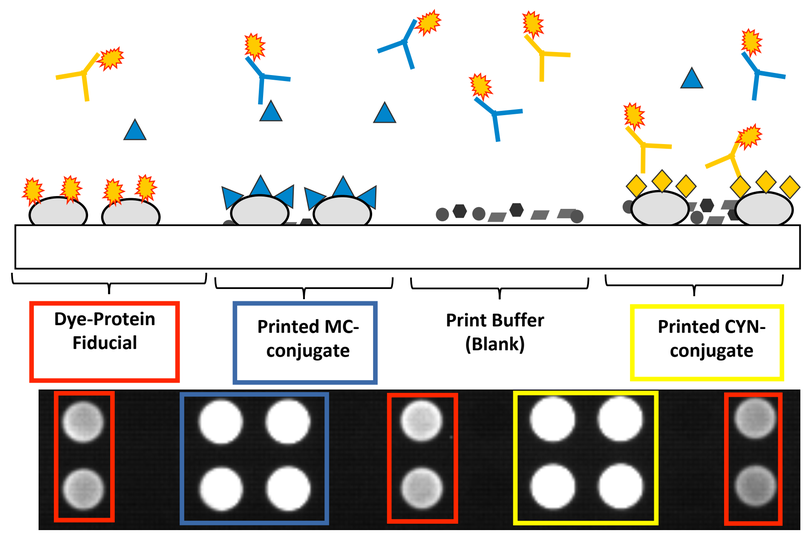
MBio LIGHDECK® TECHNOLOGY

- Fluidic cartridge-based multiplexed immunoassays
- Novel planar waveguide-based illumination
- Fluorescence imaging detection
- Spatial multiplexing with microarray technology
- Quantitative output through proprietary software









RESULTS ON NATURAL WATER SAMPLES

- 45 lake water samples were collected and tested on MBio platform and with a reference ADDA ELISA
  - 33 from Lake Erie
  - 12 from Colorado
- MBio results are the average of 3 measurements
- All samples were frozen and thawed 3x prior to measurement
- CYN was not detected by either ELISA or MBio in these samples
- Agreement between MBio and ADDA ELISA results for MC for Colorado and Western Basin of Lake Erie near Toledo
- In Sandusky Bay, Lake Erie, MBio reports lower concentrations of MC than ADDA ELISA, possibly due to congener cross reactivity
- Agreement of tested dilutions of CYN producing cell culture on MBio and ELISA

Colorado			
	Sample	MBio [ ]	ADDA ELISA [ ]
Active Bloom	Retention Pond A, Sample #1	16.54	20.25
	Retention Pond A, Sample #2	>80	>100
	Retention Pond B	<0.6	<0.15
No Bloom Visually Present	Aurora 1	<0.6	<0.15
	Aurora 2	<0.6	<0.15
	Aurora 3	<0.6	<0.15
	Boulder 1	<0.6	<0.15
	Boulder 2	<0.6	<0.15
	Westminster 1	<0.6	<0.15
	Westminster 2	<0.6	<0.15
	Westminster 3	<0.6	<0.15
	Westminster 4	<0.6	<0.15

CYN Producing Cell Culture CS506		
Sample	MBio [ ]	ELISA [ ]
1:100 dilution	>2.7	2.10
1:200 dilution	1.50	1.67
1:300 dilution	0.93	1.12
1:400 dilution	0.63	0.76

Western Basin, Lake Erie near Toledo		
Sample	MBio [ ]	ADDA ELISA [ ]
GR1, Week 0	<0.6	0.19
GR1, Week 4	2.38	2.11
MB18 Week 4	6.42	6.66
MB20 , Week 0	<0.6	0.22
7M, Week 0	>4.0	>5.0
7M, Week 1	<0.6	0.22
4P, Week 0	<0.6	<0.15
4P, Week 1	<0.6	0.35
CRIB, Week 1	<0.6	0.50
CRIB, Week 4	2.04	1.75
8M, Week 1	<0.6	0.29
8M, Week 4	4.52	6.38
Buoy (EW 5), Week 4	4.81	7.87

Sandusky Bay, Lake Erie		
Sample	MBio [ ]	ADDA ELISA [ ]
Environment Canada Station 1163, Week 3	1.53	2.12
Environment Canada Station 1163, Week 5	<0.6	1.85
ODNR 1, Week 1	2.68	6.49
ODNR 1, Week 3	2.81	5.43
ODNR 2, Week 1	1.80	2.75
ODNR 2, Week 3	3.30	6.13
ODNR 2, Week 5	2.55	6.05
ODNR 4, Week 1	0.61	0.71
ODNR 4, Week 5	2.01	4.15
ODNR 6, Week 1	2.02	3.11
ODNR 6, Week 3	2.56	6.15
ODNR 6, Week 5	2.86	6.15
Sandusky Channel Bells, Week 1	1.03	1.25
Sandusky Channel Bells, Week 3	<0.6	0.27
Sandusky Channel Bells, Week 5	<0.6	0.49
Sandusky Buoy 2, Week 1	2.36	4.80
Sandusky Buoy 2, Week 3	2.15	5.24
Sandusky Buoy 2, Week 5	1.56	3.21
Edison Bridge, Week 3	2.56	6.91
Edison Bridge, Week 5	2.53	6.49

See Reference 1

MQ Algae Lyse

- Rapid (10 minutes)
- Field portable, battery operated
- Effective (>95% lysis efficiency)
- Mechanical (bead beating) with a rotating whisk
- Measured toxin concentrations comparable to 3x freeze thaw cycles
- Cell lysis measured with microscopy

See Reference 1

Comparison of lysis methods with cell cultures

Organism:	*Microcystis aeruginosa*		*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%

Comparison of lysis methods with natural water samples

Organism:	*Aphanizomenon*	*Anabaena*	*Microcystis aeruginosa*
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%

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